1. The aim of the investigation was to identify the status of the tea bushes dependency on mycorrhizal association and occurrence of diazotrophic and phosphate solubilizing microorganisms in tea growing soils of Assam along with their application for the growth of tea.

2. The study showed variability in soil characters of four tea agro-climatic zones on the basis of the survey conducted on randomly selected 20 tea estates (TEs) of Assam.

3. Moreover, the finding highlighted the promising aspect of AM fungi and their interaction with nitrogen fixers and phosphate solubilizers in tea growth from the various experiment conducted under nursery conditions.

4. From the present investigation it was revealed that in Cachar region, among the five gardens, Coombergram T.E showed the highest potential of AM fungi association in the roots.

5. While in North Bank region the tea estates showed an increasing trend of colonization from 0-5 years to above 15 years having highest percent association observed in Rupajuli T.E (28- 88%).
6. Highest mycelia 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.

    Presence of Arbuscules and vesicles were more in South Bank region compared to Cachar and North bank. Hence, irrespective of age groups and different sites tea plants were colonised with arbuscular mycorrhizal fungi indicating a promising aspect of utilization as AM biofertilizer.

7. In each Tea Estate 10 dominant weeds were considered and selected on the basis of their abundance. Total 26 weed species of 16 families were studied and found to be highly colonized with arbuscular mycorrhizal fungi.

8. The percent association of AM fungi varied 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 during the study.

9. This investigation had revealed active and abundant AM fungi association in the roots of Ageratum conyzoides, Borreria hispida, Mimosa invisa, Mimosa pudica, commonly grown weed species of different tea growing areas. In the Cachar region, the most dominant weed flora were Ageratum conyzoides, Borreria hispida, Mimosa pudica, Mimosa invisa, Jussiaea suffruticosa and Scoparia dulcis. In North Bank region mostly Ageratum
conyzoides, Borreria hispida, Spermocoea ocymoides and Hedyotis verticillata were encountered. In the South Bank (a) Ageratum conyzoides, Borreria hispida, Gnaphalium indicum and Erechthites valerianaefolia. While in South Bank (b) Ageratum conyzoides, Borreria hispida, Spermacoe ocymoides and Peperomia pellucida were found to be dominant in the tea estates.

10. The investigation also inferred that soil characters like pH, % organic carbon, potassium, phosphorus and nitrogen had a profound influence on both root colonization and spore density in tea rhizosphere soil.

11. Acidic tea soil which ranges from 4.07 to 5.51 also supports arbuscular mycorrhizal association with the tea bushes which helped in uptake of nutrients in the tea ecosystem.

12. On analysis of correlation coefficient of Phosphorus with that of spore load of different regions depicted negative correlation of phosphorus irrespective of the age groups and the soil region wise. Maximum spore load recorded was 47/50 g soil while the minimum was 19/50g soil in the twenty tea estates.

13. In Cachar tea estates samples above 15 years showed highly positive correlation (r) = 0.953 in case of nitrogen with that of spore load while least correlation was observed in North Bank tea estates at 15 years age group. This variability might occur as a
result of land use and management practices of tea cultivation over the years.

14. The positive effects of the isolated AMF 12 propagules 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 as observed from the survey conducted in 20 tea estates of four agro-climatic zones.

15. Microorganisms were integrated component of any soil system. The study showed abundant occurrence of bacteria in almost all the Tea Estates. Diversity of fungal population, nitrogen fixers and phosphate solubilizers were noticed during the study period.

16. In four tea agro-climatic zones covered under the investigation revealed potential future prospects of isolating more beneficial microorganisms which would adapt to acidic soil conditions and helps in growth and proliferation of the tea bushes.

17. The analysis showed the occurrence of free-living Nitrogen -Fixers in tea soil. It proved that though this Nitrogen –fixers were considered to be present preferably in alkaline soils but they do occur in acidic soil. They mediate themselves to the suitability of the soil dynamic and improve the soil health. It confirmed the presence of beneficial nitrogen fixers which in turn mediates growth.
18. Out of 83 nitrogen fixers four active isolates namely MM Azm 26, MM Azm10, MM Azr 08 and MM Azr 05 the native isolates tested could further be evaluated by molecular technique to interpret more data in future. Since the test microbes are present and survive well in soil of tea plantation and survive the negative effect of tea root system, the seedling treated with these strains in near future on releasing to the field will help in acclimatizing in soil with promising results.

19. The nitrogen fixers fix nitrogen as well as showed nitrogenase activity as studied by acetylene reduction assay technique. The ethylene production from acetylene varied from isolate to isolate within the range 0.04 to 2.89 µmole/ml of the culture. The four isolates MM Azm 26, MM Azm 10, MM Azr 08 and MM Azr 05 could reduce acetylene within 2 hours and showed nitrogenase activity. Though, detailed experiment of the acetylene reduction assay could not be performed due to technical problems, it proved that the isolates were effective nitrogen fixers.

20. Though the number of phosphate solubilizing microbes was very low in all twenty gardens, two highly active isolate namely MM PSM 10 and MM PSM DR/BS were used for further study.

21. MM PSM 10 and MM PSM DR/BS were tentatively identified as Aspergillus niger and Bacillus sp., respectively.
22. These two phosphate solubilizing microorganisms along with four nitrogen fixers were found to be active phosphate solubilizer and could be used as phosphate biofertilizers in tea industry.

23. All the six active isolates namely MM Azm 26, MM Azm 10, MM Azr 08, MM Azr 05, MM PSM 10 and MM PSM DR/BS recorded both alkaline and acid phosphatase activity in the cell cultures, supernatant (extracellular enzyme) and the cell suspension (cell pellets for intracellular enzyme). No specific trend was noticed during different growth period.

24. The growth of these four N\textsubscript{2} fixers was also measured in terms of increase in protein content. The growth of the cultures was maximum at 96 hours incubation in all the four isolates namely MM Azm 26, MM Azm 10, MM Azr 08 and MM Azr 05.

25. Among the weeds Mimosa invisa was found to be host for mass multiplication of biotrophic AMF.

26. From the investigations it was observed that all these native microbial strains namely MM Azm 26, MM Azm10, MM Azr08, MM Azr05, MMPSM 10 MM PSM DR/BS and AMF 12 had their efficacy in single, dual and consortium in supporting growth and proliferation of tea plant. The consortium of microbial inocula if applied to the planting pit during plantation could help the seedling to acquire the nutrient specifically N and P from the soil.
27. High input of inorganic nitrogen and phosphorus may be minimized by incorporating the promising strains of nitrogen fixers, phosphate solubilizers and AM fungi which would definitely improve the plant and soil health profile.

28. The strains MM Azm 10, MM Azr 08, MM Azr05, MM PSM 10 were found to be highly effective in nursery conditions along with AMF 12. This study may be considered as the pathway for further investigation towards integrated nutrient status of tea plantation incorporating the diazotrophs and phosphate solubilizing microbes and its manipulation in the soil to promote plant growth.

29. The variability in the performance of native strains during nursery inoculation may be due to various environmental factors that may affect their growth and exert their effects on plant. To achieve the maximum growth promoting interaction between the inoculum and nursery seedlings it is necessary to develop efficient strains in field conditions.

30. After using these biofertilizers in nursery, the total nitrogen content in the tea plants ranged from 4.02- 4.96 % and total phosphorus content ranged from 0.08 – 1.58 %

31. 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.

32. 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.

33. Assessment of the AM fungi in feeder roots of nursery S3A3 plants after inoculation revealed the association of AM fungi. The percent AM fungi association ranged from 8.6 to 62.6%. It showed that under controlled condition also AMF 12 propagules were able to form association in the tender roots.

34. From the investigation, it could be concluded that the selected strains when inoculated singly, dual and consortium showed effective growth by enhanced shoot and root dry matter content as well as N and P uptake in their tissues.

35. Extensive field trial is needed before releasing these strains as potent biofertilizer. Hence based on the results of this investigation extensive field trials are being conducted with certain selected strains at Tocklai Experimental Station and a protocol for Mass Production and their Commercial utilization was undertaken.
Protocol for Mass Production and their Commercial utilization:

A protocol was developed for mass multiplication of AM fungi on feeder roots of *Mimosa invisa*, a common weed of tea plantation which was used as a green crop during rehabilitation period in the tea growing areas of North East India. The technology have been transferred to the member gardens of Tea Research Association and accepted by the clients.

The technology has been given below in brief:

For mass culturing the AM fungi, beds of one square meter size may be prepared where 500 g of the stock culture (may be obtained from Tocklai) should be mixed thoroughly with 7.5 cm (3 inches) top soil. If the soil was clay type, some sand may be mixed with the soil. Then in such beds seeds of *Mimosa invisa* may be sown and the plants should be allowed to grow. After the plants have grown luxuriantly for about 4-6 months, the above ground portion of the plants should be removed and discarded or used as mulch. The roots of the plants should be mixed thoroughly with the top 15 cm (6 inches) soil of the beds after thorough chopping. This soil-root mixture may be utilized as AMF biofertilizer in nursery sleeves and beds. The AM propagules containing soils may be mixed with potent strains of Diazotrophs and Phosphate solubilizers tested and may be made available to the tea growers for field application.
AM biofertilizers and formulations of plant growth promoting microbes (Diazotrophs and Phosphate solubilizers) produced in the production unit of Tocklai were supplied to several tea estates (Rungagora, Diffloo, Meleng, Borbam, Dolaguri, Lukwah, Hunwal, Teok and Bokahola) for commercial utilization and found to be highly effective in supporting the vegetative growth of tea plants. Initial information as given by the respective gardens under field conditions showed highly promising results.
OUTCOME AND FUTURE PROSPECT:

Application of Plant Growth Promoting (PGP) Microbes including N$_2$ fixers MM Azm 10, MM Azm26 and MM Azr 05 and Phosphate solubilizers (MM PSM 10, *Aspergillus niger*) alone or in combination with AMF 12 (Mycorrhizal propagules) during planting enhanced plant growth and nutrient uptake which can be utilize by the Tea growers both in organic and conventional tea cultivation practices.