CHAPTER 5

DISCUSSION

This investigation was focused at the occurrence of different Arbuscular Mycorrhizal fungi (AMF), diazotrophs and phosphate solubilizers in tea growing areas of Assam along with their application for the growth and productivity of tea plant.

An attempt was made to observe the percent infection (endophytic association) rate of arbuscular mycorrhizal fungi in the tea plants in twenty tea estates comprising four agro-climatic zones of Assam namely Cachar, North Bank, South Bank (a) and South Bank (b) regions. During the study, the occurrence and percent colonization of Arbuscular Mycorrhizal Fungi (AMF) of tea feeder roots were studied and found to vary from garden to garden. The chlamydospores were also found to vary quantitatively in different tea plantations.

Different researchers have conducted and verified the association of AMF in tea feeder roots at different clones of the tea plant and found that tea plants were mycorrhizal dependant plants (Tunstall, 1925, Hazarika et al., 2001; Barthakur et al., 2005; Dutta et al., 2007). But considering different age groups and their association along with residing
weed species of tea garden areas were not studied before. This study was mainly focused on the naturally occurring mycorrhizal association and rhizosphere microflora in tea plantation of Assam.

Tea is a monoculture perennial crop preferring acidic soil (pH 4.5-5.5) with negative rhizosphere effect (Barua, 1989; Pandey and Palni, 1996). The main objective in this study was to concentrate on biological means of promoting tea cultivation among the tea growers. Hence, preparing a databank on native tea rhizosphere microflora of Assam was highly in demand as no such work had been done so far in Assam tea plantation. Moreover, isolating and manipulating the native tea soil microorganisms for the plant growth and nutrient uptake was also carried out to incorporate the beneficial native isolate in the nursery and field conditions.

5.1 Survey of Arbuscular Mycorrhizal (AM) fungi in tea feeder roots of different tea estates of Assam under different age groups:

Extent of root colonization by the AM fungi varied in different tea plantation. In Cachar region the mycelial association was maximum (44-68%) at the age group above 15 years. In North Bank also the AMF colonization was maximum in root samples of age group above 15 years (40-88%). Percent infection rate was maximum 28-88% in the roots of tea plants of Rupajuli T.E of North Bank region among the twenty tea estates studied in four agro-climatic zones of Assam. But maximum
mycelial association was recorded within 6-15 years age group in Hunwal T.E of South Bank (a). While in South Bank (b) both Maud and Lukwah recorded 60% association in the age group 0-5 years. This variability may be due to the soil factors (Anderson et al., 1983), pH (Porter et al., 1972) organic matter (Ryan et al., 1994), the main factors that can influence the distribution of AM fungal hyphae and spores. Moreover, monoculture practice of tea plantation also may contribute to the distribution pattern of mycorrhizal association as observed from the data considering different age groups. Yet, the association rate of tea feeder roots ranged from 24 to 88% in the present investigation with the formation of arbuscules and vesicles.

The presence of arbuscules, the functional unit of AM fungi was found to be maximum in the age group of 6-15 years and above 15 years in both the Cachar and North Bank regions. Presences of arbuscules were observed more in young plants than older plants as senescent of arbuscules was more frequent. The results of the present investigation tally with the work of Bonfante Fasolo, (1978), though in tea plantation above 50 years the plants are considered to be old in terms of leaf harvest (Barua, 1989; Karmakar and Banerjee, 2005).

Out of five tea estates, in Cachar four tea estates showed 44% mycelial association at the age group of above 15 years while 28% was recorded in North Bank at the age group of 0-5 years which was very
unique to the study conducted. This may be due to the changes in soil fertility (Hayman, 1982b) or the suitability of AM fungi species of a region to infect the host plant in a particular climatic condition when the samples were collected.

A particular tea clone grown in the tea estates may also be the reason behind it. The present findings are in accordance with Mosse, 1973b who stated that mycorrhizal dependency of different host species like Leguminoseae and Gramineae and even different genotypes of the same species determined the percent root colonization rate.

Trindade et al. (2006) studied the association of AM fungi in Papaya crops and stated that in specific agro-systems, with similar edaphic and climatic characteristics, it is possible to find a pattern of arbuscular mycorrhizal (AM) fungi occurrence for a defined crop (Johnson et al., 1991) and for crops from different sites and ages (Nemec et al., 1981). Hence, the attempt to study the pattern of AM fungi occurrence for tea plantation under different age groups was a mere introductory work to interpret the possibility of AM fungi infection rate from different sites and age groups.

Among the twenty tea estates, 15 tea estates recorded vesicles and nine tea estates recorded the presence of arbuscules in the age group 0-5 years. While 17 tea estates showed vesicles formation and 8 tea estates recorded arbuscules formation in the age group 6-15 years. Above 15
years also 17 tea estates recorded formation of vesicles and 8 tea estates recorded the formation of arbuscules. Presence of arbuscules and vesicles were more in South Bank region compared to Cachar and North bank region. Occurrence of arbuscules was more in the age group 0-5 years (Bonfante-Fasolo, 1978). However, vesicle formation depends on environmental conditions such as high or low phosphorus levels. The result was in accordance with work of Smith and Read, (1997). Moreover, not all AM fungi form vesicles within roots (Gerdemann and Trappe, 1974; Abbott and Robson, 1978, Abbott.L.M.,1982) and hence in some of the tea estates negligible formation of vesicles might be due to this reason.

Since, arbuscules are the preferential site for fungus-plant metabolite exchanges (Cox et al., 1975) the detection of more arbuscules in the age group 0-5 years i.e 40% and above 15 years (32 %) in Nahortoli T.E of South Bank (b) region describes the fact that during the tender stages of growth and as well as in the mature tea plants, the fungus-plant metabolite exchanges took place which proliferates the growth and uptake of nutrient naturally. Moreover, soil of South Bank region was found to harbor more AM fungi association compared to Cachar and North Bank.

Morita and Konishi, (1989) also reported that the average percentage of tea root colonization of lightly fertilized soil was two times higher than heavily fertilized fields. Different rhizosphere
microorganisms and mixed AM fungi species present in the soil strata as well as the input of chemical fertilizers to the tea estates over the years might be another reason of variation of pattern of root colonization in the present study. But from this study it was observed that irrespective of age groups and agro-climatic zones tea feeder roots were associated with Arbuscular Mycorrhizal Fungi (AMF) as integrated component which helps the tea plant in nutrient uptake from the soil.

Study of percent association of AM fungi in tea feeder roots under different age groups was done to mitigate the goal of achieving suitability of soil and AM fungi infection (endophytic association) rate in tea plants below 5 years of planting. Presence of 24 - 44 % mycelial association in the tea plants below 5 years of plantation was an indication of suitability of the host –fungus relationship in low phosphorus available soil.

The occurrence of chlamydospores varied from region to region which may be due to seasonal variation at the time of sampling. From the survey it revealed that spore load in Cachar region ranged from 19 to 45 per 50 g soil (August and July). While in case of North bank spore load was in between 19 to 47 in the month of September and October. While South bank (a) showed 19 - 43 spore load/ 50 g soil during February to March and in South bank (b) 19- 39 spore load /50 g soil was detected in the month of September to November. The results of the present study were not much deviating from the findings of Hayman, (1970); Barthakur
et al. (1987); Chakravarty and Talukdar, (1997); Hazarika, (2000); and Bouamri, (2006). No consistent correlation between Chlamydospores with soil P or N concentrations were apparent in the different age groups. Regardless, AM fungi were active across a range of soil P and N concentrations and soil pH levels and this may be attributed to more than one mycorrhizal species present in tea rhizosphere (Barthakur et al., 1987; Robson and Abbott, 1989; Hazarika, 2000).

5.1.1. Role of soil phosphorus, nitrogen on AM fungi association:

The present investigation also tried to emphasize on whether the soil chemical parameters had significant relationship with spore population. Results revealed that the nitrogen and phosphorus had a significant effect on mycorrhizal colonization. High phosphorus and nitrogen level in soil always hamper the mycorrhizal colonization and subsequent spore formation. From the Karl’s Pearson correlation analysis it was observed that spore density of four agro-climatic zones showed “high negative correlation” with phosphorus and the maximum was recorded \( r = -0.741 \) in 0-5 years age group of Cachar tea estates, whereas it was positively correlated with nitrogen \( r = 0.973 \) at 0-5 years age group in North Bank region among the analyzed data. Results obtained was unique for this region but tally with other works elsewhere (Khan, 1972; Mosse, 1973a; Hayman, 1975; Sinegani et al., 2005). Not only the soil P and N influenced the root colonization and spore load
significantly but also the soil pH, organic carbon, potassium investigated during the study may had a correlation with it.

Sometimes the correlation coefficient may not necessarily measure any true causal relationship between two variables although it was an index of causality. It may simply be a result of the influence of one or more other variables or both of the variables under study. A significant positive correlation between AM Fungi was the effect of higher N-content. The simultaneous increase in the rate of fertilizer applied and the chlamydospores. So, in general the correlation coefficient could be interpreted in the light of other factors like soil, environmental conditions etc.

The soil physico-chemical parameters studied at different sites taking different age groups differ in their availability for uptake by the plants grown in that particular portion of tea plantation. Variation was noticed in the same tea garden soil samples in different age groups though not in a very fluctuating manner. Data recorded from different agro-climatic soil zone depicted variability of physico-chemical properties. Available phosphorus to the plants in all the regions varied and revealed low content of available $P_2O_5$. Hence, AM fungal species have demonstrated an adaptive capability for tolerating a range of soil acidity as discussed by Robson and Abbott, 1989; Abbott and Robson, 1991.
This kind of soil survey taking different age groups of the same plant species grown in a monoculture practice in a particular area with heavy load of chemical fertilizer to maintain the soil acidity was not carried out before in Assam tea plantation. Hence, future prospects of determining AM fungi diversity in monoculture cropping system during different succession of plantation would be a pathway on the basis of this preliminary findings. However, this aspect was highlighted by different workers on different crops (Hayman, 1978; Nemec et al., 1981; Johnson et al., 1991).

5.2 Survey on percent association of Arbuscular Mycorrhizal (AM) fungi in dominant tea weeds:

The percent association of AM fungi with the certain dominant weeds of all twenty tea estates showed variation in regions and tea estates, though earlier studies by Barthakur et al., (1989); Hazarika, (2000) showed the presence of AM fungi in certain weeds only.

The percent association of AM fungi was found to vary from region to region like 48 -100 % AMF association in Cachar, 20- 96 % in North Bank 16- 92 % in South Bank(a) and 36- 92 % in South Bank (b) region in different weed species respectively. This might be due to the differences in the plant rhizosphere effect as the root zone and its surrounding soil was influenced by the microbial activity as well as the nature of organic compounds released by the plant root system (Roy et
Moreover, arbuscules, the functional units were found to be in abundance (4-88%) in most of the weed species which proved their efficiencies with the host plants. The weeds uptake the nutrient from the same nutrient pool as the tea plant but still in a way they help in rehabilitation process when tea bushes were uprooted. *Mimosa invisa, Mimosa pudida* and *Ageratum conyzoides* were common weeds in most of the tea gardens. Though the weeds are considered as an unwanted event traditionally but from this investigation it can be inferred as an essential component considering the beneficial effect of AM fungi and other rhizosphere microbes for their impact in nutrient management.

5.3 Occurrence of rhizosphere microflora in the tea plantation and their evaluation:

Microorganisms are the integrated component of any soil system. The rhizosphere is a dynamic environment and as such the colonization of this area by mycorrhizal fungi integrates this fungus as part of the soil microflora which is capable of contributing to the stability of the rhizosphere (Fillion *et al.*, 1999). In Assam tea soil quantitative assessment of functional groups viz diazotrophic bacteria and phosphate solubilizing microbes were not done in a broad way. Literature pertaining to this survey was scanty though general occurrence of bacteria and fungi were studied before in tea soil (Patgiri and Bezbaruah, 1990; George & Singh, 1990; Pandey and Palni, 1996). Mazumdar *et al.* (2007) had done
some pioneering works using *Pseudomonas* isolates for biochemical characterization and testing their effect on tea leaf cuttings. But, study regarding the native tea isolates and incorporating them as bio-inoculant was an initiative in this present investigation.

In the present study occurrence of bacterial population was more in Cachar region when compared with North Bank region. Bacterial occurrence was more in South Bank region and the soil of this region harbor significantly high bacterial load in comparison to Cachar and North Bank. Among the four agro-climatic zones South bank (a) and South bank (b) regions recorded abundance of bacterial cfu load than the Cachar and North Bank regions. Fungal cfu load was also more in South Bank region compared to Cachar and North Bank regions. The presence of Actinomycetes in almost all the tea estates was an indication of the soil suitability of harboring different rhizospheric microflora.

The most important finding of this study was the occurrence of functional groups i.e the nitrogen fixers like *Azospirillum*, *Azotobacter* and phosphate solubilizer in most of the tea estates covered under the investigation. But still their occurrence differs from each tea estates. In tea estates like Phulbari, Gabroo Purbat and Khongea low bacterial cfu load was noticed while in case of fungi Harchurah, Nahortoli and Rungajaun T.Es recorded lowest fungal cfu load. In Maud T.E which is an organic tea garden bacterial and fungal cfu load was significantly high.
but presence of Actinomycetes was not detected. These variations in the occurrence of soil microorganisms in different tea estates may be attributed to plant age from which sampling was done. As this may happen at a certain plant developmental stage, specific root exudates was released and soil microorganisms may need time to adapt to the present conditions in the rhizosphere before an effect was exerted on the bacterial numbers (Marschner et al., 2001).

*Azospirillum* and *Azotobacter* like isolates from the tea rhizosphere were also reported by Baby *et al.* (2002). Earlier it was considered that acidic soil do not proliferates *Azospirillum* and *Azotobacter*, but this work confirmed its presence in acidic soil. In the present investigation emphasis was given to isolate the phosphate solubilizers along with nitrogen fixers for initiating plant growth promoting aspects from the indigenously isolated strains.

New and Kennedy, (1989) surveyed *Azospirillum* spp on the roots and associated soil of wheat grown in eastern Australia and found that significant proportion of strains isolated from the roots had a minimum pH for growth that was higher than the associated soil. It suggested that the wheat roots provided an ecological niche protecting against soil acidity. Hence, in this study the tentative *Azospirillum* isolates from the tea feeder roots survived and showed efficacy in growth when applied as soil inoculants in the nursery. The investigated strains were also an endophyte and so its growth was very prominent when sub-culturing was done. Though, tentative
Azospirillum was also isolated from tea rhizosphere soil it could not be further evaluated after primary screening as it was not able to adapt itself to the artificial environment. The experiment highlighted that Azospirillum, Azotobacter were present in tea soil though sometimes it was not encountered. Presence of Azotobacter in the tea soil was recorded low comparatively to the other functional groups in the present investigation might be due to its preference of alkaline soil as seasonal changes during collection.

Plant species and soil type interact with each other, as one factor can influence the other (Marschner et al., 2004). Plant species, which can influence rhizospheric organisms and plant related factors such as plant age and root zone location also, had its effect on rhizosphere. In tea plantation, age factor of the planting materials and different weed flora, shade trees determined the microbial community, though the elaborate study on it was not taken up in this line.

Survivality and the occurrence of Diazotrophs, Phosphate solubilizer and Actinomycetes in the acidic tea soil and its influence on the plant growth is a vital concern at present. Hence, preparing a databank of native beneficial tea rhizosphere microflora of Assam tea plantation was highly in demand. This survey was conducted over twenty commercial tea gardens of Assam and around 120 different isolates were recorded.
A few of the potential strains were evaluated for different studies during this tenure both in laboratory and in nursery conditions. Among 120 isolates six “tentative Azospirillum” strains MM Azm 01, MM Azm 03, MM Azm 05, MM Azm 06, MM Azm 10, MM Azm 26, MM Azm 42 and MM Azm 66, two “tentative Azotobacter” strains MM Azr 08, MM Azr 05, four phosphate solubilizers MM PSM DR/BS, MM PSM 10, MM PSM 11, MM PSM 06 were found to be effective and deposited to Tocklai Experimental Station, Jorhat for further studies.

Emphasizing studies on occurrence of native nitrogen fixers and phosphate solubilizers in the vicinity of tea rhizosphere zone was done for evaluating and generating data on microbial manipulation in soil in response to plant growth particularly in acidic soil conditions. Microbiologist had reported that plant rhizosphere was favorable for the survival and multiplication of nitrogen-fixers, phosphate solubilizer and actinomycetes (Singh, 2001) in accordance with seasonal variations. This study highlighted the vast microbial diversity to be explored for formulation of different bio-fertilizer.

5.4 Investigations of diazotrophs and phosphate solubilizers and phosphatase activity:

After preliminary screening of 120 different isolates, study was confined on six isolates namely MM Azm 26, MM Azm 10, MM Azr 08, MM Azr 05, MM PSM 10 and MM PSM DR/BS. MM Azm 26, MM Azm
10, MM Azr 08 and MM Azr 05 were found to be effective in fixing atmospheric nitrogen by 112,129,153 and 89 µg/ml respectively and showed nitrogenase activity during acetylene reduction assay and hence considered as potential nitrogen fixers isolated from the acidic tea soil.

Another two isolates MM PSM 10 (identified as Aspergillus niger by National Centre for Fungal Taxonomy, NCFT, New Delhi) and MM PSM DR/BS were found capable to solubilize calcium phosphate and on repeated sub-culturing the isolates formed clearing zones. The strain Aspergillus niger (MM PSM 10) and MM PSM DR/BS (tentative Bacillus spp.) showed solubilizing index 4 and 2.5 respectively and were considered as effective phosphate solubilizing microorganisms among the strains tested. MM PSM DR/BS also could fix 83 µg/ml atmospheric nitrogen. Species of Bacillus were known to dominate the rhizosphere of established tea bushes in the Himalayan region (Pandey and Palni, 1997). But, in North East India, more work is needed to confirm the dominance of a particular strain.

As tea prefers acidic soil, the phosphorus uptake by the plants become more apparent. The solubility of phosphate was inhibited by the presence of iron and aluminum in acidic soils which leads to fixation of phosphorous, making it unavailable to the plants (Kochian et.al, 2004; Ponmurugan and Gopi, 2006). Acidic conditions enhance the presence of trivalent cation Al^{3+} (Lidon and Barreiro, 2002; Kochian et al., 2005),
which was the most toxic of all Al available to plants (Delhaize and Ryan, 1995; Hoshino et al., 2000). The decrease in root growth was one of the initial and most evident symptoms of Al-toxicity at micromolar (µM) concentrations in plants (Rengel and Zhang, 2003), inducing reduced capacity for water and nutrient uptake.

Hence, an alternative strategy for high P acquisition and utilization in acidic tea soil of Assam was in demand. In Assam tea plantation reports on work on mineral nutrition in tea plants by native soil microorganisms were very scanty. Role of native phosphate solubilizing microorganisms in tea soil in this study was perhaps in the initiative phase of introducing PSM as growth promoter determining its phosphatase activity, though Patgiri and Bezbaruah, (1990) initiated the work of exploring it.

Salehi and Hajiboland, (2008) studied the effect of extremely low level of P supply in the nutrient solution on growth, P uptake and utilization in five different tea seed stocks in Iran. They suggested that P deficient tea plants did not develop any strategy for higher P uptake, neither in terms of changes in root architecture nor induction of high affinity P uptake systems. This investigation hence, focused on efficient remobilization of P, increased production and secretion of phosphatases enzyme and association with arbuscular vesicular mycorrhiza as observed in other areas and plants (Vance et al., 2003; Kochian et al., 2004).
Assay of both alkaline phosphatase (Alp) enzyme and acid phosphatase (Acp) enzyme were conducted using six selected strains and found that all of them responded well to the phosphatase enzyme test. During the investigation no such specific trend of amount of enzyme released was noticed in alkaline and acid phosphatase activity of the isolates MM Azm26 and MM Azm 10 (nitrogen fixer’s). Since the strain was isolated from tea feeder roots it may have adapt itself to the activity of the tea rhizosphere effect over the years (New and Kennedy, 1989).

Moreover the growth of isolates was slow in comparison to other bacteria in the first two to three days as they slightly preferred alkaline condition to grow in soils (Lakshmi kumari et al., 1976). But as it was tea rhizosphere isolate it showed variability in both alkaline and acid phosphatase activity in supernatant (extracellular enzyme) and cell-bound enzyme (cell suspension). The isolates mediated itself to its condition of growth. Hence, it could be concluded that the strains though possess both alkaline and acid phosphatase activity it varied from time to time and depend upon its growing condition.

Similar observation was drawn from the experiments conducted on another two nitrogen fixer MM Azr 08 and MM Azr 05. In MM Azm 26 alkaline and acid phosphatase enzyme release was maximum at 96 hours incubation in culture cells (crude enzyme), while in MM Azm 10 alkaline (pH 9) phosphatase enzyme was maximum at 24 hours incubation and in
acid (pH 4.5) maximum phosphatase enzyme liberated was at 72 hours incubation. The protein content of the cultures increased with that of the incubation time in all the six tested strains which showed good growth under P solubilizing conditions.

MM Azr 08 produces maximum alkaline phosphatase at 72 hours and acidic enzyme at 96 hours period. Likewise in MM Azr 05 maximum alkaline and acid phosphatase enzyme was secreted at 96 hours of incubation. These four nitrogen fixers apart from fixing atmospheric nitrogen secreted both alkaline and acid phosphatases enzyme activity at Nutrient broth culture which means these strains could adapt to different nutrient parameters and could solubilized phosphorus from tea soil if applied. The findings of the investigation tally with the work of Kucey, 1988a; Yadav and Dadarwal, 1997. The solubilization of different types of insoluble phosphate varied with the type of microorganisms, the type of phosphates available, media conditions and available carbon sources. In this investigation also slight modification of the Eivazi and Tabatabai, (1977) method was done and hence under the present experimental conditions both alkaline and acid concentration of the strains were differed.

The experiment conducted with another two isolate identified as phosphate solubilizer Aspergillus niger (MMPSM 10) and MM PSM DR/BS (tentative Bacillus spp.) subjecting to phosphatase enzyme assay
revealed a gradual increase of alkaline phosphatase enzyme from 24 hours incubation to 96 hours (culture cells). The maximum alkaline enzyme was recorded at 96 hours incubation in MM PSM 10 and in acidic condition at 72 hours in both nutrient and Pikovskaya’s broth.

In case of MM PSM DR/BS the maximum alkaline and acid enzyme were liberated at 72 hours incubation in both nutrient and Pikovskaya’s broth. The liberation of the phosphatase enzyme depends upon the pH, the insoluble phosphate as well as the experimental conditions. The present findings tally with the recent work of Nopparat et al., 2007; Balamurugan et al., 2010). Their results also showed variability in liberation of both acid and alkaline enzyme at different growth periods from 3rd to 7th day culture which tally with the findings of present investigation from initial 24 hours incubation to 96 hours.

The results of the phosphatase enzyme activity in the four nitrogen fixers and two phosphate solubilizers in nutrient broth showed wide range of variation in P – solubilization. The investigation revealed that the strains had the ability of phosphate solubilization and showed phosphatase activity in absence of TCP as the sole source of bound phosphate. Earlier works stated that there is positive correlation between phosphate solubilization capacity and phosphatase activity (Singh and Kapoor, 1994; Ponmurugan and Gopi, 2006).
Study of phosphate solubilizing microorganisms in tea soil of Assam was scanty specially on the prospect of phosphatase enzyme released by the indigenous microbial isolates and so these strains could be used as alternate source of phosphate biofertilizer in the tea cultivation and also a step could be initiated towards standardizing the phosphatase activity of the soil microorganism zonal wise for implementing region specific strains as inoculants in tea estates for phosphate mobilization. Moreover, N\textsubscript{2} fixers like MM Azm 26, MM Azm 10, MM Azr 08 and MM Azr05 showed both N\textsubscript{2} fixation as well as phosphate solubilization thereby giving dual benefit.

5.5 Nursery trial on interaction of rhizosphere microflora and AM fungi:

Experiment were carried out with six native rhizosphere microbes and unidentified AM propagules in permutation combinations under nursery conditions for beneficial effects. Treatments were conducted with inoculation of single, dual and consortium to observe their effect on different parameters of plant growth where significant increase in plant growth and dry matter content were observed over untreated control.

As observed by other workers (Sparling and Tinker, 1975; Powell, 1976) the different mycorrhizal plants (e.g Mimosa invisa) root fragments served as the source of effective AM fungi. Hence, the M.invisa plant
propagules were used in the present study to colonize tea feeder roots instead of only spores.

Root colonization was one of the most important steps in the interaction of bacteria and host plants. These bacteria must grow on, in or around the roots for the colonization of plant roots, which was of primary importance for an effective plant-microbe interaction (Kloepper and Beauchamp, 1992). Nevertheless, effects of nutritional conditions of the plants on root colonization or bacterial effectivity are still not fully understood. The study on interaction of AM fungi along with diazotrophs and phosphate solubilizers as growth promoters in tea plantation (Zhi, 1993; Rajgopal and Ramrithinam, 1997; Hazarika, 2000) was very scanty as compared to other crops.

During the single inoculation of four nitrogen fixers (MM Azm 10, MM Azm 26, MM Azr 08, MM Azr 05), two phosphate solubilizers (MM PSM 10, MM PSM DR/BS) and native AM fungi propagules (AMF 12) significant growth was recorded in all the treatments. The difference observed in shoot height, girth and root length was found to be significant at $P_{0.05}$ level. This was an indication that the overall significant effect of treatment obtained for shoot height, girth, and root length was largely attributed to the presence of respective inoculum. In single treatments AMF 12 responded significantly to greater root length. MM Azm 10, MM Azr 05, MM PSM 10 and AMF 12 were found to be highly effective in increasing the
shoot and root growth in the S3A3 tea plants during single inoculation. But, the effect of the other strains could not be neglected as they also promote growth above 50% over control.

Each treatment showed positive response in dry matter production of root, leaf and stem in single inoculation. MM Azm 10 and AMF 12 recorded significant growth in single inoculated treatment. The enhancement of growth parameters may be due to ability of nitrogen fixation, phosphate solubilization and AM fungi colonization (Daft and Nicolson, 1969; Gerdemann, 1975) in the roots. Similar results were obtained after single inoculation treatments carried out by several workers in different crops (Barea and Brown, 1974; El-Shourbagy et al., 1979).

The synergistic effect on plant growth following inoculation with VA mycorrhizal fungi, phosphate solubilizing microbes and nitrogen fixers was recorded by Barea and Brown, (1974); Barea et al. (1975). In the present investigation also the diazotrophs, phosphate solubilizer and arbuscular mycorrhizal fungi also acted synergistically and recorded significant growth of root length, height and girth of the plants at P<0.05 level. The dry matter content of the plants was also enhanced after treatment application and found to be significant at P<0.05 level.

Moreover, after application of microbial consortium recorded significant increase in growth rate and dry matter production in the treatments over the control. Comparative effectivity of S3A3 inoculation
with MM Azm 10, MM Azm 26 (tentative *Azospirillum* spp); MM Azr 08, MM Azr 05 (tentative *Azotobacter* spp); MM PSM 10 (*Aspergillus niger*) and MM PSM DR/BS (tentative *Bacillus* spp) as well as AMF 12 under greenhouse conditions was investigated.

The results of this experiment concluded that when plants were inoculated with the isolates showed better growth response, biomass yield and nutrient content in comparison with uninoculated control plants. Total dry weight of the plants increased in all the treatments and nutrient uptake compared to the control. Single, dual and consortium study resulted in maximum nitrogen percentage as well as phosphorus content in shoots and roots in all the treatments with microbial inoculation over the control.

The application of microbial inoculants in different combinations improved the available soil phosphorus (P$_2$O$_5$) from 13 ppm in control to maximum 80 ppm in AMF12. As a result at the same time the P- content of the tea plants in nursery also increased considerably from 5.49 to 178.04 mg/plant. This might be due to the development of a more extensive root system in association with P solubilizers and the other microbes along with AM fungi (McCully and Canny, 1988; Narula *et al.*, 2007).
The present finding highlighted that MM Azm 10 was a potential nitrogen fixer when applied as soil inoculants singly and enhanced the shoot to root ratio as well as fixed atmospheric nitrogen compared to others. The total Nitrogen (4.59%) in the tissues also increases over the control in MM Azm 10. Interesting point of this investigation was recorded in the treatment with phosphate solubilizer, MM PSM 10 (*Aspergillus niger*) having greater shoot to root ratio over control and enhanced 52.98 mg/plant phosphorus in its tissue. The treatment AMF 12 recorded the maximum phosphorus uptake (178.04 mg/plant). The ability of the isolates to fix atmospheric nitrogen, accumulation of nitrogen by nitrogenase activity and secretion of phosphatase enzyme might be the reason of better growth and dry matter production over the control in single inoculation. This investigation was in accordance with other workers in different crops (Christiansen-Weniger *et al.*, 1992; Boddey and Dobereiner, 1988; Narula *et al.*, 2007) but in case of tea plants adsorption, accumulation and assimilation of the nutrients in plant tissue is yet to be investigated in North East region incorporating only microbial fertilizer or farmyard manure to establish the mechanism of plant growth promoting factors.

In this investigation when the tea seedlings were inoculated with diazotrophs, phosphate solubilizers and arbuscular mycorrhizal fungi in single, dual or consortium recorded variability of nutrient uptake (N and
P) as well as shoot to root ratio. In the treatments with dual and consortium application root to shoot ratio was more in comparison to shoot to root ratio which in turn increases the activity of the phosphorus uptake by the plants. Moreover, the total N and P content in the tissues of the dual and consortium inoculation along with native arbuscular mycorrhizal fungi was maximum in comparison to single inoculation.

Interesting fact was that MM Azr 05 (4.96%) was able to fix atmospheric nitrogen and showed maximum nitrogen uptake singly. These findings definitely highlighted the fact that the tea plants with greater shoot to root ratio showed better vegetative growth rate above ground level and were not in stress conditions. While the treatments with better root to shoot ratio recorded enhanced belowground activity and production of dry matter content of the roots was more or less equivalent to above ground parts of the treated plants. Wilson, (1998) reviewed the probable models of shoot to root ratio in enhancing the growth of plants which is in accordance with the present findings.

Phosphorus uptake by the tea plants in dual and consortium treatments was more in comparison to single inoculation. The maximum phosphorus uptake was recorded in the dual treatment AMF 12 + PSM 10 (143.50 mg/plant). This might be due to the synergistic effect of both the arbuscular mycorrhizal fungi and phosphate solubilizer in combination as they promote solubilization of phosphorus in the soil.
In the consortium treatment AMF 12 + Azm 26 + Azr 08 recorded significant uptake of nitrogen 1174 mg/plant and phosphorus uptake 67.86 mg/plant respectively which might be attributed to the presence of two efficient nitrogen fixers and arbuscular mycorrhizal fungi (Christiansen-Weniger et al., 1992, Jiang and Sato, 1994, Baldani et al., 1997).

The survivality of the strains was also investigated and found that the isolates increased in number from initial zero days of inoculation and slowly they declined in their numbers. They multiplied and regenerated themselves in the soil for 60 days and then over time decreased their population. This was in accordance with the work of Bahme and Schroth, (1987) who reported a decline in the recoverable population of introduced bacteria over time even if the organisms were considered to be good colonizers. The recorded decline in microbial (Azotobacter, Azospirillum and phosphate solubilizing microbes) population in soil was in accordance with other results (Tripathy et al., 2001). This might be the result from the addition of much greater populations than the actual capacity of roots. Moreover, the diazotrophs, phosphate solubilizers and arbuscular mycorrhizal fungi interact among themselves and within their capacity influences the growth of the tea plants in nursery conditions.

Examination of stained roots collected from the nursery trial revealed both fungal mycelium, vesicles, and arbuscules, which are specific to AM fungi, in the young root tissue of all the treatments.
inoculated with AMF 12. Mycelium was recorded in most of the dual and consortium treatments, maximum in AMF 12 + Azm 10 (62.6%) and in AMF 12 + Azm 26 + Azr 08 + PSM 10 (60 %) in comparison to control. Vesicles, the storage and possibly propagative structures of mycorrhizal fungi, were less common in the feeder roots, maximum was recorded in the treatment AMF 12 + Azm 10 (13.3%). In some of the treatments vesicles were not recorded. Arbuscules were observed in feeder roots taken from all nursery sleeves except in the treatments AMF 12 + Azr 08, AMF 12 + PSM DR/BS, AMF 12 + Azm 10 + Azr 05 and AMF 12 + Azm 10 + Azr 05 + PSM DR/BS. In the present investigation spore load was recorded maximum in the treatment AMF 12 + PSM 10 while in others inoculated with AMF 12, diazotrophs and phosphate solubilizers in dual and consortium treatments significant variation of the spore load was encountered. This might be due to the soil available nutrients as well as synergistic effect of the inoculated microbial along with AMF 12 and their root exudates. Spore load was not correlated with that of the colonization of the feeder roots.

In theory, the inoculative potential of nursery soils where AM fungi are active would be greater where the largest numbers of young, fine roots exist and where soils was least disturbed. Under these conditions, higher inoculative potential would be expected to result from a greater abundance of extra radicle hyphae or production of resting
spores. Independently though, spore numbers in soil was an imperfect measure of the capability of AM fungi to inoculate host plants and are poorly correlated with soil colonization potential (Daniels et al., 1981; McGee, 1989; Brundrett, 1991).

The microbial interaction with the mycorrhizal colonization, assimilation and accumulation of the nutrients from the soil might affect the potentially of the individual strains and in combination to promote growth and proliferation of S3A3 tea clone in nursery conditions. The experimented S3A3 tea clone respond significantly to the overall biomass production of the plant as a whole.

From the investigation, it could be inferred that due to the inoculation of native AMF 12 propagules to the nursery sleeves, dormancy of spores was broken and it was able to infect the plant feeder roots significantly. The single, dual and consortium inoculation of diazotrophs and phosphate solubilizers along with AMF 12 significantly enhanced the shoot and root growth as well as the dry matter production of shoot and root of the experimented variety.

The tested diazotrophs and phosphate solubilizers acclimatized in the root vicinity and soil along with arbuscular mycorrhizal fungi and promote significantly uptake of nutrients particularly nitrogen and phosphorus from the soil.
The native isolates also mediate themselves to the acidic condition of the nursery soils and proliferates around the root system according to their requirement. Due to the inoculation of the diazotrophs, phosphate solubilizers and arbuscular mycorrhizal fungi credited with better uptake and mobilization of nitrogen and bound phosphate in acidic soil.

From the investigation it was revealed that the positive effects of AM fungi on tea seedling development and prolonged benefits after transplantation in the fields would gain momentum as most of the tea bushes of different age groups harbor colonization of AM fungi. Hence, from the study, it could be concluded that after rehabilitation when new plantation would be raised, by introducing mycorrhizal as well as native diazotrophs and phosphate solubilizer to the planting pit would improved the tea plant health and the soil conditions. In the present study *Mimosa invisa* was used successfully for mass culturing of AM fungi and hence this species may be recommended for mass culturing of AM fungi for commercial exploitation in the tea estates.

So, the overall concept inferred from this investigation was that the native arbuscular mycorrhizal fungi as well as the diazotrophs and phosphate solubilizers from tea rhizosphere would enhance growth and yield if applied to the planting pit. Moreover, future prospects of incorporating the strains AMF 12, MM Azm 10, MM Azm 26, MM Azr 05 and MM PSM 10 in tea cultivation was very promising.