CHAPTER 1

INTRODUCTION

1.1 Tea plantation in general:

Tea \(\textit{Camellia sinensis} (\text{L}) \text{ O. Kuntze}\) is a perennial crop. Like all other crops it also has different strata of associated facts related to its proliferation and production. Ranked with the most popular beverages, tea is believed to be discovered as a beverage around 300 B.C in China. In 1830 it was first introduced in India with seeds and plants imported from China. But in Assam the indigenous Assam variety was reported by Robert Bruce in 1923 (Barua, 1989).

Wight in 1962 proposed a concise description of the China and Assam varieties of tea, according to him the three main varieties of tea are

a) China variety: \(\textit{Camellia sinensis}\) (L) O.Kuntze

b) Assam variety: \(\textit{Camellia assamica}\) (Masters) Wight and

c) \(\textit{Cambodiensis} / \text{Southern form of tea: Camellia assamica}\) sub sp. \(\textit{Lasiocalyx}\) (Planch.MS).

The three races of tea differ in their liquor characteristics according to Wight and Gilchrist, (1961). These three races had some distinguishing anatomical and chemical characters that differentiate them from each other.
Tea, being the most commonly consumed hot beverage worldwide, at present more than 45 countries spread over all continents except North America within latitude range 45° N to 34° S where tea cultivation is groomed (Barua, 1989). The most important tea-producing nations are by percentage of world production i) India: 25.63% ii) China : 25% iii) Kenya : 10.25% iv) Sri Lanka : 9.65% v) Turkey: 6.42% vi) Indonesia : 5.31% and Others: 18% which is for a world production of 3,200,000 tonnes in 2004. (Data source: Food and Agriculture Organization of the United Nations). Among the different plantation crops, tea is considered to be the most important crop in our country. It is the second biggest foreign exchange earner and is exported to about 80 countries (Karmakar and Banerjee, 2005).

Tea is cultivated in two distinct regions of India. The North East tea belt situated between 22-27°N latitudes and 88-95°E Longitudes while the South Indian tea belt located at 8 –13° N latitude. The North East tea belt contributes 75% of the total Indian tea production with the plantation area of about 2, 28,260 hectare. Assam contributes 55 per cent of the country’s tea production (Rajkhowa et al., 2005). There are about 1000 tea estates in Assam besides having thousands of small tea gardens. The North East Indian tea belt lies in different landscapes viz. Brahmaputra and Barak Valley in Assam, North Bengal Plains of Dooars and Terai regions of West Bengal as well as Darjeeling Hills. In the Brahmaputra valley, tea is grown on both the banks of the river on flat land between 50 m and 120 m (160
ft - 400 ft) above Mean Sea Level (MSL). In South India, tea is grown on the slopes of Western Ghat Mountains and adjoining plateaus at elevations ranging from 800 to 2000 ft above MSL (Karmakar and Banerjee, 2005).

Climate with a wet, hot summer and dry cool winter are the characteristics of the tea growing areas of North East India. Each crop needs micro as well as macronutrients for its proper growth and development. Tea is no exception. Tea requires about 3-5% N, 0.5-1.0 % P$_2$O$_5$, 2-3% K$_2$O as major nutrients. It also requires about 0.5-1.0 % Sulphur, 2-3% Calcium, 1-2% Magnesium, 20-50 ppm Zinc etc. as secondary nutrients. Deficiency in any of the above nutrients affects deleteriously on the growth, production and quality of the teas (Barua, 1989; Kalita, 1992).

Tea among the other perennials is unique because only its vegetative parts – “the two leaves and the bud” - are commercially exploited. Tea is also maintained as a low bush in a continuous phase of vegetative growth though it may grow as tall as 10-15 m. However, in commercial plantations, tea is maintained as a bush of about 1 m tall, having a flat-topped foliage canopy (De Costa et al., 2007).

Growth habit is of great significance in tea productivity, which is linked to bud and shoot growth at the same time higher productivity is possible to achieve only by biological manipulation of the growth processes. In tea plantation vegetative propagation of the plant, planting,
pruning, application of fertilizer, weed control, pest management, drainage system etc. play a unique role in growth and productivity. (Barua, 1989). Bushes are the assets of the tea estate-owners as they provide green leaves which are the basic raw material for made/green tea. The composition of assets i.e. bushes are classified into three age groups viz. tender [below 5 years], economic [5-50 years] and old [above 50 years] (Barua, 1989; Karmakar and Banerjee, 2005).

1.2 Commonly grown weed species in tea fields of North East India:

Infestation of weed affects the tea plantation of North East India and crop losses both in terms of yield and quality of tea. Weed control in tea estates is a cause of concern (Rajkhowa et al., 2005). Around 130 common weeds of tea estates were identified of which include 28 monocots (16 grasses), 97 dicots and 5 fern species (Dutta, 1972). Weeds are unwanted plants in cultivated crops but to define it is difficult as one species in a particular place may be unwanted while it may have importance in another place. The term weed is applied to all those plants for which man has found no use and which in their evolution have developed such power of aggression, persistence, or reproduction to make them a menace to the best possible development of crop (Nelson, 1946). Weeds have both beneficial and harmful effects and they are classified
according to the duration of their life cycle into three categories i.e. annual, biennial and perennial.

Some of the very common weeds in tea plantation are Achyranthes aspera, Ageratum conyzoides, Borreria hispida, Capsella bursapastoris, Cassia tora, Chrysopogon aciculatus, Drymaria cordata, Drymaria spp., Erechthites valerianaefolia, Gnaphalium indicum, Hedyotis lineate, Impatiens raylei, Leonurus sibiricus, Leucas aspera, Leucas linifolia, Melastoma malabathricum, Mikania micrantha, Mimosa invisa, Mimosa pudica, Oxalis corymbosa, Peperomia pellucida, Phyllanthus nirurii, Pouzolzia indica, Rungia repens, Scoparia dulcis, Solanum khasianum, Solanum nigrum, Spermacoce ocymoides, Stellaria media, Zehneria umbellate etc.

Beneficial aspect of the weed flora is to be explored so that the competition between the cultivated crops and the weed for nutrient uptake could be reduced. Some of the plant species may be used as alternate source for nutrient uptake from the nutrient pool. The growth of Albizzia species is reliable indicators of land suitable for growth of tea (Mann, 1935; Mann and Gokhale, 1960). Accordingly, Albizzia species are used as shade trees in tea plantation. Melastoma malabathricum, a shrub considered to be weed in tea plantation is also a reliable indicator for successful utilization of the area for tea cultivation. These plants like tea
accumulate aluminum. Hence, both the beneficial and harmful aspect of the weed in tea cultivation is considered.

1.3 *Arbuscular Mycorrhizal fungi (AMF):*

Arbuscular Mycorrhizal (AM) fungi was first coined by German Botanist Albert Bernhard Frank in 1885 (Powell and Bagyaraj, 1984). Frank was also considered to be the first to have distinguished between ectotropic and endotropic mycorrhiza (endomycorrhiza). At the present era, worldwide attention is been given to this interesting association of the fungus with the feeder roots of a plant. Mycorrhiza is a Greek word which literally means “fungus root”. This fungus root association is a symbiotic one (endophytic association) in which young feeder roots show no sign of damage even when densely infected or associated (Bonfante and Anca, 2009). The term association should be used instead of infection, though the term infection is being commonly used.

Vesicular Arbuscular Mycorrhizae are characterized with arbuscules and vesicles in the roots of the infected plant. Arbuscules are considered to be the functional unit of the AM fungi. They are present in the inner layers of the cortical parenchyma. Arbuscules are formed due to the penetration of the intercellular hyphae into the cortical cells giving rise to a complex hyphal branching like small bushes. Researchers considered it to be the fungus plant metabolite exchange site. Its presence indicates
the AM fungi infection (= association) in a root. All endophytic fungi belonging to genera *Glomus, Gigaspora* and *Acaulospora* form arbuscules (Gerdemann and Trappe, 1974). At the later stage of infection (actually association) the arbuscules was disintegrated in the cells and is digested by the host plant. Life span of arbuscule is limited to a few days normally 4-5 days (Cox and Tinker, 1976). Vesicles are also one type of AM propagules able to initiate AM symbiosis. They are globose bodies and can be intercellular or intracellular within roots. According to the extensive research it was found that most AM fungi do form the vesicles within the roots. From the cytological point of view the vesicles (mostly rich in lipids) are considered to be the resting organs and their numbers frequently increase in old or dead roots (McLennan, 1926). AM fungi also do not always exhibit host specificity and even a single species can infect and establish symbiotic relationship with a diverse group of plants. It is remarkable that even a single AM fungi species can have a worldwide distribution (Gerdemann and Trappe, 1974).

1.4 Arbuscular Mycorrhizal Fungi (AMF) and its interaction with Rhizosphere Microflora:

Rhizosphere the term first coined by L. Hiltner in 1904 is the zone (about 5 to 40% of the soil in the rooting zone depending upon crop root architecture) under the influence of the root where the majority of the soil microorganisms reside. Rhizosphere microflora is the identification of
particular plant species. So, the rhizosphere microflora is credited with certain beneficial effects in crop nutrition and growth. They are responsible for mineralization of organic matter and release of nutrient elements for plant use, solubilization of insoluble phosphate and making it available to the crop, protection of the host plant against root pathogens, production of growth stimulating substances, fixation of nitrogen, stimulation of ammonification, nitrification and overall improvement in nutrient uptake and so on.

Among the rhizosphere microflora free-living diazotrophs have a unique place in nutrient mobilization as they are able to fix atmospheric $\text{N}_2$ with the production of available N for the plants. Free-living diazotrophs are classified into three types aerobic, anaerobic and facultative anaerobic depending on the growth and survival of the organisms in the presence or absence of oxygen. Genera *Azotobacter* and *Azospirillum* (microaerophilic) comes under the species of aerobic bacteria.

In 1901, Dutch microbiologist M. W. Beijerinck (Beijerinck, 1901) was the first to isolate and describe *Azotobacter chroocooccum* and *Azotobacter agilis*. *Azotobacter* cells are polymorphic, rod to coccoid shaped according to different species. *Azospirillum* was also first described by Beijerinck in 1925 under the name *Spirillum lipoferum*. But Tarrand *et al.* (1978) changed the nomenclature of this organism and was
designated as *Azospirillum*. In the recent years both *Azotobacter* and *Azospirillum* received attention in crop production and soil fertility status. In most of the plantation crop like rubber, tea, coffee, application of *Azotobacter* and *Azospirillum* revolutionized the productivity of the crops according to the data obtained by several workers (Kumari and Balasubramanian, 1993; Baby *et al.*, 2002). Now a days, phosphate solubilizing microbes also occupy a unique place in the area of microbial research. Phosphate solubilizing microorganisms played a significant role in increasing P-availability by solubilizing the insoluble inorganic phosphorus in soil and makes it available to plants. Research on phosphate solubilizing microorganisms’ dates back to 1960s. Recently research is going on towards enhancing the availability of native soil P by different crops (Rodriguez and Fraga, 1999; Vassilev *et al.*, 2001; Barea *et al.*, 2005).

One of the pioneers in mycorrhizal research in India, Bagyaraj (1984) demonstrated that AM and rhizosphere microorganisms can mutually influence each other and this can result in synergistic interaction. Ahanthem and Jha (2006) studied the influence of dual inoculation of *Glomus* and *Azospirillum* on growth, mycorrhizal infection and nutrient acquisition of rice at two sources and four regimes of inorganic phosphorus fertilizer. They stated that generally mycorrhizal plants had more shoot phosphorus than non-mycorrhizal ones.
Misra (2001) states that mycorrhizal colonization also helps in promotion of rhizospheric microorganisms including those responsible for mobilization of nutrients such as phosphate solubilizing microorganisms (PSM) and symbiotic and non-symbiotic nitrogen fixers. Mycorrhizal colonization also allows the introduced population of beneficial soil microorganisms in the form of inoculants like *Azotobacter*, *Azospirillum* and PSM to thrive well.

### 1.5 Association of AM fungi and rhizosphere microflora in tea:

The vesicular arbuscular mycorrhiza (AMF) is a balanced mutalistic symbiosis in which (Hayman, 1974) both partners can be benefited. It incorporates a three-way interaction between soil, plant and fungus in which the movement of phosphate is of prime importance after solubilization. Phosphate moves in three stages –uptake by the soil based hyphae, transfer into the root based mycelium via the connecting link (entry points) and release into the plant. The interactions are also influenced by environmental conditions. AM fungi are ubiquitous in geographic distribution and associated with majority of agricultural crops. Association of mycorrhiza is well documented in legumes, cereals, pulses, oilseeds, vegetables, commercial crops (tobacco, cotton, sugarcane etc.) and many tropical plantation crops like rubber, coffee, tea, olive oil, palm etc (Hayman, 1982a).
Tunstall (1925, 1930) reported the association of AMF in tea feeder roots for the first time. In the recent years various workers (Barthakur et al., 1992, 1994, Hazarika et al., 2001, Chakraborty et al., 2004 and Dutta et al., 2007) have studied the possible beneficial aspects of AM fungi and rhizosphere microflora in tea plantation. AMF and other beneficial rhizosphere microbes are forming a balanced ecological niche in the tea rhizosphere (Pandey and Palni 1996; Palni et al., 1998, Baby et al., 2002).

1.6 Biofertilizer:

The outstanding achievement in crop sciences during the last decades is the development of high yielding cultivars. The advanced agronomic technology of using fertilizers has revolutionized the production of many short, medium and long duration crops. But the recent global concern of the energy crisis, rapid depletion of nonrenewable energy sources and pollution caused by chemical fertilizers are of added factors. As a consequence of this, microbiologists are emphasizing on the use of biofertilizer to exploit potential alternative sources of plant nutrients to retain the indigenous soil microflora and soil health.

Biofertilizer are ecofriendly and have been proved to be effective and economical alternative of chemical fertilizers with lesser input of capital and energy (Mubeen et al., 2006). The potentiality of using AM
fungi as biofertilizer along with other rhizosphere microorganisms is a hope for a sustainable agricultural output. AM fungi have been shown to stabilize soil aggregates which are valuable in agricultural land (Smith and Read, 1997). Though, the potentiality of AM fungi as biofertilizer is well documented (Barea et al., 2004) the work on scientific use of AMF in tea cultivation is scanty.

Barea et al. (2005) stated that the specific management of mycorrhiza/bacteria interactions, through the manipulation of appropriate mycorrhizospheres, should be one of the main objectives of applied research in the future. The use of microbial inoculants must take into account the importance of retaining microbial diversity in rhizosphere ecology and in achieving realistic and effective biotechnological applications (‘rhizosphere technology’). Further studies must address the consequences of the interactions between microbes in the rhizosphere under field conditions to assess their ecological impacts and biotechnological potential in near future.

Tea, since its cultivation, had gone through different strata of changes. Use of pesticides for control of pests and diseases along with the application of fertilizers to increase leaf harvest is a part and parcel of tea industry at present. No doubt, over the decades the synthetic pesticides and chemical fertilizers have improved agricultural production, but indiscriminate and excessive use of chemicals for plant protection and to
get higher yield hampered the tea ecosystem and also cause threats to human health. As a result organic tea is of great demand, other countries will not purchase tea which would contain traceable amount of pesticide and/or chemical fertilizers.

But, the scenario is now changing and the agroscientists and researchers are deriving new naturally occurring substances or organisms which are readily biodegradable, species specific and harmless to non-target organisms. Demand for organic tea cultivation has certainly opened new vistas in the direction of soil health and plant growth and quality parameters. Needless to say, the bio-agricultural inputs like biofertilizer, vermiculture etc. can contribute significantly to crop yield and quantity without hampering the native soil ecosystem. Use of microbial and endomycorrhizal inocula as biofertilizer instead of synthetic molecules will definitely reduce the harmful effect of pesticide toxicity.

So, in the recent era of globalization and advance research methodology, tea industry has undergone tremendous changes in relation to its nutritional inputs, pest and disease management, conservation of microbial biodiversity and introduction of biological methods of dealing the problems. Researches in the field of rhizosphere microflora have opened the door for initiating plant growth promoting factors to manipulate from the existent flora. (Paula et al., 1992; Dutta et al., 2007; Esmaeil et al., 2008; Zaki et al., 2009). Vesicular Arbuscular Mycorrhiza
(VAM) now known as Arbuscular Mycorrhizal Fungi (AMF) is also a niche area of research in tea plantation. Workers in the past had cited the presence of AM fungi in tea of North east India (Barthakur et al., 1992, 1994, Hazarika et al., 2001).

With this background, the beneficial aspects of AM fungi, N$_2$ fixers and phosphate solubilizers on tea plantation were taken up.

1.7 Justification of the work:

As tea thrives in acid soils, the problem of phosphate nutrition is too apparent. NPK are the major mineral elements required for the vigor of the tea plant. The importance of phosphorous in the growth process is well documented. It is an integral part of the cells vital centre—the nucleus. Leaf phosphorous is believed to influence the photosynthetic rate. Deficiency in phosphorous hinder the conversion of starch to sugar in the plants and as maximum available phosphorous is obtained at around pH 6-7, which arose a problem in tea plant as it thrives in acidic soils.

Apart from this, the interactions between the tea plant, soil nutrient and the microorganisms is the new quest in the area of research for its vital application and utility. Hence, mineral nutrition and manipulation of rhizosphere microflora for efficient release of
nutrients needs more attention for achieving better quality and productivity avoiding chemical substitute.

So, the present investigation on the interactions between Arbuscular Mycorrhizal (AM) fungi and the other rhizosphere microflora i.e., diazotrophs and phosphate solubilizing microbes as efficient plant growth promoter along with its impact on growth and vigor of the tea plant will definitely help in understanding the nutrient absorption characteristics of different native microflora in tea ecosystem.

Subsequently, as we strive to optimize the performance of all crops, increasing the value of inoculating soil with other microorganisms or promoting the activity of native microorganisms through management practices was also explored. Moreover, an attempt was undertaken to manage the indigenous Arbuscular Mycorrhizal (AM) fungi in such a way that the efficient native fungi are enhanced. So, the manipulation of this symbiotic association as well as consortium to attain a beneficial economic strategy and to introduce a new direction in the field of nutrient management particularly for the organic tea growers of the industry was undertaken.
1.8 Aim of the Work:

Hence, the proposed study “Studies of Arbuscular Mycorrhizal (AM) Fungi in Tea, *Camellia sinensis* (L) O. Kuntze and Effect of their Interactions with Rhizosphere Microflora” aims at exploring new techniques of introducing and popularizing the AM biofertilizer with efficient strains of native diazotrophs and phosphate solubilizers in such way as to provide the planters an economic easy to use biofertilizer. The objectives were

1. To assess the occurrence of Arbuscular Mycorrhizal (AM) fungi in tea growing areas of Assam.
2. To quantify and isolate tea rhizosphere microflora mainly diazotrophs and phosphate solubilizers.
3. To observe the interactions between the diazotrophs and Phosphate solubilizers with Arbuscular Mycorrhizal (AM) fungi in response to tea growth.
4. To develop a protocol for utilization of plant growth promoting microbes in tea nursery.