In tea soil, the population level of *Azospirillum* was higher than PSB. The population level of these organisms was influenced by age of the tea bushes, nature of the soil and cultural practices. The soil temperature, moisture level, pH, atmospheric temperature and rainfall influenced the soil microflora (Pandey and Palni, 1999). Tea roots secrete root exudates that contained antimicrobial metabolites which also influenced the quantity and quality of rhizosphere microflora (Pandey and Palni, 1996). Plantation crops like arecanut, cashew, cocoa, rubber, cardamom and sapota grown in acidic soils colonized *Azospirillum* in their root system (Govindan and Purushothaman, 1985; Hu *et al*., 2006). Gajendiran and Mahadevan (1989) isolated nitrogen fixing bacteria from tree species of *Tectona grandis*, *Mangifera indica*, *Helicterus isora*, *Themeda cymbaria* and *Anogeissus*. Nair and Subba Rao (1977) found that mixed cropping system stimulated the population of nitrogen fixing bacteria in the rhizosphere of coconut. Population level of PSB also varied with soil type, climatic conditions and cropping history (Kucey, 1983 and Gupta *et al*., 1986). Yadav and Singh (1991) opined that the large variation in the population of *Azospirillum* and PSB in different soils was due to the difference in organic carbon content and types of crops (Gaind, 1987).

The population level of both *Azospirillum* and PSB were higher in the rhizosphere soil compared to the non rhizosphere soil and also there was a clonal preference. Increase in microbial population in the rhizosphere zone was attributed to the influence of factors like root exudates and decaying root materials. Rhizosphere effect indicated the affinity of microorganism to its host plant. Johri *et al*. (1999) found greater number of PSM in rhizoplane and rhizosphere than root free area of *Prosopis juliflora* and in rhizosphere of
wheat, maize, cow pea, groundnut, soyabean, green gram, and rye grass (Yadav and Dadarwal, 1997). Rhizosphere is the zone of increased microbial activity immediately around the roots of higher plants (Harmastini et al., 1990).

Among ten *Azospirillum* strains, eight were identified as *Azospirillum lipoferum* and the remaining two as *A. brasilense*. Among PSB strains, seven were identified as *Pseudomonas putida*, two as *P. fluorescens* and remaining one as *Bacillus megaterium*. This indicated that *A. lipoferum* and *Pseudomonas* spp. were dominant species/genera in tea soils. Isolates from jackfruit, breadfruit, sweet potato, turmeric were identified as *A. lipoferum* and that from coconut, cocoyam, elephant foot yam and sugarcane as *A. brasilense* (Govindan and Purushothaman, 1985).

RAPD profile of selected *Azospirillum* and PSB strains was studied with random primers and developed dendrogram. Diversity within certain species of nitrogen fixing soil bacteria including *Azospirillum* (Fani et al., 1993) and *Bacillus* (Mavingui et al., 1992) has already been reported. The dendrogram constructed based on the dissimilarity coefficient showed grouping of the isolates into different clusters based on the existence and occurrence. The dendrogram of different isolates of *Pseudomonas* was constructed using RAPD markers showed 60 per cent dissimilarity among *Pseudomonas* associated with pearl millet, cotton and paddy (Loganathan et al., 1999). It was well known that organic soil had higher diversity than sandy soil due to their physico-chemical complexity (Torsvik et al., 1996).
Biology of *Azospirillum* and PSB

Tolerance to pH and temperature, resistance to antibiotics, fungicides, and pesticides, utilization of carbon, nitrogen and amino acid sources varied within the selected strains of *Azospirillum* and PSB. These variations are mainly due to the nature of strains and differences in the species of respective genus. The pH ranges for the optimum growth of *A. amazonense*, *A. lipoferum* and *A. brasilense* strains isolated from variety of habitats were found to be 5.7 - 6.5, 5.7 - 6.8 and 6.0 - 7.3 respectively (Baldani *et al.*, 1986b). Acidic pH was favourable for the growth as well as solubilization of P by PSB (Sperber, 1958a; Molla *et al.*, 1984). In the present study it was found that the preferential temperature for the growth of *Azospirillum* and PSB strains was 15 to 35ºC above and below which the growth was retarded. The optimum temperature for growth of *Azospirillum* was reported to be between 32 to 40ºC (Day, 1977), while PSB preferred a temperature range of 30 - 35ºC (Gaind and Gaur, 1999).

Similarly, a variation was noticed among the selected strains on their tolerance level to various antibiotics, pesticides and fungicides. Similar observations were made by a number of workers (Gadkari, 1987; Bezbaruah, 1999; Rai, 1986). Balandreau (1986) observed variation in the resistance level of microorganism belonging to same species isolated from comparable habitats or even from the same habitat. Dobereiner and Baldani (1979) reported that *Azospirillum lipoferum* and *A. brasilense* showed low level of resistance to streptomycin. Mishra *et al.* (1979) reported that *S. lipoferum* was able to tolerate up to 500 ppm of streptomycin, 100 ppm of novobiocin and 10 ppm of kanamycin. *Azospirillum amazonense* strains were resistant to penicillin but relatively tolerant to chloramphenicol and
erythromycin (Magalhaes et al., 1983). Govindarajan and Prushothaman (1984) screened 80 isolates of *Azospirillum* with respect to tolerance of antibiotics and found that almost all isolates had low level of tolerance to streptomycin.

Agrochemicals especially pesticides and herbicides had adverse effect on *Azospirillum* growth (Gadkari, 1987) and incorporation of insecticides in the growth media caused either cell disruption or formation of cyst-like bacteria. The pesticides generally reduced the population counts and inhibitory effect varied with different pesticides in tea soils (Bezbaruah, 1999). On the other hand, application of herbicides in very low concentration significantly augmented the proliferation of aerobic non-symbiotic nitrogen fixing bacteria in the rhizosphere but in higher concentration the effect was negative (Debnath et al., 2002). They pointed out that the microorganisms utilized the herbicides and their degraded products for their growth and development.

There was a marked difference in the carbohydrate utilization between the species of *Azospirillum*. *A. lipoferum* grew on D-glucose but *A. brasilense* did not (Goebel and Krieg, 1984; Loh et al., 1984; Martinez-Drets et al., 1984). Konde (1984) reported that *Azospirillum brasilense* grew well in succinate, glycerol, D-fructose, D-gluconate, D-galactose, and L-arabinose but not on D-glucose, while *Azospirillum lipoferum* grew well in succinate, glucose, and mannose and α-ketoglutaric acid. *Pseudomonas fluorescens* utilized variety of carbon compounds as energy source. Among the carbon sources tested, glucose was found to be best followed by galactose for both tricalcium phosphate and rock phosphate solubilization (Dave and Patel, 2003).
The nitrogen sources were poorly utilized by *Azospirillum* strains, while nitrogen compounds supported the growth of PSB strains. It was observed that *Azospirillum* isolates fairly utilized both inorganic and organic nitrogenous compounds (Konde, 1984). *A. brasilense* preferred organic acids such as malate, succinate, lactate and pyruvate, (Burris *et al.*, 1978; Dobereiner, 1983a).

Dave and Patel (2003) experimentally proved that among various N sources tested, ammonium sulphate and ammonium nitrate were best for the solubilization of rock phosphate and tricalcium phosphate and urea and asparagine were the least for *Pseudomonas fluorescens*. Sodium nitrate, calcium nitrate and potassium nitrate were moderate sources of N for P solubilization. In contrary, the findings of Gaur and Sachar, (1980) showed asparagine as the best nitrogen source for P solubilization by three strains of *Pseudomonas striata*.

All the amino acids supported the growth of *Azospirillum* and PSB strains. The preference of amino acids differed from strains to strains for both *Azospirillum* and PSB. Amino acids such as L-cystine and L-tyrosine supported good growth of both *A. brasilense* and *A. lipoferum* (Konde, 1984).

There was a variation in the ammonia excretion by *Azospirillum* strains. The time required for the maximum production of ammonium was 12th day and then declined. It was found that three promising strains of *Azospirillum* were able to release ammonia in the culture medium which was influenced by the incubation period (Kundu, 1987). He suggested
that this may be due to cell lysis or impairment in assimilation. Hartman et al. (1983) reported that during early stages of growth, these organisms utilized the fixed ammonia present in the vicinity for their own growth but subsequently released into the medium. Excretion of ammonia is a desirable character of diazotrophic bacteria which contribute towards plant productivity (Anand et al., 1999).

Kundu (1987) observed that ammonia excretion took place only after the exhaustion of carbon sources. Variation in medium composition showed remarkable effect on release of ammonia. He concluded that Azospirillum under limited carbon availability released a part of their fixed nitrogen as ammonia and it was varied with strains to strains.

Poly-β-hydroxy butyrate is a common reserve food material in the bacterial world which imparts tolerance to higher temperature (Tal and Okon, 1985). Present study showed the existence of wide variation in the production of PHB by different Azospirillum strains. Similar observation was reported by Purushothaman and Vijila (1989). They have observed the accumulation of PHB in the Azospirillum cells that grown at 50°C confirming its involvement in imparting temperature tolerance.

A positive correlation was observed between amount of EPS production and in vitro N fixation. The strains M 2 and AN 45 produced higher amount of EPS and possessed higher N fixation. Similar observation was made on Azospirillum isolated from Pennisetum americanum L. (Garg et al., 1996) and opined that Azospirillum produced higher concentration of EPS and helped in the colonization of host roots. This view was supported by Michiels et al. (1989) and they have observed that the anchoring of Azospirillum on root
surface was increased by the production of EPS. Wani et al. (1976) reported that the EPS production was higher in stationary culture compared to shake culture and production was positively correlated with population level of particular strain. A very strong correlation was observed between EPS production and nitrogen fixation by N-fixers.

PSB strains produced more EPS compared to Azospirillum. Among PSB, it was maximum with MP 5 (Pseudomonas putida) and least in WP 11 (Bacillus megaterium). On the contrary, Tank and Saraf (2003) reported higher EPS production in Azospirillum isolate compared to Pseudomonas and Bacillus isolates. It has been proved that EPS production provides several ecological advantages to the organisms (Garg et al., 1996).

Production of siderophore was not significantly varied between Azospirillum strains. Mori et al. (1992) observed that almost all azospiral strains produced siderophores for the conversion of nitrogen to ammonia. Production of siderophores was influenced by the environmental factors and media composition, especially carbon source and trace elements (Sharma and Johri, 2003). It was also reported that the production of siderophores positively correlated with nitrogenase activity and culture conditions (Shah et al., 1993).

Among PSB strains, Pseudomonas spp. produced more amount of siderophore compared to Bacillus megaterium (WP 11). This finding strongly supported by Chincholkar (2000) that among large diversity of bacteria, pseudomonads were characterized by the production of siderophores. This report was further supported by Meyer and Abdallah (1978) and Cox and Adams (1985) that Pseudomonas fluorescens, P. putida and P. aeruginosa produced different types of siderophores. It has been experimentally proved that among other
microorganisms, fluorescent pseudomonads are known to produce higher amount of siderophores (Sharma and Johri, 2003; Subba Rao, 1977) and this was attributed to their biocontrol potential.

Siderophores act as growth factors and/or antibiotics. Siderophore B10 inhibits the growth of *Erwinia carotovora* which causes the soft rot disease of potato. Siderophore pseudobactin deprived *E. carotovora* of Fe by scavenging the available element in the vicinity and thus reduced disease severity by minimizing the virulence of the pathogen. Similar response was observed with *F. oxysporum* in flax seedlings and *Gaemannomyces graminis* in wheat infected by take-all disease (Subba Rao, 1995).

*Azospirillum lipoferum* M produced catechol–type siderophores under iron starved conditions that exhibited antimicrobial activity against various bacterial and fungal pathogens (Shah *et al.*, 1992). Twenty seven *Azospirillum* strains produced bacteriocins that inhibited the growth of several pathogenic bacteria (Tapia-Hernandez *et al.*, 1990).

Plants inoculated with *Pseudomonas* suppressed the soil born pathogen (*Fusarium oxysporum*) and increased the seedling survivability up to 90 per cent (Subba Rao, 1995). Presence of siderophore suppressed the plant pathogens *Yersinia pestis, Pseudomonas aeruginosa, Vibrio vulnificus* etc. (Litwin *et al.*, 1996). The mechanism of suppression of pathogen is that the siderophore binds to the Fe$^{3+}$ that is available in the rhizosphere and there by effectively prevent the growth of pathogenic in that region (Suslow, 1982).
The nitrogenase activity was greatly affected by biotic and abiotic factors. Addition of carbon sources had a positive impact, while nitrogen sources had a negative impact. Inorganic nitrogen sources caused significant reduction in nitrogenase activity, while organic sources such as amino acids and protein either stimulated or did not significantly inhibited the activity (Rao and Venkateswarlu, 1982). Das and Mishra (1983) observed that acetylene reduction was best with fructose and malate than succinate, pyruvate and lactate. Further, isolates of A. lipoferum exhibited a higher nitrogenase activity compared to A. brasilense in nitrogen free medium (Han and New, 1998).

The optimum pH range for nitrogen fixation was 4.5 - 6.5. Karthikeyan (1994) isolated acid tolerant strains of Azospirillum that could fix nitrogen at pH levels of 4.5 to 6.0. A decrease in the nitrogenase activity was recorded when the pH was raised to 7.8. An optimum pH of 7.1 to 7.4 was recorded by Okon et al. (1977a) for growth and nitrogenase activity. Charyulu and Rao (1980) found that isolates originated from low pH (< 4.0) had lower nitrogen fixation than those from soils of higher pH up to 6.6.

Azospirillum required an optimum temperature of 32 - 40°C for nitrogen fixation (Day and Dobereiner, 1976; Jagnow, 1982), a little fluctuation with in this range would not alter nitrogen fixation (Albrecht et al., 1977; Dobereiner, 1980). Temperature less than 32°C and above 45°C inhibited the nitrogen fixation (Neyra and Dobereiner, 1977). Azospirillum brasilense, A. lipoferum and A. halopraeferens showed optimum level of acetylene reduction activity at 25, 30 and 40°C respectively. It was found that the temperature above 35°C inhibited both nif gene expression and acetylene reduction activity (Tripathi and Klingmuller,
1992). In the present study, optimum temperature for nitrogenase activity of *Azospirillum* was found to be 20 - 30°C. The organism was able to tolerate up to 40°C above which the activity was retarded drastically.

There was a positive correlation between the population level and acetylene reduction up to $1 \times 10^8$ after which there was a reduction. The population level lower than $1 \times 10^5$ and above $1 \times 10^8$ reduced the acetylene reduction. This reduction may be due the physiological requirement of the organism. This indicated the minimum requirement of the population to bring out the biological properties to a significant level.

There was a wide variation among the strains in the acetylene reduction activity in the roots. Only limited information is available on the rates of AR activity on the roots of dicotyledonous plants (Neyra and Dobereiner, 1977). Acetylene-reduction activity of *Azospirillum* in association with wheat root was between 1 - 5 and 1 - 10 n mole C$_2$H$_4$ mg dry roots$^{-1}$ h$^{-1}$ and the activity was strictly dependent on live bacteria in roots (Han and New, 1998). *A. brasilense* was found in association with the root population at $8 \times 10^7$ cells per gram dry weight. At this population level, the acetylene-reduction activity was found at the maximum of $2.8 \mu$ mole C$_2$H$_4$ g dry roots$^{-1}$ h$^{-1}$ (Okon *et al.*, 1983).

The nature of soil greatly affected the nitrogenase activity. The activity was higher in Anamallais and Vandiperiyar soil with their respective strains. Biological nitrogen fixation in a plant-soil system was strictly dependent on the soil O$_2$ tension and the concentration of mineral N (Alexander *et al.*, 1987). Charyulu and Rao (1980) reported that nitrogen fixation by *Azospirillum* widely varied with soil type, soil organic matter and N content. It was
noticeable that the strains with the lowest nitrogen fixation were associated with light
textured and low pH soil.

Organic matter (OM) is a natural substrate for saprophytic microorganisms and
provides nutrition to plants indirectly through the activity of soil microorganisms. In soil rich
in organic matter, the nitrogenase activity was higher compared to those with low OM
content (Subba Rao, 1995). This finding was further supported by Van Berkum and Bohlool
(1980) that nitrogenase activity of *Azospirillum* was greatly affected by the difference in
organic matter content. It has also been reported that the higher organic matter content of the
soil increased the activity of beneficial microorganisms (Iswaran and Chhonkar, 1971).

The carbon and nitrogen sources greatly affected the phosphate solubilization of PSB.
P solubilization differed between strains. This may be due to the nature of organic acids and
enzymes produced by strains. Based on the nature and quantity of organic acid produced by
PSB, the P solubilization differed between the strains. Glucose was the best carbon source for
almost all strains. Likewise, ammonium sulphate and ammonium nitrate were the best
nitrogen sources for these organisms. The form of carbon sources greatly affected the
phosphate solubilization *in vitro and in situ* and was more active in presence of hexoses and
pentoses or disaccharides (Patil *et al.*, 2001). PSM produced significant quantity of organic
acids as metabolic by-products depending on various carbon sources (Bhattacharyya and
Jain, 2000). Dave and Patel (2003) found that all the inorganic nitrogenous compounds
supported phosphate solubilization more efficiently than the organic compounds.
The nature of phosphate sources affected the solubilization by PSB strains. Among them, Ca$_3$(PO$_4$)$_2$ was the best phosphate source for all PSB strains followed by FePO$_4$, Mg$_3$(PO$_4$)$_2$ and AlPO$_4$. Among various phosphate sources, solubilization was comparatively low with aluminium phosphate (AlPO$_4$) than other phosphate sources. The solubilization of different types of insoluble phosphate was varied with the type of microorganisms, the type of phosphates available, media conditions and available carbon sources (Yadav and Dadarwal, 1997; Kucey, 1988b). Similar results were obtained by Dave and Patel (2003) where PSB solubilized different types of P sources in the liquid medium. However, most of the strains did not show a higher P solubilization with AlPO$_4$ and FeSO$_4$ compared to Ca$_3$PO$_4$.

**Mass multiplication**

In fermentor, the growth was rapid compared to flask culture. This may be due to the controlled pH, temperature, aeration and nutrient supply which facilitated the rapid growth of culture in a fermentor. A viable count ranged from $10^9$ to $10^{10}$ ml$^{-1}$ is preferred for the preparations of bioformulation (Dadarwal et. al., 1997). And this population was attained within 3 - 5 days in the case of fast growing organisms such as *Azospirillum*, *Pseudomonas* and *Bacillus* sp. In the case of slow growing organisms, it took about 6-7 days to reach such counts (Dadarwal et. al., 1997). Most of the laboratories the practice is in using logarithmic or late logarithmic phase culture with fermentation period of 30 - 72 h or 10 - 15 day old culture (Sadasivan and Neyra, 1985).
The shelf life could be improved by paying attention to the physiological stage of the cells eg. high content of PHB, cysts and flocculates. The cells in the form of cysts will survive better because the cysts have storage materials and growth promoting substances (Sadasivan and Neyra, 1985). In PSB, the shelf life could be increased by using the strains in the sporulating stage. The sporulating culture was mixed with carrier materials which enhanced the shelf life of the organism (Thangaraju, 1996; Anandham et al., 2007). The quality of bioformulations was also determined by their shelf life. The shelf life of *Azospirillum* and PSB was higher in composted coir pith followed by vermicompost (Rajannan et al., 1996; Ramalingam and Ranganathan, 2001). Gaind and Gaur (1990) reported that at storage temperature of 28ºC and 35ºC and the RH had little effect on shelf life of the organisms used. Moisture content of carrier material influenced shelf life of PSB (Gaur and Gaind, 1984). Shelf life of beneficial organisms was decreased in paddy straw. This may be attributed to the production of phenolic acids composition of organic material (Gaur and Pareek, 1972). *B. polymyxa*, a spore former, could not withstand the high temperature and low moisture status of the carrier which may be due to its ability to produce polysaccharide extracellularly (Rolz and Leon, 1988).

Based on the *in vitro* and *in vivo* experiments, Muthukumarasamy et al. (1996) suggested that vermicompost can be used as an alternative carrier material to lignite. In addition to these, composed coir pith is a good carrier for bacterial as well as fungal inoculants (Baby, 2004). Finely ground vermiculite supplemented with nutrient sources and moisture could be used for the preparation of bacterial inoculants. An inoculant was formulated by Fages (1992) that consisted of dried micro-encapsulated bacterial cells
dehydration in a polymer matrix. In this formulation, the number of viable cells decreased to $10^9$ cfu g$^{-1}$ from $10^{12}$ within 90 days of storage.

Present study revealed that composted coir pith is a good carrier for bacterial strains. There was a variation in the response of seedling and clones with respect to the carrier material. The quality of bioformulation is mainly depending on the nature of carrier materials. Among different carrier materials the population level of bioinoculants was higher in composted coir pith followed by vermicompost and the proliferation was lesser in organic manure and lignite. Composted coir pith kept the viability of microbes for a longer period by providing organic food base to the organism and retaining the moisture content. Dadarwal et al. (1997) reported that characteristics of a good carrier material are water holding capacity, pH, particle size, sticking ability on seeds and total surface area provided for absorption of microbial cells per unit weight. The composted coir pith has all these characters and the superior performance of composted coir pith formation may be attributed to this. The formulation should contain $1 \times 10^7$ cfu g$^{-1}$ of carrier material in the case of bacteria and $1 \times 10^6$ for fungi (Baby, 2004).

The nature of carrier material, shelf life and inoculum potential are important in the quality of bioinoculants. The mass production technology for biofertilizers involves careful selection of the microbial strains, a low cost growth medium and a suitable carrier material for a long shelf life. Quality of bioinoculants is one of the most important factors deciding their performance. A good carrier material is one which can keep up the viability of microbes
for a longer period by providing organic food base to the organisms and retaining the moisture content.

The survival of *Azospirillum* and phosphate solubilizing bacteria was better in press mud compared to peat, lignite, and composted press mud, but in the field the survival of *Azospirillum* was better in composted pressmud applied soil (Rajannan *et al.*, 1996). Govindarajan (1987) studied the survival of *Azospirillum lipoferum* in peat, coir pith and peat-coir pith mixture and found that the peat supported the maximum proliferation of *Azospirillum* than other carriers. On the other hand, Sureshkumar (1996) found that pressmud was a superior carrier compared to peat and lignite. The survival of *Azospirillum* and phosphate solubilizing bacteria was better in press mud compared to peat, lignite, and composted press mud, but in the field the survival of *Azospirillum* was better in composted pressured applied soil (Rajannan *et al.*, 1996).

**Nursery and field performance of biofertilizers**

Application of *Azospirillum* and PSB increased the plant growth and soil nutrients under both nursery and field conditions. The response varied with time, dosage, frequency and mode of application, bacterial strains and type of planting material.

Amendment of nursery soil with bioformulation increased its pH as well as EC. Among the three clones tested, rooting was poor in UPASI-3, while the other two exhibited 90 to 100 per cent rooting. In UPASI-3, the cuttings exhibited club callusing with poor root development in most of the treatments. This may be due to the increased pH and EC of the soil. UPASI-3 is known to be highly sensitive to pH and soil texture (Venkataramani and
Sharma, 1969). Interference of pH on rooting success in organic manure/biofertilizer treatment in nursery has already been reported (Victor, 2000). The per cent success in other two clones could be due to their tolerance to pH. The seedlings were unaffected by the change in pH confirming their higher tolerance to pH.

The response to bioformulation was more prominent when it was incorporated in the growing zone than in the rooting zone. In addition to this, application at different time interval indicated that the response was prominent when applied two months after putting out cuttings. Tea being highly sensitive to pH, exposure of cuttings directly to higher pH and EC resulted in callusing and subsequent inhibition on the root development. In the case of treatment where in bioformulation was applied two months after putting out of cuttings had normal callusing and rooting. This further confirms the interference of pH on rooting of cuttings. Since the biofertilizer was applied at the time of root initiation, easy colonization of them on the root system was possible resulting in quick and prolonged response. Fallik et al. (1988) and Chakraborty et al. (2002) found that the time of application of Azospirillum influenced the root growth in maize. They observed a difference in the root surface area between pre-emergence (at the time of sowing) and post emergence (after the appearance of the second leaf) treatment.

The response to biofertilizer application was varied with different clones. This may be due to the difference in the nature of colonization, root exudates produced by plants, nature of the root system and their genetic make up. Plants and bacteria closely interact influencing their activity. Kundu et al. (2002) were of opinion that the genetic make up of
plants determines the composition of root exudates. Michiels et al. (1989) suggested the chemotactic response to organic acids as one of the factors determining host-specificity in colonization.

The biofertilizer application increased the yield in both high and low yielding fields. The response to biofertilizer application was more when the soils were optimally fertilized. A significant increase in yield was achieved by the application of biofertilizers with intermediate rate of N-fertilizer (Okon and Labandera-Gonzalez, 1994; Bashan et al., 2006). Application of biofertilizer can be adjusted based on the OM content of the soil, which is an integrated approach for sustainable productivity. In tea soil, OM content was positively correlated with the population level of Azospirillum. Baby (2002) reported that Azospirillum population was higher with increase in amount of OM content.

In the agricultural field, higher level of organic matter content increased the plant growth and development. High level of OM content improved the soil physical nature and it was positively correlated in the improved plant growth and yield of crop plants (Rajagopal and Ramarethinam, 1997). Higher OM content in the soil facilitated the survival of plant growth promoting organisms such as Azospirillum, Rhizobium, Azotobacter etc. (Tilak et al., 1979). Organic matter helps to maintain high viable count of beneficial microorganisms (Tilak and Singh, 1994). High level of organic matter content increased the water holding capacity and neutral pH for better survival of the microorganisms (Date, 1968).

Organic matter influences physical, chemical and biological properties of soil despite its small proportion in the soil. It commonly accounts for at least half the cation exchange
capacity of the soil and is perhaps responsible for more than any other single factor for the stability of the soil. Moreover, it supplies energy for microorganisms whose activities are of vital importance in soil dynamics (Surendra Mohan, 1995).

The response of tea plant to bioformulation was more when applied by placement compared to broadcasting. This was reflected in the yield response as well as population buildup of Azospirillum and PSB. In placement, the bacteria can be introduced near to the root zone facilitating their easy colonization on the feeder root. The higher response to placement may be attributed to this factor. However, the method of application should be practical, economical and easy to accomplish for the farmer. Seed dressing, placement in furrow, broadcasting are the common methods of application. All these methods provided positive results. However, the volume of inoculants needed to achieve optimal results depends on the cell concentration (Okon and Labandera-Gonzalez, 1994; Ellis, 1995; Jat and Shaktawat, 2001).

The split application of biofertilizers (two splits in a year) was superior to one time application with respect to yield, population buildup and soil nutrient status. The time of application of biofertilizers is crucial as the bacterial inoculation should be at a time when plants need it (Bashan, 1986a). The use of biofertilizers for winter wheat had limited value until obtaining an understanding on the factors influencing the rhizosphere competence of bacterial inoculants (Kucey, 1988a). Further, an optimum dosage of biofertilizer is needed to bring out the desired result. In tea, 25 kg/ha was found as optimum. In maize, an optimal density of Azospirillum was not required to promote yield. However, it was found that the
optimal density is indispensable up to the emergence of the radicle (Jacoud et al., 1998). Application of 75 per cent of recommended dose of N:P (40:20 kg/ha) along with biofertilizers (2-4 kg/ha) significantly increased the grain and straw yield of sorghum-horsegram cropping system (Kapulnik et al., 1981a; Morgenstern and Okon, 1987; Parasuraman et al., 2000). In wheat, a significant increase in the yield and P uptake was observed with integrated use of rock phosphate and PSB @ 30 - 35 kg/ha (Narayanasamy et al., 1981; Dubey et al., 1997).

The dual inoculation of *Azosphirillum* and PSB increased the N-metabolism and P-metabolism in tea plants and soils under nursery condition. Dual inoculation of *Azosphirillum* and PSB improved the N and P metabolism and growth hormones compared to control. In *Azosphirillum* treatments an increase in total N and nitrogenase activity and in PSB treatments increase in available P and phosphatase activity were noticed irrespective of the carrier material. Dual inoculation further showed an increase in plant growth hormones in soil and population build up of the respective organisms. The positive benefits from inoculation were attributed to several mechanisms such as nitrogen fixation, phytohormones production, enhanced nutrient uptake (Wani, 1990; Barea et al., 1983; Kapulnik et al., 1981b; Fernandez et al., 2007) and phosphate solubilization (Frietas and Germida, 1990). Padmavathi et al. (1991) investigated that the dual inoculation of *Azosphirillum lipoferum* and phosphate solubilizers greatly helped in the uptake of nitrogen, phosphorus, zinc, copper and iron in the shoots of foxtail millet under field conditions.
*Azospirillum* strains produced several plant hormones. The major hormone produced were indole -3- acetic acid (IAA) and indole -3- butyric acid (IBA) (Fallik *et al*., 1989) and other hormones were detected at much lower, but biologically active level. These hormones include indole -3- lactic acid (Tien *et al*., 1979), indole -3- methanol (Crozier *et al*., 1988) and other unidentified indole compounds (Hartmann *et al*., 1983), cytokinins (Horemans *et al*., 1986) and several gibberellins (Bottini *et al*., 1989). Tien *et al*. (1978) found that *A. brasilense* was able to produce gibberellins and cytokinin like substances in the liquid culture (Vlassak and Reynders, 1978). *Azospirillum brasilense* was found to produce high amounts of IAA (Horemans *et al*., 1986), however, it depended on the species and strains (Hartman *et al*., 1983; Omay *et al*., 1993). Further, the IAA production was proportional to the bacterial population in the medium, when other factors were not limiting. Likewise, phosphate solubilizing bacteria also produced growth promoting substances such as auxins, gibberellins and cytokinins, which improved the plant growth and stimulated the microbial development (Sattar and Gaur, 1987; Sullia, 1968).

The application of bioformulation also increased the soil nutrient level such as total N and available P. In *Azospirillum* and PSB treatments, the total N and available P were increased. This increase in the soil nutrient level was responsible for plant growth and development. *Azospirillum* inoculation increased plant growth, dry weight, total nitrogen content and yield of cereal and forage grasses (Nur *et al*., 1980; Kundu, 1988). In addition to morphological effect, there was an increase in biochemical characters (Wani *et al*., 1985). *Azospirillum* inoculated plants showed increase in the level of total nitrogen in different parts of the plant (Saxena *et al*., 1990). Inoculation with nitrogen fixing bacteria always increased
leaf NRA suggesting a greater supply of NO$_3$ to the plants over uninoculated control. The increased NO$_3$ uptake may relate to increased root development in response to production of hormones (Tien et al., 1979; Tilak and Subba Rao, 1987). Ferreira et al. (1987) reported that wheat plants inoculated with *Azospirillum* showed greater activity of the nitrate reductase enzyme in the leaf compared to uninoculated plants.

**Role of biofertilizers in organic farming**

Organic farming is best suited for perennial plantation crops like tea, which provide proper shade and cover to the soil and keep the soil microbial activity constant. To keep the soil microbi ally active, there should be enough organic matter and hence an organic farming system will be ideal (Baby, 2003). One way of achieving organic farming is through integrated nutrient management rather than sole reliance on chemical fertilizers. For sustainable productivity, chemical fertilizers, organic manures and biofertilizers should be given as complementary to each other in a balanced way (Baby, 2002).

In the present study, application of organic inputs such as compost, neem cake, wood ash and rock phosphate along with biofertilizers gave higher yield. On the other hand, in treatment with organic manures wherein biofertilizers were not given, there was a reduction in the yield. Inoculation of biofertilizers along with organic manure gave better results than their individual application and combination of two or three bioinoculants (Rajagopal and Ramarethinam, 1997; Behera et al., 2007). Agronomists have visualized potential of organic farming for increase in growth and flavor quality of tea.
Organic inputs increased the soil N and P content. The population buildup of *Azospirillum* and PSB was higher in the treatments supplemented with organic manure. It was proved that application of organic manures increased the biological properties of soil that increased the populations build up of beneficial microorganisms (Subba Rao, 1995). These organic inputs are facilitating good habitats of beneficial microorganisms in the soil environment.

The activity of beneficial microorganism in soil was enhanced by the application of organic inputs. It will maintain as well as improve the population level of agriculturally important microorganisms (Calvaruso et al., 2007). Baby (2006) opined that the organic input as biofertilizers reduced the application of chemical fertilizers considerably in the fertilizer schedules. A reduction of 15 - 20% of nitrogenous fertilizers and even up to 50% of phosphatic fertilizers was possible by applying biofertilizers.

Integrated fertilizer management with finger millet (*Eleusine coracana*) revealed that seed inoculation of *Azospirillum* gave an additive effect over farmyard manure (FYM), nitrogen and phosphorus application alone and the effect of *Azospirillum* was found to be equivalent to 20 kg N/ha through urea. The combined application of *Azospirillum* and nitrogen gave better effect than *Azospirillum* with phosphorus. However, rock phosphate (RP) recorded growth and yield on par with single super phosphate (SSP). Highest dry matter, ear heads/m², length of finger, NPK uptake and grain and straw yield were obtained by *Azospirillum* + N₈₀ followed by *Azospirillum* + N₆₀ (Chakraborty et al., 2002). Integrated fertilizer management with wheat increased the yield and reduced nitrogenous fertilizer
requirements up to 40 kg/ha. This amount is economically important and indicated the higher potential of grass-bacteria system (Parasuraman et al., 2000). Under field condition, application of organic fertilizers and biofertilizers (phosphate solubilizing bacteria and *Rhizobium*) and inorganic fertilizers (phosphorus and sulphur) gave better yield response in fenugreek (*Trigonella foenum-graecum*).

In cotton, combined inoculation of *Azospirillum*, *Pseudomonas striata* and 50 % urea and 100 % P as rock phosphate gave better response, nutrient uptake and population buildup of beneficial microorganisms (Prathibha et al., 1994). In sugarcane, application of pressmud @ 20 kg/ha with *Azospirillum*, increased the percentage of germination of setts, length of cane and number of tillers. In rice, treatment with the inoculation of *Azospirillum* along with pressmud@12.5 kg/ha and N:P:K @ 100:50:50 kg/ha recorded maximum yield (Sundaram, 1991). The combined inoculation of bioinoculants such as *Azospirillum* and PSB with 50 % of N and P recorded higher microbial population, number of bolls/plants and kapas yield of rice fallow cotton (Thamizh Vendan et al., 2000; Kundu and Gaur, 1984).

Organic matter like compost on decomposition releases humic acid (HA) which acts as a growth stimulator for plants. HA facilitates the uptake of nutrients and increase the growth performance of plants. It also enhances the microbial activity and improves soil health (Gaur et al., 1971).

Organic farming is to reduce the chemical inputs and sustain crop production. Organic tea cultivation is at the conservation of ecology and natural habitat without polluting soil, air and water and yet maintaining sustainable tea production. In organic, naturally
occurring, mined products and bulky and concentrated organic manures such as compost, neemcake, wood ash, bone meal, fish meal, rock phosphate, biofertilizers and biodynamic formulation are used for nutrition and maintenance of soil fertility. Organic matter influences physical, chemical and biological properties of soil despite the small proportion present in the soil.