Chapter II

Review of Literature
II .1. Diseases of Tea

Tea is prone to many diseases during its long life span which extends over 60 years. Watt and Mann, (1903) was the first to give a comprehensive account on the pests and diseases of tea in their memorable book “Pests and Blights of Tea Plants” where they enumerated only a dozen diseases, though they mentioned that type of disease appeared to be growing in numbers and virulence every season. Mann and Hutchinson (1904) described some tea specific diseases. Fetch (1923) described several fungi parasitic on tea and brought out a comprehensive book on “Fungi and Diseases in Plants” from Ceylon. Butler and Bisby (1937) reported 30 species of fungi on tea from India. Besides these treatises, many regional works are also available from India (Tunstall, 1930), Sri Lanka (Gadd, 1949), East Africa (Goodchild, 1952), Mauritius (Ramlogun, 1971), Japan (Hamaya, 1981), Peoples Republic of China (Sudoi and Langat 1992; Tzong Mao Chen and Shin Funchen, 1982), which describes in detail about the fungi and bacteria associated with tea plants.

Tunstall (1942) described specific diseases of tea and Tunstall and Sarmah (1947) described the stem diseases. Different aspects of tea diseases have also been discussed by Agnihothrudu (1963); Satyanarayana and Barua (1975). Agnihothrudu (1963) reported 389 species of fungi on tea, out of which over 190 occur in NE India. A few more species has been added by Chandra Mouli and Ravi Kumar (1988); Rattan and Pawsay (1981). A number of the fungal pathogen causes foliar diseases of tea, some of which cause serious damage to the existence of the tea plantations.

II.1.1. Foliar Diseases

The foliar diseases are extremely important economically as even slight damage to the tea leaves reduces the quality and quantity of tea production. Black rot is a major disease of tea capable of causing a significant depreciation in the tea yield and quality in
India (Banerjee, 1993). Black rot of tea is also a problem in Indonesia, India and Sri Lanka (Chandra Mouli, 1999). In NE India, two species viz., *Corticium invisum* Petch and *Corticium theae* Bernard are found causing black rot disease in tea (Tunstall, 1940). In NE India, black rot was recognized for the first time in 1914 in a garden in the Surma Valley and the first serious outbreak was recorded in Upper Assam in a garden in Lakhimpur district in 1918 (Tunstall, 1940). Satyanarayana and Barua (1983) noted that black rot occur frequently in the plains in NE India. *Pestalozzia theae* Sawada is a common pathogen of tea in NE India and frequently occur in association with brown blight, resembling the latter in appearance (Tunstall and Bose, 1920). The most common leaf diseases viz., leaf blight, leaf spot and leaf rot occur in many tea growing countries (Eden, 1976; Hainsworth, 1952; Petch, 1923; Sarmah, 1960). Of them, brown blight (*Colletotrichum camelliae*) and grey blight (*Pestalozzia theae* Sawada) though ubiquitous, are neither serious nor reported to cause epidemics. Brown blight disease can only gain entrance through a wound of tissue that in some other way may have been weakened (Baxter, 1974; Bertus, 1974; Dicken and Cook, 1989).

In Assam, blister blight was first reported in 1968 as having been prevalent for 10 years. It attracted little attention till the fungus responsible moved in 1908 out of Assam Valley and caused a severe attack in Darjeeling (Eden, 1976). Sarmah (1960) indicated that the fungus probably thrives on wild tea in the Himalayan forest hill and the spores are carried down to the plains by wind. At present it is still a major problem of Darjeeling (Ghosh Hazra, 1991). Blister blight (*Exobasidium vexans* Massee) by far the most important leaf disease, is known to occur in almost all the tea growing areas in India, Sri Lanka and Japan. In Japan and Taiwan, tea leaves are also affected by reticular blight caused by *E. reticulatum* Ito and Sawada (Dutta, 1995; Chandra Mouli, 1999).
The leaf spots caused by *Cercospora theae* (Cav.) Breda de Hann. has been occasionally found to be severe in Japan (Hamaya, 1981). Anthracnose (*Gleosprium theae-sinensis* Miyake) is a serious leaf disease in Japan. In Taiwan and China, though widely distributed, this disease is not so severe. A type of black rot caused by *Cerotobasidium* species has also been reported from Japan (Chandra Mouli, 1999).

Leaf scab caused by *Elsinoe theae* and sooty mould (*Meliola* species and *Caprodium theae* Boed) occur commonly but neither of them are considered to be a serious problem. Certain other foliar fungi, such as *Piggotia theae*, *Hendusonia theicola*, *Phoma theicola*, *Cladosporium* species *Phyllosticta* species, *Discoria* species, *Septoria* species are reported by Petch (1923).

Red rust causes serious die-back of the stem of young tea during its formative years. In Cachar district of Assam, it was probably one of the most serious causes of decline and closure of old tea gardens (Tunstall, 1940). Sarmah (1960) observed that the spores of red rust are common almost throughout the year and mostly come from collateral hosts which are abundant. Red rust has been reckoned as one of the most problematic secondary diseases of tea in NE India (Agnihothrudu, 1963). Red rust caused by *Cephalurose parasitica* (Karst.) a parasitic green alga and birds eye spot (*Cercospora theae* Car and Caurzi) occur in tea in several tea growing countries. Ten-fifteen per cent crop loss due to the disease was recorded in Assam and the predisposing cause was bad drainage system (Satyanarayana and Baruah, 1983).

**II.1.2. Stem Diseases**

Among the major stem diseases, wood rot is reported from India, Sri Lanka and Kenya. Collar canker (*Phomopsis theae* Petch) and dieback (*Leptothyrium theae* Petch) are frequently reported from India and Sri Lanka. Collar canker is also reported from Kenya. The branch canker (*Macrophoma theicola* Petch), apart from India, Sri Lanka,
Indonesia and Taiwan is also known to occur in Kenya and Malawi. Thorny stem blight (*Tunstallia aculiata* (Petch) Agnihotrudu) is reported world wide (Chandra Mouli, 1999).

Tunstall and Sarmah (1947) listed *Poria hypobrunnea* Petch as a commonly occurring disease associated with the decay or dead woody tissues in NE India. *Phomopsis theae*, which causes collar canker and *Macrophomina theicola* produces stem canker. These are important diseases that could inflict heavy economic loss. *Leptothynium theae* is reported only from Sri Lanka (Gadd, 1949) and India (Venkata Ram, 1960). Species of *Nectria* and *Septobasidium* occur only on woody branches (Tunstall and Sarmah, 1947). Similarly, the pathogen causing thorny stem blight (*Tunstallia aculeate*) and wood rot (*Hypoxylon* species) inhabits old stem portions of the tea bushes, and can cripple the bush frame once it establishes in the stem tissue (Venkata Ram, 1974).

Horsehair blight disease caused by *Marasmias equicrinis*, common in Japan, Sri Lanka and India, has recently been reported from Taiwan (Hu, 1984). The disease is mild and does not pose serious problem.

* Corticum invisum and *C. theae* occur under highly humid conditions and the pathogen is known to over winter in twig as sclerotia (Tunstall and Sarma, 1947). Bacterial shoot blight *P. syringae* pv. *theae* (Hari) Young, Dye and Wilke is of common occurrence in Japan (Hamaya, 1981).

**II.1.3. Root Diseases**

Butler (1918) observed that root diseases in tea are known to originate from parasites within the roots of the plants in natural vegetation and as such they constitute a major problem of tea cultivation.

Root diseases are a common feature wherever tea is grown on land which has previously been under jungle. This is attributed to the presence of tree stumps that are
left behind while clearing the jungle and which serve as a source of infection for long periods. Root diseases spread slowly and since their effects are not immediately visible, they are able to secure a firm hold on the tea bushes before their presence is detected and control measures adopted. Their importance in reducing crop is nevertheless great, not only because they cause the death of the entire bush, but also because of the long time taken before the bushes that are replaced come into production.

The above ground symptoms of all the root diseases are similar. They are always associated with water shortage, but they also occur at times when the soil is not deficient of water. If a whole bush or part of it suddenly dries up and dies with all its leaves attached, it is almost always a case of root disease. The leaves often turn a coppery brown and stay attached to the branches for a few days before they fall off. The roots of the bushes should be dug up and examined carefully for identification of the disease (Sarmah, 1960).

In old tea, the chief source of infection lies in the stumps of shade trees. Numerous secondary infections can occur as a result of accidental dispersal of infected material. In addition, infections can also take place by means of wind-borne spores. When the disease is at an advance stage, fructifications of the causative fungus frequently develop on the affected stumps of tea. The spores released from these fructifications settle and germinate on freshly cut shade tree stumps and the fungus ramifies through the tissues and invades the entire root system. The fungus then spreads from the roots of these shade tree stumps to neighboring tea roots. Instances of direct infection of tea by wind-borne spores are very rare (Dey, 2001).
Unlike other diseases, root diseases are difficult to control, as by the time their presence is detected, it is too late. Therefore, attention must be focused on preventing the spread of the disease to healthy tea plants once its presence has been detected.

The first step is to remove all the dead or dying bushes as soon as they are detected, with all their roots and burn them on the spot, irrespective of the cause of death. Under no circumstances the stump should be left in the ground or below ground. Any jungle or shade tree stump in the diseased patch must also be dug up and burnt, and its lateral roots should be traced and pulled out as far as possible. No roots, living or dead and no woody material which might become a food base for pathogens should be left in the soil.

In order to stop the spread of root diseases, it is not sufficient to remove the visibly affected bush, because, by the time it is diagnosed, adjacent bushes, though not showing symptoms, may already be infected. It is advisable, therefore, to remove healthy looking bushes surrounding the infected patches for examination until two rows of healthy bushes have been removed.

**Primary Root Diseases**

Primary root disease is one in which the fungus attack itself is the direct cause of death of the bush. Even the most vigorous and healthy bushes may be attacked and killed. The common symptom of primary root diseases are wilting and dying of foliage, but the withered leaves remain attached to the branches for sometimes, before they drop off. Sometimes, a patch of tea infected by a primary root disease may be recognized by the presence of a dead branch or branches on the side adjoining the focal centre, which is near a completely dead bush or a vacancy. Primary root disease may kill one to nine
bushes depending on the spacing and planting arrangement, at a time in any patch (Sarmah, 1960).

Primary root diseases are very common in tea plantations raised after clearing natural growth and therefore all the cases have the legacy of the jungle. They generally spread through soil or by root contact. When an apparently healthy tea bush dies suddenly, with the withered and dried leaves remaining attached to the bushes, the most usual cause of death is primary root disease. A bush may also be killed completely by a secondary root disease, so it is important to ascertain the casual agent before replacing it with an in fill. It is essential to be able to recognize the difference between the common, primary and secondary root diseases. If every death is attributed to attack of primary disease, it may result in unnecessary removal of a healthy bushes. On the other hand, if a primary disease is mistaken for a secondary one and no uprooting is done, the dead bush may spread infection to its neighbor and ultimately severe loss may occur. Description of the some common primary root diseases are as given below:

**Brown root rot disease** (*Fomes lamoensis* (Murr.) Sacc. and Trott.)

It is commonly prevalent in low elevation tea growing areas particularly in hilly region. Petch describes this disease as the earliest known root disease of tea. It is prevalent in all the tea growing areas of Sri Lanka but seldom causes any serious trouble. The progress of the disease is usually slow and, although the fungus produces external mycelium, it does not appear to spread to any appreciable distance through the soil. Apparently the disease passes from one bush to another along the roots which happen to be in contact. The organism causing brown root rot gains entry through the roots. Infection also occur by infected material coming into contact with the healthy plants (Satyanarana, 1979). The disease is more common on tea in sandy soil and characteristic feature of brown rot is the presence of brown mycelium on the root
surface to which soil, sand and stone particles remain encrusted (Banerjee, 1993). The mycelium cannot be easily knocked or washed off. The mycelium of the fungus acts like a cement and binds the soil to the roots. The mycelium can be seen as tawny brown threads often collected into woolly masses between the soil particles. In old cases, these masses of mycelium acquire a black, hard covering, sometimes with a brown powdery outer layer. When the bark is removed, there is usually a layer of white or brownish mycelium on the surface of the wood. The wood rarely shows much evidence of decay, especially if the bush has been dug up as soon as it was dead, but in advanced cases it is permeated with yellow-brown sheets, which assume a honeycomb structure and appear as a network of lines when the wood is cut. At this stage, the wood of the diseased root becomes soft, spongy and crumbling under slight pressure. Since infection spreads mostly through diseased root materials, dead tea bushes and stumps of the shade trees are the potential sources of the disease. *F. lamoensis* infection cannot pass from one root to another, unless the roots are in close contact. Since the infection spreads mostly through diseased root materials, dead tea bushes and stumps of felled jungle or shade trees are potential sources of the disease. The disease development is rapid and once established, it spreads to adjacent plants through root-to-root contact. After removal of the diseased plant, the pathogen can survive in debris remaining in the soil for more than 10 year (Sarmah 1960).

**Charcoal stump rot (*Ustulina zonata* (Lev.) Sacc.)**

Generally lightning is the pre-disposing factor for this disease. The disease is capable of causing sudden death of the bushes. W fan shaped patches of mycelium on the wood surface below the bark, the wood is transversed by typical double black line, carbonaceous brittle fructifications at the collar region are the symptoms of the attack. Charcoal stump rot is the most common of all the root diseases of tea in NE India. It
was also reported from South India and Ceylon on tea and a large number of alternate hosts are also recorded. In South India, it is invariably seen on areas affected by lightning (Satyanarayana, 1979). The organism, is soil-borne and enter the tea plant through roots. Infection also occurs through wound in thick branches by air borne spores. On the collar region of the affected tea bush, the fungus produces a black, hard effuse fructification which is wavy on the surface (Satyanarayana, 1980).

This disease is sometimes more difficult to identify than the other root diseases because roots attacked by *Ustulina* have no visible mycelium on the surface. But if the bark is removed, large white or brownish-white, fan shaped patches of mycelium are found overlying the wood. The fans may frequently be fused to form a thin continuous sheet, but the fan structure is usually recognizable. The wood is often hard and permeated by black sheets which appear as black lines when they are cut. Such lines are found in wood killed by other fungi also, but in the case of *Ustulina* the lines are duplicated as if drawn with a faulty nib which draws two parallel lines simultaneously. Fructifications are produced in abundance on dead *Grevillea* and *Albizzia* stumps and frequently at the collar of diseased tea bushes. They are somewhat ovoid in shape and initially thin and plate like, greyish white in colour and dotted with black spots. When old they are charcoal black and brittle and hence the name "Charcoal Stump Rot" for the disease (Sarmah, 1960).

*Ustulina* usually attacks tea by spreading to its roots from the roots of decaying stumps. Its prevalence is due to the felling of shade trees like *Grevillea robusta* and *Albizzia moluccana* without prior ring-barking and leaving the stumps to decay on the site. Very often diseased or dead bushes are found in the vicinity of decaying stumps. In some instances, a row of dead bushes can be traced along the course of a lateral root. Unlike *Poria* the fungus cannot pass from one bush to another unless the roots are in close
Plate 1(c): Brown root rot disease infected roots

Plate 1. (A) Typical tea garden of Barak Valley, (B) Brown root rot disease infected area and (C a,b) Brown root rot disease infected roots
contact. The disease therefore occurs most frequently on solitary bushes (Satyanarayana 1979).

**Root splitting disease (*Armillaria mellea* (Vahl ex Fries) Kummer)**

Root splitting disease is the most devastating primary root rot disease occurring at high altitudes. It was recorded and diagnosed from Sikkim and later from other gardens in Darjeeling (Satyanarayana *et al.*, 1980). This disease is identified by cracks on the bark; compact, white fungal growth in the crevices; thick white layer of mycelium between the leaves and the wood and the presence of thin root like or shoe lace like rhizomorphs which are either black or brown in colour. The fungus can withstand adverse conditions and when conditions are favourable spreads freely through the soil. Fruiting body of the fungus is rarely seen.

**Black root disease (*Rosellinia arcuata* Petch)**

Black root rot is one of the predominant root rot disease of tea plant, it is caused by the fungus *Rosellinia arcuata* Petch considered as serious primary root disease in India as well as N.E. India (Chandra Mouli, 1999). This disease is characterized by black woolly mycelial growth on the bark of the infected root and collar region with the presence of radiating ‘stars’ of white mycelium between the bark and the wood. Black lead shot like bodies are sometimes to be seen on collar. The mycelium grows freely through the surface soil and organic matter. The spread of the disease is very rapid in damp weather.

**Red root disease (*Poria hypolateritia* Berk)**

Red root disease is the most common and destructive, affecting the tea bush. It also affects coffee. The mycelium of the fungus in early stages of infection is of white strands and it later attains bright red colour from which the name Red Root Disease is derived. The mycelial strands fuse with one another to form a sheet in advanced stage of
infection and the mycelial shoots become dark, almost black in colour. The affected root is invariably encrusted with soil and stones. When such roots are washed in water with vigorously rubbing and held under light, the characteristic light red mycelium can be seen. Affected roots lose weight and become spongy. As the symptoms on host are manifested slowly, it is rather difficult to diagnose the disease in the early stages. It makes about 2-5 years for the tea plant to succumb to this malady. Symptoms of leaf yellowing, unthriftiness and lack of vigorous growth can be noticed among young plants invaded by this pathogen. Most frequently infection occurs by direct contact of diseased material with the roots of the plant (Sarmah, 1960).

**Xylaria root disease (Xylaria species Roger)**

The occurrence of this disease was first identified in 1971. Affected tea roots were covered by typical, black ribbon like, compact strands of fungal hyphae. The growth of mycelium is superficial and the affect of the fungal growth on the host tissue is confined more or less to the bark of the roots. The disease occurs in isolated patches and high casualty is noticed soon after drought.

**Secondary root diseases**

Secondary root diseases are those in which the fungus generally seen in association with the root is only of secondary importance, the prime cause of bush decline being something else. The fungus in this case invades the roots, which are already weakened by other factors and perhaps accelerate the death of the infected plant. The causes of secondary infection may be a mechanical injury to the plant, droughty condition, lack of drainage or hail storm or lightning. One has to study the symptoms carefully to decide whether the pathogen entered after occurrence of the pre-disposing factor or not. The following are some of the common secondary root diseases of tea.
Diplodia root disease (*Botryodiplodia theobromae* Pat.)

Diplodia root disease, though very commonly infects root only when the soil temperature is high (Venkata Ram, 1960) and the root starch reserves are depleted (Gadd, 1949; Petch, 1923; Venkata Ram, 1960). It is due to continuous hard plucking and pruning the bushes after a rush crop. Prolonged drought and a severe attack of pest and diseases may also be the cause of the attack. The common symptom of the Diplodia Root disease is root surface covered with small, groups of grayish black to coal black hairy cushions, giving a sooty appearance. Other symptoms are weak appearance of frame and presence of unhealthy leaves, failure of bushes to recover after pruning. New shoots after pruning may also exhibit die back (Venkata Ram, 1960).

Violet root rot (*Sphaerostilbe repens* B. & Br.)

Violet root rot is a disease of tea bushes and occurs due to the attack of a parasitic fungus on roots *Sphaerostilbe repens* and very common in North-east India. It is mainly occurs in water logging conditions. The symptoms of a disease are very characteristic leaves become turn yellowish and the fresh green color is lost. As the condition becomes aggravated the bushes began to die in a specific fashion the leaves drop of in fresh state without withering and dying. Violet, blue –black patches can be observed in roots on examination. The most characteristic feature of the disease is the sour rancid vinegar like smell of the roots (Sarmah, 1960). It is found most commonly on heavy soils particularly where there is flooding, back feeding of drains, collapsing or blockage of drains; poor soil aeration also favors the disease occurrence.

III.1.4. Chemical control (Fungicides) of root diseases of tea

Root diseases are insidious and losses caused by them staggering. They are a legacy of jungles that spread to tea fields which are planted after clearing the jungle. The diseases
are transmitted to young tea plants whenever their roots come in contact with the left
over roots of jungle trees harbouring the pathogenic organisms. Once the infection is
established, the diseases spread to the neighbouring plants by root contact and aerial
dispersal of spores (Chandra Mouli, 1999). Due to non availability of suitable
fungicides to control primary root disease of tea, some advances have been made and
some excellent control of brown root rot disease are achieved by chemical method,
mostly soil fumigation (Vapam, shell D-D, Chlorobromo propane formalin and
Nemagon) (Shanmuganathan, 1964). Though root disease attack is confined mostly to
the top 75 cm of root system, injection of the fumigant 30 cm deep gave effective
control. Methyl bromide was found to be useful in controlling certain primary root
diseases (Shanmuganathan and Redlich, 1965; Venkata Ram, 1972b). Certain
phytosanitary measures are useful in addition to fumigation of the affected area. A
trench of 45 cm wide and 120 cm deep may be made as an immediate measure of
isolation, while trenching two rows of apparently healthy bushes for Red Root Disease
should be included in the patch. The patch may be put under a non-host, such as
Guatemala grass, to rehabilitate the area for future planting (Arulpragasam, 1986). Soil
fumigation by a chemical, Durofume® which is a mixture Methyl bromide and Ethylene
dibromide is recommended to control the primary root diseases. Before taking up the
fumigation, all dead and dying branches are to be cleared in addition to the dead bushes.
The soil is leveled and loosened with a digging fork and the patch to be treated is
divided into strips of 9 m x 1.2 m. A polythene tent is erected over the strip and a
dispenser is thrust in the centre of the tent. Methyl bromide/ Durofume® canister is
placed in the receptacle of the dispenser horizontally and the polythene tent is sealed.
The canister is hit gently to puncture and release the gas. The boiling point of Methyl
bromide is 4°C and being three times heavier than air, sinks into the soil when released.
The tent should not be disturbed for 7 days. The requirement of Methyl Bromides is $40/m^2$. Soil fumigation with Methane sodium or carbon-di-sulphide at the rate of 172 ml per square meter can also be done. Before taking up the fumigation, all dead and dying bushes are cleared and the soil is leveled. Holes of 30 cm deep at 30 cm interval are made and the chemical is poured into them and covered with soil. The quantity of chemical to be poured into each hole is 8 ml of Methane sodium and 10 ml of carbon-di-sulphide. Light watering is done immediately after application. Treatment is very effective if it is done during April/May or November/December. Rehabilitating the threatened area with tea has to be done only after 6 months. A cost:benefit ratio of 1:14 has been achieved with soil fumigation as against conventional (trenching) treatment (Satyanarayana, 1980). Black root rot was effectively controlled by soil drenching of the affected area either with mancozeb or carbendazim (Chandra Mouli and Parthiban, 1991). Arulpragasam et al. (1987a) suggested rehabilitation of the diseases patches with grasses (*Tripsacum laxum*) for two years followed by tea plantation. Dirufuran has however, been banned of of its possible contribution to the environmental problem i.e. ozone layer depletion. This is not available in the market any more.

Efforts on disease control are mainly tried by flooding, fumigation with NH$_3$ of fields and application of fungicides. But the recurrent rate after termination of treatments and their economic feasibility are still a problem. Continuous application of synthetic compounds deteriorates the soil fertility and crop production.

Microbial control of fungal and insect pests is in great demand in agriculture at present. The interest in microbial pesticides has expanded because of problems, such as insect pest resistance, emergence of secondary pests, and toxic residues, that have developed with use of the broad spectrum chemical pesticides. Moreover, recent successes of microbial pesticide have greatly encouraged their usage. Insect pests cause a wide
variety of damage to plants. In the tropics it is virtually impossible to cultivate without resorting to pesticide application for controlling insect pests. Excessive use of chemical pesticides has posed serious problems of environmental contamination through the interference in the food chain. Apart from this, the resistance of pests to these chemicals is known to increase over time (Burges and Hussey, 1971). Thus there is a continuous need for safer, ecologically tenable pesticides.

Fungicidal protection is the prime strategy in the control of plant diseases (Maloy, 1993). In tea variety of fungicides with different modes of action is being used for the control of leaf diseases such as blister blight, grey blight, brown blight, red rust and anthracnose (Muraleedharan and Chen, 1997). According to Horikawa (1986), 12 of 31 fungicides registered in Japan were found to be effective controlling gray blight of tea. Differences in the emergence of resistant isolates to benzimidazole fungicides between of P. theae and P. longiseta was demonstrated in Japan (Oniki et al. 1986). Protectant fungicides are used as prophylactic chemicals, as they act outside the plant prior to infection by the pathogen.

Continuous application of synthetic compound has resulted in the reduction of biodiversity of flora and fauna, particularly the non-targeted beneficial microorganisms present in the phyllosphere/rhizosphere. It also often eliminates the natural enemies, outbreak of secondary pests, development of resistance to pesticides and contamination of food and the eco-system. Bio-pesticides, as a component of integrated pest and disease management are cheaper and eco-friendly than synthetic pesticides and fungicides. So, it is the need of the hour that we must resort to safer agricultural practices for the improved tea of productivity. The interest towards organic agricultural produce is mounting to catch up benefits of present day market. Also the future
agricultural exports face the risk of rejection if they are not free of pesticide residue. Use of naturally occurring microorganisms which can suppress/control the growth of pathogen/disease will be a viable alternative choice. Hence, nowadays interest has been shifted to environmentally safe and economically viable means for crop improvement, more often referred to as ‘Biocontrol’. In this regard, a lot of efforts are being made around the globe to develop commercial formulations with microbial strains, particularly with bacterial and fungal strains.

III.1.5. Soil microorganisms in tea plantations

From a microbiologist's point of view an established tea rhizosphere provides an excellent site for studying microbial interactions under natural conditions in a specific environment, more so due to the perennial nature of the tea plants (Pandey and Palni, 1999). The rhizosphere of young tea plants and that of a number of other perennial plants, of different ages, growing in established tea fields, appeared to stimulate microbial growth (Pandey and Palni, 1996). As in all life forms, the microbes are dependent on water, excess water encourages the activity of anaerobic forms whereas, well-aerated soils in tea encourage aerobic forms which promote oxidative reactions such as nitrate and sulphate formation (Bezbaruah, 1999). Indirect evidence to the importance of soil microorganisms in tea cultivation, appeared nearly 90 years back when Watt and Mann (1903) recommended organic sources of fertilizers such as vegetable matter, dung and oil cakes. Tea soil microbiology was actually initiated in 1901 with the report of mycorrhiza on tea roots (Webster 1956). Tunstall (1925,1930) made a detailed study of mycorrhiza on tea roots. Satyanarayana and Venkataramanan (1979) and Venkataramanan and Satyanarayana (1978, 1979) also reported mycorrhiza in tea. Hadfield (1960) initiated a study on tea rhizosphere and its effect on soil algae.
Apart from these, planting of leguminous shade trees and herbs to improve tea soil fertility through root nodules (Wight 1958), tea plantation soil microbiology has been confined to the study of pathogenic and non-pathogenic soil fungi. Agnihotru (1959) also reported that the rhizosphere bacteria of tea bushes infected with charcoal stump rot (Ustulina zonata) contained a higher diversity of bacterial strains than the rhizosphere bacteria of uninfected plants. Agnihotru (1960) also listed the fungal flora in tea plantation soils of Tocklai, Jorhat, Assam, India.

II. 4 The Rhizosphere Microorganisms

Studies have unequivocally proved that the saprophytic microflora of the rhizosphere includes both deleterious and beneficial organisms that have the potential to influence plant growth and crop yields significantly (Dube, 2001; Gupta et al., 2001). They classified rhizosphere microorganisms as beneficial (symbiotic) or harmful (pathogenic) or having no effect on the plant (neutral). Salt (1979) came out with the concept of minor pathogen, which are either saprophytes or parasites and are confined to juvenile tissues such as root hairs, root tips and cortical cells. The major pathogens (or tissue pathogens) on the other hand, penetrate the stele and disrupt the phloem, causing major disease symptoms. The minor pathogens are classified into two groups-one effects plants by their metabolites without parasitizing plant tissues and are designated as deleterious rhizosphere microorganisms (DRMO) or more often deleterious rhizobacteria (DRB) and the other that parasitize plant tissues are named as parasitizing minor pathogens (Schippers et al., 1986).
III.1.6. BENEFICIAL RHIZOSPHERE BACTERIA

The beneficial rhizosphere bacteria are no more limited to the symbiotic organisms. A new world has opened up with the discovery of non-symbiotic beneficial bacteria called plant growth promoting rhizobacteria or PGPR (Kloepper and Schroth, 1978).

Bio control of soil borne pathogens through Plant growth promoting Rhizobacteria (PGPR):

Over the last decades, a great diversity of rhizospheric microorganisms has been described, characterized, and in many cases tested for activity as biocontrol agents against soil borne pathogens. Use of microorganisms such as plant growth promoting rhizobacteria serves as an ideal alternative as their application results in the physiological and chemical stimulation of plant roots resulting in rapid emergence, higher chlorophyll content and enhanced stature (Lynch, 1976). A number of bacterial strains belonging to the genera Bacillus, Proteus and Pseudomonas have been screened for their ability to inhibit the growth of the pathogens F. lamoensis and U. zonata in different culture medium in vitro, nursery and field condition (Mishra, 2007). Selection of potential strains for crop improvement and/or control of plant pathogens through in vitro antibiosis study has been done by several workers (Chakraborty et al., 2005d; Dileep Kumar, 1999; Dileep Kumar and Bezbarua, 1997; Deka Boruah and Dileep Kumar, 2002; Sarmah et al., 2005; Utkhede and Rahe, 1983, Mishra 2007). Although the in vitro antibiosis test does not always correlate with the suppression of soil-borne plant disease, but because of the magnitude of the rhizosphere population and the lack of a more reliable method, in vitro screening of organisms is a valuable tool to select the potential strains (Cirvilleri et al., 1999; Cook, 1993).
**Plant Growth Promoting Rhizobacteria**

The studies on PGPR emerged from the work on the biological control, pursued at the University of California, Berkeley during 1970's to develop a control for the *Erwinia* soft-rot and black leg pathogens of potato. The fluorescent pseudomonads isolated from the rhizosphere of potato, that colonized potato roots and showed antibiosis against the erwinias were picked up for the study. These *Pseudomonas* strains did not show good performance in disease control at the field level but interestingly, they stimulated plant growth in green house as well as in the field trials (Burr et al., 1978). This prompted research on PGPR independent of biological control (Suslow et al., 1979). These findings were confirmed by Kloeper et al. (1980a), and extended to sugar-beets (Suslow and Schroth, 1982a) and radish (Kloepper and Schroth, 1978). Schorth and Hancock (1982) summarized results of their work on PGPR from field studies and reported that the fluorescent Pseudomonads increased the yield of potato by 5-33%, of sugar-beet 4-8 tonnes per hectare, and root weight of radish, 60-144%. Recent studies have confirmed their ability to induce disease resistance in host plants due to the production of certain enzymes and compounds like salicylic acid, jasmonic acid and siderophores which are discussed below in detail (Chakraborty et al., 2002; Maurhofer et al., 1998; Zehnder et al., 2000). There are several ways by which plant growth promoting bacteria can affect plant growth directly, *e.g.* by solubilization of minerals such as phosphorus, production of siderophores that solubilize and sequester iron or production of plant growth regulators (hormones). In some soils, 2,4-diacetylphloroglucinol (2,4-DAPG) producing fluorescent *Pseudomonas* species are largely responsible for the natural suppression of take-all known at take-all decline (Weller et al., 2002). Other antifungal metabolites, the phenazines, are produced via the shikimic acid pathway by the bio-control strains of *P. fluorescens* 2-79 and *P.*
chlororaphis 30-84. These compounds have a major role in the suppression of take-all by both the strains (Bull et al., 1991 and Pierson and Thomashow, 1992) and they have contributed to the long-term survival of the bacteria on the roots (Mazzola et al., 1992).

Indirect growth promotion occurs when PGPR promote plant growth by improving growth restricting conditions. This can happen by producing antagonistic substances or inducing resistance to pathogens. A bacterium can affect plant growth by one or more of these mechanisms and also can use different abilities for growth promotion at various times during the life cycle of the plant.

**Mechanism of PGPR:**

PGPR brings the physical and mechanical strength of the cell wall as well as changing the physiological and biochemical reaction of the host leading to the synthesis of defense chemical against the pathogen. The success of a plant in warding off invading pathogens relies primarily on its ability to build a line of defense for protecting cell walls against the spread of the pathogen (Benhamou et al., 1996a). It is also known that PGPR can modify the cell wall structure in response to pathogenic attack (Benhamou et al., 1996b). *P. fluorescences* strain 63-28 with treatment of pea seeds has resulted in the formation of structural barriers against the *Pythium ultimum* (Benhamou et al., 1996a).

Application of PGPR resulted in the biochemical or physiological changes in the plants. Many microorganisms are capable of producing auxins, cytokinins, gibberellins, ethylene, (ET), or abscisic acid (ABA). Ethylene, a hormone produced in all the plants, mediates several responses to the developmental and environmental signals in plants. Its involvement in plant growth when excreted around the roots has also been reported. Normally induction of systemic resistance (ISR) by PGPR is associated with the accumulation of PR proteins (pathogenesis related proteins) (Maurhofer et al., 1994;
Viswanathan and Samiyappan, 1999b) and synthesis of phytoalexin and other secondary metabolites (Zdor and Anderson, 1992). Root colonization of bean by fluorescent bacteria is correlated with induction of PR proteins and systemic resistance against Botrytis cinerea (Zdor and Anderson, 1992). It is also reported that microorganisms can produce siderophore which are ferric iron specific ligand without which the organisms are unable to retrieve iron (Khan and Zaidi, 2003; Winkelmann, 1986).

Production of antibiotic by soil microorganisms play an important role in the biological control of plant pathogens. Microbial antagonism includes three types of activity, antibiosis, competition and parasitism and predation. Antibiosis is the inhibition or destruction of one organism by the metabolic product of another (Cook and Baker, 1983). Metabolic products include not only antibiotics but also lytic enzymes, volatile compounds, and other toxic substances. Antibiotics are low molecular weight organic compounds that are produced by microorganisms and are deleterious to the growth and activity of other microorganisms (Fravel, 1988). Sometimes production of antibiotic correlates with biological control. Chaetomium globosum produces chaetomin and suppressed Venturia inaequalis which causes apple scab. In seven strains of Chaetomium globosum, antibiotic production in vitro positively correlated with suppression of the pathogen on apple seedlings (Cullen and Andrews, 1984). Agrobacterium radiobacter K84 produces agrocin 84 and suppressed Agrobacterium tumefaciens which causes crown gall (Kerr, 1990).

Defense related enzymes other than PR proteins, siderophore and antibiotic are also involved against the plant disease includes PAL, POD, PPO. Phenylalanine ammonia lyase (PAL) (EC 4.3.1.5) is currently the most studied enzyme. Cinnamic acid, one product of the enzyme reaction, is the precursor of a large variety of secondary
constituents, some of which, e.g. lignin, may account for an appreciable proportion of the total carbon in a plant. Not the least reason for the interest in this enzyme is, perhaps, the fact that it is relatively stable and very easily assayed by spectrophotometry (Koukal and Conn, 1961; Zucker, 1965), with $[^{14}\text{C}]$-phenylalanine (Subba Rao et al., 1967) and even GLC (Vaughn and Anderson, 1971). Current interest in this enzyme, discovered by Koukal and Conn in 1961, is reflected in the discussions by Yoshida (1969) and Zucker (1972). Polyphenol Oxidase (PPO) is a copper containing enzyme which is known as catechol oxidase, catecholase, diphenol oxidase, phenolase and tyrosinase (Martinez and Whitaker, 1995). In plant both soluble and membrane bound PPO with predicted molecular weight range from 57 to 62 KDa have been reported and they are basically chloroplastic located enzymes (Mayer, 1987; Tamuly and Roy, 1995). The PPO gene is encoded in the nucleus and translated in the cytoplasm, the pro PPO formed is then transported to the chloroplast where it is cleaved by a protease, producing the active form (Martinez and whitaker, 1995). The molecular weight of tea PPO has been reported to be $144 \pm 160$ KDa measured by sedimentation and diffusion (Gregory and Bandall, 1966). On gel filtration the molecular weight has been reported to be as 148 KDa (Halder, 1993) and 150 KDa (Chen, 1986). The pH optima for tea PPO have been reported to be ranging from 4.6 to 6.5 (Gregory and Bendall, 1966). Peroxidase (POD) (EC 1.11.1.7) is a hemoprotein that catalyses the oxidation of substrates in the presence of $\text{H}_2\text{O}_2$. Peroxidase (POD) has a broad specificity towards its substrates including mono and diphenol regardless of orientation and many others (Dix et al. 1981; Sesto and Huystee, 1989). POD activity increases during the growth restricted state of senescence. The greatest proportion of the POD activity following injury appears to be associated with the action bound ionically to the cell wall which becomes heavily lignified following injury (Huystee and Cairns, 1980). Its metabolic
role varies with the tissues in which it is found. Since POD may lead to the formation of quinones, all the products which can arise as a result of reaction of PPO, the flavanoid ‘A’ ring has been expected to be vulnerable leading to polymer formation by the action of POD (Dix et al., 1981). Huihau et al., (1986) reported three isozymes of tea POD. But on starch gel electrophoresis seven lain bands having POD activity have been detected by Tirimanna (1972), of which at least three were positively charged. During the process of tea fermentation, remarkable changes in their activities were observed. Tea POD occurs as insoluble form, large part of which can be solubilised in homogenate by addition of detergents such as tween 80 (Takeo and Kato, 1972).

III.1.7. Fungal Biocontrol Agents:

Biocontrol fungi (BCF) are agents that control plant diseases. These are beneficial organisms that reduce the negative effects of plant pathogens and promote positive responses in the plant. They do control diseases and in addition have other benefits, including amelioration of intrinsic physiological stresses in seeds and alleviation of abiotic stresses. They can also improve photosynthetic efficiency, especially in plants. There are several soil fungi which also increase nitrogen use efficiency in plants. Several mechanisms are involve to control plant diseases, some are due to mycoparasitism, production of antibiotics, and competitions for nutrients in the rhizosphere (Chet, 1987; Harman, 2007; Harman et al. 2004; Vinale et al. 2008). Most of the early work on biocontrol of plant diseases one by Trichoderma spp. During the process of mycoparasitism, the fungi first locates target hyphae by probing with constitutively produced cell wall degrading enzymes (CWDEs) coupled with very sensitive detection of cell wall fragments released from target fungi (Harman et al. 2004; Viterbo et al. 2002; Zeilinger et al. 1999). Expression of fungitoxic CWDEs is
induced, and these diffuse towards the target fungi and attack even before physical contact (Brunner et al. 2003; Viterbo et al. 2002; Zeilinger et al. 1999). This detection stimulates increased the directional growth towards the target fungus. Once the fungi come into contact, the BCF attach and may coil around and form appresoria on the surface of the host (Inber et al. 1996). Enzymes and antibiotic substances produced that kill and / or degrade the target hyphae and permit penetration of the BCF. Both the enzymes and antibiotic are strongly antifungal and are synergistic in their action (Chet et al. 1998; Howell 1998; Lorito 1998; Schirmbock et al., 1994).

Many Trichoderma spp can colonize plant roots of dicots and monocots (Harman et al. 2004). During this process Trichoderma hyphae coil around the roots, form appresoria-like structures, and finally penetrate the root cortex (Yedidia et al. 1999). Contact with pathogenic and nonpathogenic microorganisms triggers a wide range of defense mechanisms in plants. Two main mechanisms are recognized: systemic acquired resistance (SAR) and induced systemic resistance (ISR). SAR is involvement with the signal molecule salicylic acid (Durrant and Dong 2004) whereas ISR known to result from colonization of roots by certain nonpathogenic rhizosphere bacteria (van Loon et al. 1998). Trichoderma grows intercellularly in the root epidermis and cortex and induces the surrounding plant cells to deposit cell wall material and produce phenolic compounds. Most of the BCF can produce gibberellins as part of modulation of plant defenses in the roots (Schafer et al. 2009). Other mechanisms are employed by the plant to restrict the growth of this fungus roots, and recent studies implicate a β-glucosidase, PYK10, and perhaps germins in this process (Schafer et al. 2009; Sherameti et al. 2008) as well as salicylic acid (Stein et al. 2008). When inside plant roots, fungi have access to plant nutrients, which allow them to proliferate. Moreover they significantly enhance the root growth in many cases (Druege et al. 2007; Harman 2000; Harman et al. 2008;

From the above detailed review on the various aspects of plantation crop (tea), pathogen, beneficial microorganisms (bacteria and fungi) and fungicides which can be summarized in this chapter as Integrated management of brown root rot disease of tea which has drawned much attention in the recent time. In the present study isolation of some beneficial bacterial /fungal strains and some systemic fungicides screened have showed to be effective against the brown root rot fungus studied in detailed for the disease assessment, disease control, growth promotion and biomass during the nursery/field experiment.
STUDY AREA:
Geographically Assam can be divided into two major physical regions - the Barak Valley and the Brahmaputra Valley. Total geographical area of Assam is 78,550 sq km where, Barak valley constitute 8.9% percent of the geographical of Assam. Geographically Barak valley is surrounded by N.C. Hills and Jaintia hills in North, East by state Manipur, south by Mizoram and west Tripura and Sylhet district of Bangladesh. The climate of Barak valley is sub-tropical, warm and humid. The average rainfall of this area is 3180 mm with average rainy days of 146 per annum. The rainfall is caused by South-west monsson, which begins in the early June and continues up to October. The valley, however experience pre-monsoon rainfall in the month of March and April. Temperature in this valley ranges from 12.2°C to 35.4°C. The temperature range is moderate and winter is less severe than in the other parts of the state. Relative humidity is higher (above 90%) than that in the afternoon time due to foggy weather in the winter. Monthly variation of relative humidity in the morning ranging from 92-98% and in the afternoon varying from 43-78%
Soil type of Barak valley is entirely alluvial in origin and is composed of pebbles, sand, silt, clay and sometimes a mixture of sand and clay containing decomposed vegetables matter. There are some major soil classes in the valley are (a) Old riverine alluvium soil, (b) Old mountain alluvium soil, (c) Non laterised soil, (d) Laterised soil and (e) peat soil.

EXPERIMENTAL SITE
Experiment was carried out under the agro-climatic condition of Barak valley of Assam, india, in the experimental nursery of Ecology and Environmental Science Department, Assam university, silchar and Roskandy tea estate, cachar, Assam, during the year 2008.
to 2012. The experimental site was situated between 26°47’N latitude and 94°12’E longitude at an elevation of 86.60 m above mean sea level. Hot and humid summer, dry and cold winter is common features of this region. The details of the materials used and methods followed for conducting the experiments are described hereunder.
Plate-1. Map showing the study area in Barak valley (South Assam)