2.1 AN OVERVIEW OF TEA

Tea, a hot beverage with good aroma, is made from tender leaves of the tea plant (Saravanakumar et al 2007). The tea plants that give the ‘cup that cheers’ belong to the family Camelliaceae, which contains 82 species of the genus Camellia. Taxonomists attribute the heterogeneous origin of present day commercial tea plants to three distinct taxa. Tea plants are evergreen members of the Camellia family that are native to China, Tibet and northern India. There are three main varieties of the tea plants. The small leaf variety, Camellia sinensis (L.) O. Kuntze), thrives in the cool, high mountain regions of China and Japan. The broad leaf variety, Camellia assamica (Masters) Wight, grows best in the moist, tropical climates found in Northeast of India, Assam. The intermediate leaf variety, known as ‘Cambod’ Camellia assamica ssp. lasiocalyx (Planex ex Watt) Wight grows well in moderate temperatures with rainfall. This variety is extensively cultivated in southern India, especially in the Western Ghats region of Tamil Nadu, Kerala and Karnataka states. This variety produces dark green, shiny leaves and small, white blossoms (Hudson et al 1998).

According to an old Chinese saying, “superior tea comes from high mountains”. The high altitude and mountain mists help shield crops against excessive sunlight and provide the appropriate temperature and humidity to allow the leaves to develop slowly and remain tender (Barua 1965). As with wine, the quality and taste of a particular tea is influenced both by the
environment (soil, climate, and altitude) and the tea maker (who decides when and how leaves are harvested and processed) (Verma and Chandramouli 1995).

2.2 TEA CULTIVATION IN SOUTHERN INDIA

Tea bushes cultivated in southern India mostly belong to the 'Assam jats' and 'China hybrids'. These plants are hardy, multi stemmed; slow growing evergreen shrubs which if allowed to, can grow up to 2.5 m in height. It takes four to six years for the plants to mature. The leaves are small, dark green, elliptic-oblong or ovate-oblong, lamina sparsa appressed and pubescent, with densely pubescent midribs, and have obtusely serrulate to double serrate and flat to wavy margins. Under cultivation the bushes are regularly pruned and trained to maintain them as low spreading bushes to ensure that maximum crops of young shoots can be plucked with easy harvesting (Hudson et al 1998).

The root systems of a tea bushes can be divided into main roots, subsidiary roots and feeder roots covering primary, secondary, tertiary root architecture. The lateral roots give rise to a surface mat of feeding roots which lack root hairs when mature. Root systems vary in tea bushes depending on genetic makeup and soil environment. Starch is stored in roots. The plucking season begins in March and closes by November, and the cold winter months of December to February are a period of dormancy, where the yields are low (Hajra 2001).

2.3 BIOTIC AND ABIOTIC FACTORS FOR TEA PLANT GROWTH

Tea is grown under a regime of air temperature that varies between 8°C and 35°C. In southern India, the extension growth ceases at monthly mean
maximum and minimum temperatures of 19.4°C and 12.4°C; respectively in November. Flushing growth commences near the end of March, when mean maximum and minimum temperatures exceed 21°C and 14°C; respectively. Tea is basically a rain-fed crop. It grows well in areas where annual rainfall varies from 1150 to 6000 mm. Tea should not normally be grown in areas where the rainfall is below 1150 mm, unless irrigation is available. Relative humidity of 80-90% is favourable during the growth period of tea plants. Below RH of 50%, shoot growth is inhibited, and below RH of 40%, growth is adversely affected (Hajra 2001).

Tea plants require a warm and humid climate, and the ideal ambient temperature is 18°C to 29°C, with well-distributed rainfall (>1400 mm per annum). Well drained sandy loam soils with pH of 4.5 to 5.5 are considered ideal for successful plantations. Tea soils are highly weathered, acidic and of low fertility status. In tea soils of southern India, overall content of nitrogen and organic matter varies, respectively, from 0.07 to 0.09% and 1.0 to 1.2%. The critical values have been fixed at 0.1% for nitrogen and at 1% for organic matter. The optimum amounts of available phosphorous and magnesium and base saturation is low. The minimum level (critical limit) of nutrient status of tea soils should be 10 mg/g for phosphorus, 80 mg/g for potassium, 25 mg/g for magnesium and 90 mg/g for calcium (Ellis and Nyirenda 1995).

2.4 ROLE OF FERTILIZERS IN TEA PLANTS
fertilizers, either in soils or in flush shoots. Fertilizers are of key importance, and the costliest input for increasing the sustainable productivity of tea (Verma and Palani 2011).

2.5 PLANT GROWTH REGULATORS IN TEA PLANTS

Growth is a multifactor-dependant rhythmic phenomenon. Besides the availability of nutrients, plants require growth regulators for their growth and development. These are available/synthesized endogenously in the plant systems. Under favourable conditions, the levels of endogenous growth promoting substances are greater than that of inhibiting substances, which results in accelerated growth and development of the plants (Gensheng 1991). In certain circumstances, levels of inhibitors increase, tilting the promoter: inhibitor ratio, which arrests the plant growth or the plant enters in dormancy (Kumar and Manivel 1998), leading to crop loss.

Under these circumstances, external application of plant growth regulators (PGRs) tilts the ratio towards the promoters, and this enhances growth and development in crop plants. All the PGRs available in the market are either synthetic or extracted natural products from plants or microbes. Commonly applied PGRs in tea plants are amino acid formulations, mixtures of nutrients, hydrolyzed proteins, tricontanol, humic acids and seaweed extracts (Mandal et al 2007). This PGRs application in tea plants is mainly for budji (dormant epical buds) reduction and leaf productivity.
and warm, humid climate of tea growing areas are highly conducive for disease development (Verma and Chandramouli 1995). A large number of pathogenic organisms, collected from different parts of plants, occupy this ecological niche (Bore 1996).

Tea plants are rain fed and commercially grown in humid zones with moist environments. The plants are pruned into bushes and are being harvested from the second year onwards at regular intervals of 7-20 days throughout the year. Hence, the microclimatic conditions and monoculture habitats results in a wide range of phytopathogens, which can cause severe losses. These have been estimated to be 25-33% during 2001 to 2010 (Ponmurugan et al 2010). Among the microbial infestations in tea plants, fungi play major roles in tea diseases, which in turn affect the capital losses. From earlier reports of Agnihothrudu (1964), more than 385 fungal species have been associated with tea plants, and 82 are pathogenic and associated with various diseases infecting roots, stems and leaves. Several fungal diseases associated with tea roots are listed in the Table 2.1.

<table>
<thead>
<tr>
<th>Name of the root disease</th>
<th>Causal agent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Root splitting</td>
<td>Armillaria mellea</td>
</tr>
<tr>
<td>Black root rot</td>
<td>Rosellinia arcuata</td>
</tr>
<tr>
<td>Botryodiplodia root rot</td>
<td>Botryodiplodia theobromae</td>
</tr>
<tr>
<td>Brown root rot</td>
<td>Fomes noxius</td>
</tr>
<tr>
<td>Black root rot</td>
<td>Corticium theae</td>
</tr>
<tr>
<td>Charcoal stump rot</td>
<td>Ustulina deusta</td>
</tr>
<tr>
<td>Red root rot</td>
<td>Poria hypolateritia</td>
</tr>
<tr>
<td>Purple root rot</td>
<td>Helicobasidium compactum</td>
</tr>
<tr>
<td>Root rot</td>
<td>Cylindrocladium camelliae</td>
</tr>
<tr>
<td>Tarry root rot</td>
<td>Hypoxylon asarcodes</td>
</tr>
<tr>
<td>Violet root rot</td>
<td>Sphaerostilbe repens</td>
</tr>
<tr>
<td>White rot</td>
<td>Fomes lignosus</td>
</tr>
<tr>
<td>Wood rot</td>
<td>Hypoxylon serpens</td>
</tr>
<tr>
<td>Xylaria root rot</td>
<td>Xylaria sp.</td>
</tr>
</tbody>
</table>
2.7 RED ROOT ROT DISEASE OF TEA PLANTS

Among different root diseases, red root rot, brown root rot and black root rot are commonly occurring diseases in southern India. In particular, red root rot, caused by the fungal pathogen *Poria hypolateralitia* Berk. ex Cooke, is an important disease in tea plantations, causing heavy crop losses. Red root rot is a serious root disease of tea in Sri Lanka, and the disease occurs at the elevations between 750 and 2000 m above mean sea level (MSL). These elevations include regions with different climatic conditions (Shanmuganathan 1997). Red root rot is important because it affects entire root systems of plants, resulting in sudden death of bushes and very considerable crop losses, which may be as great as 43% (Baby 2001).

*Poria hypolateralitia* is a fast spreading, slow killing pathogen, and the pathogen may be identified by the mycelial strands in which the fungus forms on exterior of roots, which appear white and soft. Later, the fungus forms into compact, tough, red or dark red cords. The pathogen spreads to other bushes by interweaving with adhering soil. After washing with water, the infected soil disappears exposing blood red mycelium. In advanced stages, the old cords of mycelium change colour to black. Infected root wood finally becomes spongy and sodden. Very rarely, frutification conidia form at the collar area of infected plants seen. This pathogen also attacks coffee, rubber and *Greviella*.

According to earlier history and disease incidence in Ceylon, close examination of *P. hypolateralitia*-infected root systems revealed that the pathogen colonized top 76 cm of soil. The fungus invades practically all lateral roots, and main stump, and causes soft rot. Sterile fructifications are frequently formed on the collar of dead bushes. Infection occurs both by contact between healthy and infected roots and by growth of mycelium
through soil. The majority of infection probably arises by way of lateral roots (Mulder and Redlich 1962).

In the absence of air-borne conidia, the only known source of the pathogen is mycelium that eventually spreads through soil and contacts neighbourhood bushes for subsequent infection. Besides this, planting of shade trees susceptible to *P. hypolateritia* has long been abandoned. It appears unlikely that shade tree roots can still serve as sources of inoculum for emergence of disease. There are two ways by which the pathogen gains entry into hosts. These are in young plantations through infected roots left over during uprooting of old tea plants, and through accidental spread of infected material by man in both young and old plantings. The former can be prevented by fumigating all infested patches in the old tea areas before uprooting. Moreover the dispersal of infected roots within a tea plantation is difficult to prevent (Kerv and Vytilingam 1966).

The earlier recommendation involved the diseased plants being grubbed by winching before the affected land is fumigated. The fumigant has to deal with only residual inoculum. Much inoculum is dispersed during the initial digging up operations. But such dispersal would be unimportant, if uprooting was carried out after fumigation with methyl bromide, as the pathogen would be dead in roots and taken out freely after treatment. Methyl bromide was applied with 1-2 lb/100 ft$^2$ for red root rot control (Shanmuganathan and Fernando 1997).

*In vitro* studies on three isolates of *P. hypolateritia* obtained from three different climatical areas of Sri Lanka revealed that there was a significant difference in their growth, among different media tested. All the isolates showed optimum growth on potato dextrose agar (PDA) at 25°C, and pH values from 3.5 to 5.5. All the isolates secreted one of polygalacturonase, and three forms of β-glucosidase. Hence the detection of these isolates with
different characteristics must be taken in to consideration for future disease control, when developing new clones and new planting programmes in tea industries (Wijesundera and Kulatunga 1993).

Red root rot is a destructive and economically important disease in terms of direct capital losses in tea estates (Muraleedharan and Chen 1997). The disease affects mainly root systems, and later disturbs major foliage especially mother leaves and maintenance foliage. This directly affects the active sites of photosynthesis and supply of food materials from the actively growing harvestable shoots (Rajkumar et al 1998). It has been reported that red root rot in tea plants can also be caused by *Ganoderma philippii* in China (Chen and Chen 1990).

At present, the management strategies for red root rot are solely based on sanitation, wherein infected tea plants, plus one or two rows of apparently healthy bushes in the surrounding areas are uprooted and burnt out. The susceptible and tolerant nature of tea plants to red root disease was reported to be related to water stress and cultural operations, like tipping, pruning, mechanical shear harvesting and careless manual plucking methods (Ponmurugan et al 2010).

### 2.8 PLANT GROWTH PROMOTING RHIZOBACTERIA: AN ALTERNATIVE APPROACH FOR CHEMICAL FERTILIZERS

The term PGPR was first defined by Kloeper and Scroth (1978), to describe soil bacteria that colonize the roots of plants and promote plant growth. The first clear indication of improved plant growth and biological control of root pathogens was from seed bacterization with *Pseudomonas* rhizobacteria strains. Kloeper et al (1980) coined the term PGPR to include
bacteria inhabiting root and rhizosphere soil, which have the ability to increase plant growth.

In the context of increasing international concern for food and environmental quality, the use of PGPR for reducing chemical inputs in agriculture is potentially important (Ashrafuzzaman et al 2009). Nitrogen and phosphorus are essential nutrients for plant growth and development. Microorganisms are important in agriculture in order to promote cycling of plant nutrients, and reduce the need for chemical fertilizers as much as possible. Plant growth-promoting rhizobacteria can exert beneficial effects upon plant growth. Nitrogen fixing and phosphate solubilizing bacteria may be important for plant nutrition by increasing nitrogen and phosphate uptake by the plants, and playing significant roles as PGPR in the biofertilization of crops (Cakmakci et al 2006).

2.9 MECHANISM OF PLANT GROWTH PROMOTING RHIZOBACTERIA AND ITS SIGNIFICANCE

The mechanisms involved in growth promotion when agronomic crops are inoculated with rhizobacteria include, increase in the nitrogen fixation, the production of auxin, gibberellins, cytokinin, ethylene, the solubilization of phosphorus and oxidation of sulfur, increase in nitrate availability, the extracellular production of antibiotics, lytic enzymes, hydrocyanic acid, and increased in root permeability (Enebak and Carey 2000). Of these ACC deaminase activity, siderophore production, enhanced biological nitrogen fixation and enhanced uptake of essential plant nutrients could be the most likely explanations for growth promotion.

Plant growth promotion has been widely reported for numerous species of Gram negative bacteria (Babalola et al 2003). The phenomenon has been extensively studied in numerous species of PGPR including *Bacillus* and
Pseudomonas. Treatment of plants with PGPRs has increased the seed germination, seedling vigour, emergence, plant stands, root growth, shoot growth, total biomass of the plants, seed weight, early flowering, and grain, fodder, and fruit yields (Van Loon et al 1998, Ramamoorthy et al 2001).

2.10 FLUORESCENT PSEUDOMONAS AS PLANT GROWTH PROMOTING RHIZOBACTERIA AND BIOCONTROL AGENTS

Among the different PGPRs, fluorescent Pseudomonads are considered to be the most promising group of PGPR involved in biocontrol of plant diseases (Moeinzadeh et al 2010). The genus Pseudomonas encompasses a diverse and ecologically significant group of bacteria. Fluorescent Pseudomonads are Gram negative, rod shaped, and chemoheterotrophic bacteria with polar flagella. They are characterized by production of yellow green iron chelating low molecular weight siderophores (pyoverdines or pseudobactins) in culture, that fluoresce under UV light. They are common rhizosphere inhabitants and are the most studied group within the genus Pseudomonas. They can be visually distinguished from the other Pseudomonas species by their ability to produce water soluble yellow/green pigments.

Fluorescent Pseudomonads are well recognized as PGPR, phosphate solubilizers and as biocontrol agents against plant pathogens. The occurrence and distribution of fluorescent Pseudomonads have been studied by many workers, and these organisms have been reported as rapid and aggressive root colonizers of cereals, pulses, oilseeds and vegetable crops, and from rhizospheres of rice, wheat, pigeon pea, ground nut and chilli crops. Rao et al (1999) isolated fluorescent Pseudomonads from the rhizoplane and rhizosphere of pea and soybean plants.
2.10.1 *Pseudomonas fluorescens* as Plant Growth Regulator and Phosphate Solubilizer

Indole-3-acetic acid (IAA) is a phytohormone which is known to be involved in root initiation, cell division and cell enlargement (Ponmurugan and Gopi 2006). Plant growth promoting rhizobacteria, including fluorescent Pseudomonads are capable of surviving and colonizing the rhizosphere of all field crops. They promote plant growth by secreting auxins, gibberellins and cytokinins. The intrinsic ability of high level of IAA production by fluorescent Pseudomonads is a general characteristic. This bacterial IAA plays a major role in the development of host plant root systems.

Phosphate solubilization occurs from carboxlic acids synthesized and released by microorganisms. Gupta et al (2002) described *Pseudomonas* species as potent phosphate solubilizers. This has high tricalcium phosphate solubilizing ability. Haque and Dave (2005) studied ecology and diversity of phosphate solubilizing microorganisms in soil under organic and non-organic farming, in virgin and barren soils of Gujarat, and found *Pseudomonas* spp., to be most prevalent among the bacterial isolates obtained.

Phosphate solubilizing *Pseudomonas* strains was considered as possible inoculation tools for phosphate deficient soils that may use results from laboratory assays, green house experiments as well as field trials (Henri et al 2008). It was previously reported that *Pseudomonas* spp. have high potential to solubilize phosphorus which in turn provides useful supply of the soluble form of phosphate to plants (Rodriguez and Fraga 1999).

2.10.2 Siderophore and Hydrogen cyanide Production by *Pseudomonas fluorescens*

Fluorescent Pseudomonads are characterized by the production of yellow-green pigments termed pyoverdines which fluoresce under UV light
and function as siderophores. These are low molecular weight, extracellular compounds with high affinity for ferric iron, to take up iron from the environment. Their mode of action in suppression of disease was thought to be solely based on competition for iron with respective pathogens. Tripathi and Johri (2002) reported production of iron chelating hydroxymate type siderophores as one of the mechanisms for \textit{in vitro} inhibition of the plant pathogens \textit{Colletotrichum dematium}, \textit{Rhizoctonia solani} and \textit{Sclerotium rolfsii}.

Hydrogen cyanide (HCN) inhibits the electron transport, and energy supply to cells is disrupted leading to organism death. This compound inhibits proper functioning of enzymes and natural receptors by reversible mechanism of inhibition. HCN also inhibits the action of cytochrome oxidase. Ramette et al (2003) reported that HCN is a broadspectrum antimicrobial compound involved in biological control of root disease by many plant-associated fluorescent Pseudomonads.

\section*{2.10.3 Antibiosis and Production of Antimicrobial Metabolites}

Antibiotics are generally considered to be organic compounds of low molecular weight produced by microbes. Antibiosis plays an active role in the biocontrol of plant diseases, and often acts in concert with competition and parasitism. Production and release of certain toxic/inhibitory metabolites by antagonistic bacteria have been recognized as major factors in suppression of root pathogens. Mishra et al (2005) reported that two strains of fluorescent Pseudomonads, isolated from tea rhizospheres and rhizoplanes of rice, showed \textit{in vitro} antibiosis against \textit{Fomes lamoensis} and \textit{Ustulina zonata}, the causal agents respectively, of brown root rot and charcoal stump rot of tea. Application of these strains resulted in suppression of both the diseases under gnotobiotic and nursery conditions.
Several strains of *Pseudomonas* spp. have been shown to produce wide arrays of antibiotics, which include 2,4-Diacetyl phloroglucinol (2,4-DAPG), HCN, kanosamine, phenazine-1-carboxylic acid, pyoluteorin, oomycin A, pyrrolnitrin, pyocyanin, viscosinamide, tropolone and cyclic lipopeptides, as well as several other uncharacterized moieties (O’ Sullivan and O’Gara 1992).

Surjit Sen et al (2006) reported that in dual culture, significant growth inhibition of *Sclerotium rolfsii* by *Pseudomonas* was observed. Mycelial growth of the fungus was restricted near bacterial streaks in dual cultures. Increase in incubation period was proportionate to growth inhibition of *S. rolfsii* up to 6 days. Microscopic study of mycelia from interacting zones showed hyphal shrivelling, and mycelial deformities such as swelling, fragmentation, short branching, and, finally, cell lysis.

### 2.10.4 Induced Systemic Resistance

Induced resistance is defined as enhancement of the plant defensive capacity against a broad spectrum of pathogens and pests, which is acquired after appropriate stimulation. The resulting elevated resistance due to an inducing agent upon infection by pathogen or by rhizobacteria is called induced systemic resistance (ISR) or systemic acquired resistance (SAR) (Hammerschmidt and Kuc 1995, Van Loon et al 1998). A large number of defense enzymes that have been associated with ISR include phenolics, phenylalanine ammonia lyase, tyrosine ammonia lyase, chitinase, β-1,3-glucanase, peroxidase, polyphenol oxidase, superoxide dismutase, catalase, lipoxygenase, ascorate peroxidase and proteinase inhibitors (Van Loon et al 1998). These enzymes also liberate molecules that elicit the initial steps in induction of resistance, including phytoalexins and phenolic compounds. ISR by PGPR has been achieved in several crop species (Ramamoorthy et al 2001), against a broad spectrum of fungal pathogens.
2.10.5 Bioformulation of *Pseudomonas fluorescens*

An important area of biological control is the development of formulations that would allow for viable microbial activity for long periods of time (Manju et al. 2013). Mass multiplication of PGPR in suitable media, and development of a powder formulation was first carried out in earlier studies. A dried powder formulation of PGPR especially is important for seed treatments and soil applications. The survival of PGPR in dried formulations and the effectiveness of methyl cellulose in powder formulations for coating sugarbeet seed have been well documented. Talc-based formulations of *P. fluorescens* isolated from the rhizosphere of different crops have been developed (Vidhyasekaran and Muthamilan 1995). It has been confirmed by previous research that talc formulations consisting combination of both *P. fluorescens* and *T. atroviride* were most effective for reducing dry root rot incidence caused by *Macrophomina phaseolina* in greengram under glasshouse and field conditions, as compared with other single treatments and untreated controls (Thilagavathi et al. 2007).