Chapter VIII

General Discussion
VIII.1. Isolation of brown root rot pathogen (*Fomes lamoensis*) from different areas of Barak Valley

The basic information on the tea disease is derived from the work of Watt and Mann (1903), Petch (1923), Butler (1918). In North East India several workers such as Tunstall (1940), Tunstall and Sarmah (1947) and Sarmah (1960) described the diseases associated with tea. Agnihothrudu (1964) reported a total of 209 genera comprising 389 species of fungi on tea, out of which over 190 occur in NE India. Brown root rot disease is primary root disease of tea commonly prevalent in the low elevation tea growing areas, particularly in the tea garden of the hills/hillocks. Among the fungal pathogens, the higher fungi, namely the *Ascomycetes*, *Basidiomycetes* and *Deuteromycetes* comprised 73 genera. Among the major tea pathogens 37% caused various leaf diseases, 36% caused stem diseases and 27 % caused root diseases (Chandra Mouli, 1995). Petch (1923) described brown root rot disease as the earliest known root disease of tea. In Barak valley region it is the most dominant root pathogen compared to others. 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 are happen to be in contact. The organism causing brown root rot gains entry through the roots. Infection also occurs by infected material coming into contact with 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 stony particles remain encrusted (Banerjee, 1993). In the present work the isolated fungus was confirmed as *Fomes lamoensis* which was compared with the isolate received from ITCC (IARI), New Delhi.
VIII.2. Isolation and identification of bacterial/fungal strain from various tea growing area of Barak Valley.

For the isolation of bacterial/fungal strains, soil samples were collected from different tea Estates of Barak Valley. The pH shown in different samples ranged from 4.08 to 5.48. The Urease activity revealed that the collected soil samples are having fairy good activity of microorganisms (Bezbaruah 1999). A total 114 numbers of bacterial strains were isolated. Out of this 27 numbers of bacteria were selected for identification. From the morphological study most of the bacterial strains, their forms observed are circular, margins are entire with maximum raised elevation. Most of the bacteria are found to have white pigmentation with cocci shape and they are found to be gram positive. Results from biochemical test showed that PM 12, PM 13, PM20, PM43, PM 83, PM93, PM98, PM105 and PM 114 are having the capability to produce starch hydrolysis, whereas only PM 43, PM105 PM 108 and PM 114 could produce catalase. The best three bacterial strains (on the basis of in vitro screening against the pathogen) i.e. PM 43, PM 105 and PM 112 were identified as *Bacillus cereus*, *Pseudomonas aeruginosa* and *Pseudomonas aeruginosa* respectively. The identification of the bacterial strains was confirmed from the Institute of Microbial Technology (IMTECH), Chandigarh.

The fungal strains were isolated and identified with the help of reference books (Gilman,1956; Barnet and Hunter, 1972; and Subramaniam, 1971; Agarwal et al. 2002). The genera *Aspergillus* and *Trichoderma* were found to be the most dominant in all the soil samples collected from the tea growing areas of Barak Valley.
VIII.3. *In vitro* antibiosis study of the isolated bacterial/fungal strains against brown root rot pathogen

Seven numbers of bacterial strains belonging to the genera *Bacillus* and *Pseudomonas* were screened for their ability to inhibit the growth of the pathogen *Fomes lamoensis* in King's medium B, Nutrient agar and Potato dextrose agar medium. The strain PM 105 showed the best inhibition of the pathogen followed by PM 43 and PM112 respectively. These three strains were selected for the growth promotion and disease suppression studies under nursery condition. 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 Bebaruah, 1997; Deka Boruah and Dileep Kumar, 2002; Sarmah *et al*., 2005; Utkhede and Rahe, 1983; Morang *et al*. 2011). Many rhizobacteria including fluorescent Pseudomonas are known to secrete a variety of antifungal molecule under *in vitro* and *in situ* condition (Cook *et al*. 1995). Johri *et al* 1997 reported *in vitro* antagonism of pathogenic fungi and field performance by bacteria recovered from the rhizosphere of plants in India. *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Bacillus subtilis* and *Bacillus cereus* are reported to be antagonistic against phytopathogenic fungi (Verma *et al*. 2010). *In vitro* screening of organisms is a valuable tool to select the potential strains (Cirvilleri *et al*., 1999; Cook, 1993).

Biocontrol fungi (BCF) are beneficial organisms that reduce the negative effect of plant pathogens and promote positive responses in the plants. In biological control of plant pathogens using fungi, antagonists are biological agents with the potential to interfere in the life process of the plant pathogens. Antagonists may be applied to soil to a) Destroy
pathogen inoculums, b) Prevent recolonization of the treated soil by a pathogen or c) Protect germinating seeds and roots from infection. Most of the soil fungi isolated from the Tea Agroecosystem are known to survive saprophytically in nature (Deb and Dutta 1991). In the present in vitro antagonism study of fungi Trichoderma viride showed the highest inhibition against the pathogen followed by T. citrinoviride, Aspergillus niger and Penicillium sp respectively. In the colony interaction, the T. viride, T. harzianum and T. citrinoviride showed ‘F’ type’ whereas and A. niger showed ‘E’ type’ colony interaction.

Release of inhibitory substances/metabolites produced by Trichoderma viride into the host organism is known to result in direct inhibition of growth of the pathogen by disintegrating the hyphal wall resulting in the penetration, absorption and lysis of the mycelium (Chen et al. 2005, Chet et al 1998 and Elad et al 1982, 1983). Due to several adverse effect of chemical control of pest and diseases the attention on the biological control is now increased by using some beneficial microorganisms. Several soil fungi such as Aspergillus flavus, Alternaria alternaria, Penicillium aurantiogriseum, Coniothyrium minitans, Gliocadium sp and Tricoderma sp are known to be effective antagonists against the plant pathogens (Royse and Ries, 1978; Sinaga 1986; Adebajo and Bankole, 2004; Rabeendran et al., 2006). Members of Trichoderma sp are known to be active hypoparasites of several soil fungi and hence it has been used as a biocontrol agent (Ekefan et al. 2009). Control of plant diseases by the use of antagonistic microorganisms can be an effective means of controlling the plant pathogens (Cook, 1993). The result obtained in the present work conforms with the above findings reported by the different authors as described above.
Biochemical tests were performed to evaluate the changes (Chlorophyll content, PAL, POX and Total phenol) brought about by the treatment with the bacterial strains PM 43, PM105 and PM 112. All the treatments had significant effect (increase) on the chlorophyll content in tea leaves. Chlorophyll content is an indicator of how healthy is the maintenance foliage of a tea plants. As the vigorous growth is the basic objective of rearing of the young tea plants, therefore, building up of higher quantum of chlorophyll is essential for healthier leaves. Higher level of chlorophyll content was recorded in the leaves of tea plants treated with the bacterial strains. Lowest chlorophyll content was recorded in the pathogen alone inoculated (control) tea plants. Saharan & Saharan (2004) observed maximum reduction of chlorophyll (a and b) in the diseased leaves of blight infected Cluster bean as compared to its healthy leaves of the control plants at 65 and 80 DAS. A significant decrease in the total chlorophyll content is a well-established phenomenon in many crop plants due to various diseases (Gupta et al., 1987; Lodha et al., 1993; Singh et al., 1993) Nottol (2005) observed that the total chlorophyll content as well as net photosynthesis increased in tea clones with the application of N, P & K.

PAL, POD and PPO are considered as disease defense related enzymes in Induced Systemic Resistance (ISR) in plants. From the study, it was observed that there has been considerable increase in the activity of both the defense causing enzymes tested. From the observation it was found that the activity of defense related enzymes (PAL, POD and PPO) have increased rapidly upto the 30 DAT in the pathogen infected tea plants after which decrease in their level was observed from the 45 DAT onwards. At the same time, it was also observed that the tea plants treated with the pathogen as well as the bacterial strains
(PM 43, PM 105 and PM 112) were found to have shown highest enzyme activity till the 75 DAT. In pathogen alone (control) inoculated plants the enzyme activity decreased rapidly within 45 DAT. This was found to be responsible for the gradual occurrence of the disease incidence/development. Similar studies, which showed an increase in PAL, Peroxidase (POD) and Polyphenol oxidase (PPO) activity were reported by Sandeep (2004) and Krishnaveni (2005). Jebakumar et al. (2001) showed that PAL and β-1,3-glucanase activity have increased significantly after 48 hrs of infection in Phytophthora capsici in black pepper. PAL is the first enzyme of phenylpropanoid metabolism in higher plants and it has been suggested to play a significant role in regulating the accumulation of phenolics (Massala et al., 1980). PAL catalyzes the first reaction in the synthesis of a wide range of natural products based on the phenylpropane skeleton including lignin monomers and certain phytoalexins (Lamb et al., 1989). PAL is also associated with the accumulation of aromatic fungitoxic compounds (Ampomah & Friend, 1988). Relation between PAL activity and resistance, in different seedling parts, has been reported in potato (Yoshioka & Doke, 1994) and barley (Carvar et al., 1994). The rapid induction of PAL genes in resistant interaction between host and its pathogen might be due to the involvement of a signal transduction mechanism, triggered specifically as a result of interaction between the elicitor and receptor molecules, thereby showing differential transcriptional rates of PAL in compatible and incompatible interactions (Abenthum et al., 1995; Kale & Choudhary, 2001; Reinecke & Kindl, 1994). POD activity also increases due to physiological or environmental stress such as disease or injury. The enzyme activity has been found to have increased in fungal, bacterial and virus infection in some plants (Farkas and Stahmann, 1966; Lovrekovich et al., 1968; Maxwell and Bateman, 1967; Wasfy et al., 1978). Chakraborthy et al. (2005) reported significant increase in POD activity where the tea
bushes could completely overcome the damage caused by blister blight disease causing organism *Exobasidium vexans* under field conditions. Such higher POD activities have been correlated with the greater resistance to *Sphaerotheca fulginea* in muskmelon plants (Reuveni & Bothma, 1985). Although peroxidase exists in healthy tissue, the increase in activity after fungal infection is largely caused by the formation of new molecular forms, the isoenzymes (Veech, 1969; Novacky and Wheeler, 1970).

In the present work increase in accumulation of total phenols was observed in all the bacteria treated tea plants. Higher phenol accumulation was also observed in pathogen treated (control) tea plants upto 30 DAT. While sharp decline of phenol content was observed after 45 DAT in the control. Chakraborty *et al.* (2006) reported that In tea plants the total phenol increased significantly by the treatment with *B. megaterium*. This finding confirms with the result obtained in the present work suggests that the application of biocontrol agents (i.e. antagonists bacteria) can increased the activity of PAL, POD, PPO and total phenol which revealed that the plants treated with bacteria can enhances the disease defence mechanisms against the pathogen (*F. lamoensis*).

VIII.5. Chemical and Biological of brown root rot under gnotobiotic /Nursery and field condition

VIII.5.1. Chemical control

Suppressive effect of various fungicides was observed on the radial growth of the pathogen *Fomes lamoensis in vitro*. The antifungal activity of the systemic fungicides have been reported to be the result of their ability to inhibit ergosterol bio- synthesis in fungi (Annon, 1985; Dutta and Debnath 1990; Dutta, 1994 and Agnihothrudu 1990). It was also reported that the antifungal acitivity of triflumizole was observed (which act as a selective inhibitor
of dimethylation) during the egosterol biosynthesis (Mizuno 1988). Increased percent inhibition of the radial growth of the fungus was observed in both hexaconazole and propiconazole with the increase in concentration, on the other hand, no increase of inhibition was observed in case of bavistin, roko and ektino treatment with the increase in their concentration. Among the systemic fungicides, hexaconazole was found to be highly effective in inhibiting the growth of the pathogen at the lowest concentration of 10 ppm (86.66%), followed by propiconazole (37.77%). Similar results were also observed with the tea pathogens Hypoxylon serpens (Onsando, 1986), Colletotrichum gloeosporioides (Ali et al. 1993), Pestalotia theae (Dutta and Begum, 1989) and Phomopsis theae (Ponmurugan et al. 2006). It was also reported earlier that hexaconazole and propiconazole were found to be more effective even at low concentration against the tea pathogens (Sarkar et al 2010).

Systemic fungicides such as bitertanol was found to be effective in controlling gray blight of tea in Korea (Shin et al. 2000). The fungicides Carbendazim was observed to be the best in controlling thorny stem blight disease of tea and this may be attributed to its ability in inhibiting the mitosis of the organism in vitro (Mouli and Baby 2000; Kalim et al. 2000). Results from the nursery condition in the present study have also showed that hexaconazole and propiconazole at the concentration of 100 ppm have the efficacy to control brown root rot disease of tea caused by F. lamoensis.

In the present investigation from the nursery experiment it was observed that Fomes lamoensis infected tea plants showed disease symptoms with maximum disease severity. While highest disease control was observed in the plants treated with Hexaconazole+ F. lamoensis (96.66%) and Propiconazole+ F. lamoensis (93.33%). While Bavistin + F. lamoensis showed the lowest (60%) disease control with the increase in disease incidence (40%) as compared to Hexaconazole and Propiconaloe treatments at 100
ppm concentration. Evaluation of number of triazole fungicides in the recent years revealed that formulation containing cyproconazole, hexaconazole, propiconazole and tebuconazole are excellent fungicides in controlling blister blight of tea (Dutta et al. 1992, Premkumar et al. 1998, Nithyameenakshi et al. 2010). The efficacy was reported to have improved when triazole was used in combination with copper oxychloride (Chandramouli and Agnihuthrudu, 1989). Strobilurin fungicides are also reported to be effective at very low concentration, 12-20 ppm (Wong and Wilcox, 2002 and Miller and Gubler, 2004).

From the nursery experiment the effect of fungicides on the growth promotion parameters on tea was observed. In fungicide treated plants it has been observed that Hexaconaxole, Propiconazole, and Bavistin increased the shoot height and root length with the increase in concentration of the fungicides used (10,50 and 100 ppm) Higher fresh and dry weight of the shoot and root was also observed the fungicide treated plants as compared to control (F. lamoensis inoculated plants). The results obtained in the present work are similar to those of Dimond (1967) that the chemotherapeutic compound rids the host of an established infection. Dutta (1980) reported that use of fungicides increased the yield of plant height as well as yield of tomato and tea. The production of higher yield in tea has been attributed to the change in metabolism of the fungicide treated plants (Dutta, 1994). Application of systemic fungicides could control grey blight disease of tea which also increased the production due to their phytotonic effect (Sanjay et al. 2008). Fungicide benlate has also been found to cause an increase in the fresh and dry weight of Sesbania sesban (Siddiqui et al. 1997). Windham & Windham (2004) have reported that systemic fungicides, which are based on SBI (Sterol biosynthesis inhibitor) are closely related to plant growth regulators the use of which at higher than labeled rates shorten the plant internodes, which may lead to slow shoot growth. Therefore, due attention should be given
on the concentration of the SBI fungicides used, while planning the treatment of any crop plant including tea.

**Biological control**

Bacteria are indigenous to soil and the plant rhizosphere and play a major role in the biocontrol of the plant pathogens. They can suppress a broad spectrum of fungal diseases. In the present study, gnotobiotic experiment was conducted to know the disease suppression by using bacterial strains in the flask condition having Hoffland Plant Nutrient solution (PNS). Due to the application of potential bacterial strains, reduction in the activity in expressing the symptoms (drying of leaves, leaf shedding and drying of tea plants) as well as severity of the disease was observed in the *Fomes lamoensis* inoculated plants.

Individual bacteria inoculated to plants showed varying results. The results indicated that when the plants were treated with the pathogen alone, drying symptoms occurred within 20 days after inoculation. On the other hand, Plants treated with the pathogen as well as the antagonistic bacteria and bacteria alone, no symptoms were recorded till the last day observation (20 days). Mishra (2007) reported that in gnotobiotic condition drying observed in pathogen (*F. lamoensis*) treated tea plants on the 8th days onwards, while bacteria treated plants grown in pathogen infested flask survived till the last day of observation, but the plants showed minor level of wilting of leaves.

In the present investigation from the nursery experiment it was observed that *Fomes lamoensis* alone inoculated tea plants have shown early disease symptoms and maximum disease severity. The bacterial strains PM 105, PM 43 and PM112 showed 70-80% disease control. Chakraborty *et al.* (2005d) also reported reduction in the development of brown root rot disease of tea by the bacterial application under pot culture condition. From the nursery study, drying of leaves were observed from the 35th day onwards and
drying of tea plants started from 120\textsuperscript{th} day in the pathogen treated tea plants. Whereas, in tea plants treated with the bacterial strains and with the pathogen, there was a delay in the onset of drying of leaves \textit{i.e.}, from the 57\textsuperscript{th} day and no drying of tea plants were observed till the end of the observations. To counter check the results the experiment was repeated thrice. Same trend in the result was observed, which confirms the ability of these bacterial strains \textit{viz.}, PM 43, PM 105 and PM 112 in inducing disease control resistance in the treated plants. In the field study it was also observed that minimum disease severity occurred in the bacteria treated tea plants (infected with \textit{F. lamoensis}).

Since beneficial bacteria are indigenous to soil and common inhabitants of the rhizosphere, they can suppress plant diseases by protecting roots from the infection by soil-borne pathogens. The use of plant growth promoting rhizobacteria has become a common practice in many parts of the world. They dominate the rhizosphere and possess several properties that have made them as biocontrol agent of choice (Johri, 1997). Greater application of beneficial bacteria (PGPR) is possible in agriculture for biocontrol of plant pathogens and biofertilization (Siddiqui, 2006). The bacterial strains isolated from \textit{Lolium perenne} rhizosphere are reported to be capable of acting as plant growth promoting bacteria and as biocontrol agents (Shoebitz \textit{et al.} 2007). A major group of bacteria with potential for biocontrol are the \textit{pseudomonads} (Kremer and Kennedy 1996). Ishrat-Izar \textit{et al.} (1995) reported that a growth promoting bacterium \textit{P. aeruginosa} significantly reduced infection by \textit{Macrophomina phaseolina} and \textit{R. solani} on chickpeas. A talc based powder formulation developed by Vidhyasekaran & Muthamilan (1995) was found to be effective for the control of rice blast (Vidhyasekaran \textit{et al.}, 1997a) and pigeon pea wilt (Vidhyasekaran \textit{et al.}, 1997b). Effective control of rice sheath blight pathogen \textit{R. solani} was obtained when the talc based powder formulation of \textit{P. fluorescens} strain Pf was
applied to seed, foliage, root and soil (Vidyasekaran & Muthamilan, 1999). The genus Bacillus is also reported to have plant growth promotion ability and disease control potential against the pathogen ((Wahyudi et al. 2011, Chakraborty, 2012).

The nursery and field experiments conducted in the present investigation on the growth promotion in tea plants revealed significant increase in plant growth parameters, viz. number of newly emergent leaves, number of lateral branches, plant shoot height, root length, fresh weight of shoot and root and dry weight of shoot and root in the plants treated with PM 43, PM 105 and PM112 respectively. Screening of bacteria for growth promotion under gnotobiotic conditions and in vitro, production of auxins are useful approach for selecting bacteria for their plant promotion growth ability (Asghar et al. 2004). Mechanisms of growth promotion by fluorescent Pseudomonads are complex and appears to compare both microbial balance and alteration in the host plant physiology (Glick, 1995). Growth promotion occurs as a result of direct stimulation of plant growth, induction of host plant systemic resistance or suppression of plant pathogen (Dwivedi and Johri, 2003; Fakhouri et al., 2001; Lugtenberg et al., 2002; Thomashow and Weller, 1995; van Loon et al., 1998). Free-living rhizobacteria usually do not rely on single mechanism of promoting plant growth. Significant increase in plant growth parameters may be attributed to the production of plant growth regulators such as auxins, gibberellin, cytokinins, ethylene (Frankenberger and Arshad, 1995), and plant hormones (i.e.IAA) (Mordukhova et al., 1991, Kende 1993) etc.

In the nursery experiment it was observed that the tea plants treated with Fomes lamoensis alone had the highest disease incidence as compared to other fungal organism (BCF) treated plants. Highest disease control was recorded in the plants treated with the antagonistic strain of T. Viride and T. citrinoviride respectively. An extensive and
promising study has been done and reported on the interaction of biocontrol agent and plant pathogen on the commercially important crop plants (Vesseur et al., 1990). Various plant diseases have been successfully controlled through bacterial and fungal antagonists (Cook and Baker, 1983; Campbell, 1989). Most of the early work reported on the biocontrol of plant pathogen was by *Trichoderma spp*, which revolved around the direct ability of these fungi to interact with the soil pathogens (Shoresh et al. 2010). Mechanism of the mycoparasitism, production of antibiotics, and competition for nutrients in the rhizosphere are suggested to be responsible for the said control (Harman et al. 2004 and Vinale et al. 2008). 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 wall fragments released from the target fungi (Sivan and Chet 1989, Zeilinger et al. 1999). The fungitoxic CWDEs attacks even before the physical contact with the pathogen have also been observed (Brunner et al. 2003). The detection stimulates increased and directional growth towards the target fungus (Brunner et al. 2003 and Zeilinger et al. 1999). Once the fungi come into contact may coil around and form appresoria on the surface of the host hyphae (Hua and Meyerowitz, 1998). Enzymes and antibiotic/antibiotic like substances produced have synergistic action to kill the pathogen (Howell, 1998, Lorito 1998 and Schirmbock, 1994). The *in vitro* antagonism of *Trichoderma* sp. against certain primary root pathogens of tea (Baby and Chandramouli, 1996) as well as efficacy of *Trichoderma* bioformulations in controlling some of the primary and secondary root diseases (Borthakur and Dutta, 1992), and *Phomopsis* canker (Ponmurugan and Baby, 2007) diseases have been reported.

In the present study it has been observed that the biocontrol fungi (BCF) enhanced plant growth promotion. In antagonistic fungi treated tea plants number of new leaves,
number of lateral branches, shoot height, root length, Fresh weight of shoot and root and dry weight of shoot and root significantly increased in all the treatments. In the treatment T4 highest shoot height was recorded, percent increase over control was observed, whereas lowest shoot height was recorded in the control. Similarly, in root length highest % increase over control was observed in Treatment T5. From the result of the biomass study, all the BCF treated tea plants showed an increase in biomass over the control. The results obtained in the present work are similar to *Trichoderma* and *Sebacimales* species inoculation that induced root and shoot growth in *Piper dilatatum* (Barazani *et al.* 2005, Harman 2000, Harman *et al.* 2008, Harman *et al.* 2004, Harman *et al.* 2004, Peskan *et al.* 2004 and Rai *et al.* 2001).

Therefore, from the present study it can be suggested that use of beneficial bacteria/fungi and the use of minimum level of systemic fungicides as a strategy for IPM (i.e. hexaconazole, propiconazole and bavistin) could help to control the brown root rot disease of tea as well increase the productivity of tea at large.