DISCUSSION

Plants in nature have evolved a series of mechanisms by which they can defend themselves against the multitude of organisms attacking them. The versatile multicomponent defence is adequate to provide them protection against most of their potential pathogens; only a few of them can overcome this defence and cause disease (Sinha, 1995). Varieties within the host species are resistant when they possess one or more resistant gene(s) and susceptible when they lack any such genes. To account for the observed specificity and degree of variability of host parasite system, the fungal receptors must have high information content. Antigens located on the cell surface are generally involved in information transfer and/ or the maintenance of membrane integrity during the cell-to-cell interaction of host and parasites. Host pathogen interaction occurring in nature is one of the most complex biological processes and involves recognition between the host and pathogen both at the cellular and subcellular level. A cell that reacts in a special way in consequence of an association with another cell or its products does so because it acquires information, which is conveyed through chemical or physical signals in the process of recognition. Differences in physiological responses and morphological structures of various host genotypes affect their susceptibility or resistance to invasion and its consequences while similar variation in pathogens influence their growth rate and virulence (Loomis and Adams, 1983). The spatial and temporal deployment of plant defence responses involves the complex interplay of signal events, often resulting in superimposition of signaling processes (Graham and Graham, 1996). In spite of lacking immune responses like animals, plants have nevertheless evolved immune mechanisms of various types by which they can counter the advance of foreign organisms. The result is that disease tends to be specific, a given pathogen usually infecting distinct range of host plants

The present study was undertaken to study the interaction of tea and *F. lamaoensis,* the causal organism of brown root rot disease. At the onset, pathogenicity test of the Brown root rot pathogen *Fomes lamaoensis* was carried out on 10 Tocklai, 6 UPASI and 9 Darjeeling tea varieties. Among the Tocklai, UPASI and Darjeeling varieties TV-18, UP-26 and T-78 were most susceptible, respectively, while TV-26, UP-8 and BS/7A/76 were most resistant. Among all twentyfive varieties, UP-26 was most

susceptible. Since available reports indicated that the tea plants become more susceptible from 3yr upward disease assessment of plants of all ages was carried out and disease intensity was observed to be maximum in 5yr old plants and minimum in 1yr old plants.

The growth and infectivity of the pathogen depends on a number of environmental factors like temperature, pH of medium and seasonal changes. So, the effect of different cultural conditions on growth of *F. Lamaoensis invitro* was carried out. physiological studies in relation to medium, pH, incubation period was carried out. The optimum temperature for the growth of *Fomes lamaoensis* was 30°C which was in accordance to previous reports (Barthakur and Samajpati, 1985). Maximum vegetative growth was obtained with 15 days of incubation period utilizing carbon source, lactose or fructose and calcium nitrate and beef extract as inorganic nitrogen and organic nitrogen sources respectively. The pathogen was found to withstand a wide range of pH (3.0-8.0) but the optimum was 4.5-5.0 and the pathogen grew well under acidic condition (pH 4.0-6.0), which coincides with the pH of tea soil. The wood-rotting fungi exhibit maximum metabolic activity at the acidic range (pH 3.0- 7.0) in most cases (Srivastava and Bano, 1970; Brodziac, 1980 and Hong *et al.*, 1981).

During host parasite interaction even when coincidence of location and time is provided and supplemented with optimum conditions for the development of the pathogen, parasitic relationship can only be established if the host recognizes the pathogen on the one hand, and the pathogen can overcome the various defense mechanisms of the host, on the other hand whenever an intimate and continuing association of cells of host and pathogen occur it has been observed that partners of this association have a unique serological resemblance to one another involving one or more antigenic determinants. The presence of cross reactive antigens might be involved in determining the degree of compatibility in such interactions which has been reviewed by several authors (DeVay *et. al.*, 1972; DeVay and Alder, 1976; Kalyansundaram, 1978; Chakraborty, 1988; Purkayastha, 1989; Purkayastha et. al., 1991). In the present study, polyclonal antibodies (Pabs) raised against fungal and host tissues were used for determining the presence of cross reactive antigens (CRA)

between tea varieties and *F. lamaoensis* as well as for the immunodetection of the pathogen in the tea root tissues and soil. Effectiveness of each PAb was initially checked through immunodiffution tests. Since immunoenzymatic reactions like ELISA were very sensitive techniques and non-specific bindings interferes with the actual antigen-antibody reactions, IgG was purified from crude antiserum following ammonium sulphate precipitation and fractionation through chromatography on DEAE-cellulose column as described by Clausen (1988).

Enzyme linked immunosorbent assay (ELISA) has proved to be one of the most sensitive serological techniques. Since ELISA depends on number of factors and this varies from system to system it was considered essential to optimize various conditions before further tests. Hence sensitivity of assay was optimized considering two variables i.e. concentration of antigen and concentration of IgG using Direct antigen coated (DAC) ELISA. Enzyme dilution ,1:10,000, and substrate concentration, 1mg/ml, were kept constant. Positive results were obtained with very low concentration of both antigen and IgG and the sensitivity tests of Pabs obtained from different bleedings raised against different immunogens showed maximum ELISA values in 3rd followed by 4th bleedings. It was reported by Mohan (1988) that a concentration of Phytophthora antigens as low as 2ng/ml could be detected in indirect ELISA by antiserum raised against pooled mycelial suspensions of five *P. fragariae* races. Chakraborty *et. al.* (1996) also reported that antiserum raised against *Pestalotiopsis theae* could defect homologous antigen at 25ng/ml and antisera upto 1: 16,000 was effective for detections.

In indirect ELISA the presence of CRA among *F. lamaoensis* and tea varieties, using PAb raised against mycelia and cell wall antigen preparations of *F. lamaoensis* at a concentration of 40 mg/ml with tea root antigens at a concentration of 100 mg/ml. In the present study, TV-18, UP-26, T-78 showed maximum absorbance values in ELISA. Also in this study, though indirect ELISA readily detected CRA between tea root antigens and *F. lamaoensis*, much difference was not observed among the different tea varieties. CRA was also deteced in crude preparations and purified preparations from mycelia of *Phytophthora infestans* (races 4 and 1.2.3.4.7) with antisera of potato cvs. King Edward and Pentland Dell in concentrations lower than 50 mg/ml protein

(Alba and Devay, 1985) using indirect ELISA. The presence of CRA in several host pathogen interactions has also been reported by a number of previous workers, e.g. soybeen and *Myrotheciun roridum* (Ghosh and Purkayastha, 1990); groundnut and *Macrophomina phaseolina* (Purkayastha and Pradhan, 1994); tea and *Bipolaris carbonum* (Chakraborty and Saha, 1994) and tea and *Ustulina zonata* (Chakraborty *et.al.* 2002b). In order to determine whether the observed cross reactivity between *F. lamaoensis* and susceptible varieties was specific, antigen preparations from non host (*Oryza sativa, Dracaena* sp., *Leucaena leucocephala, Phyllanthus niruri*) and non pathogen (*Fusarium oxysporum, Beauveria Bassiana*) were also assayed with PAb of mycelia and cell wall of *F. lamaoensis* and showed very low reactivity in ELISA. Since the indirect ELISA tests were made under the same conditions and with at least three replicates it appears that these observed antigenic disparities have same significance in the basic compatibility of host and pathogen (Chakraborty, 1988).

Cross reactivity of the PAb raised against *F. lamaoemsis* was tested with other soil fungi. Results revealed that among all the fungi tested PAb of *F. lamaoensis* reacted to some extent with two tea root pathogens *U. zonata* and *R. arcuata*. Mohan (1989) showed that antisera raised against mycelial suspension of *Phytophthora fragariae*(PfM) reacted strongly with antigens from several *Phytophthora* species. He observed that anti-PfM could not be made specific for *P. fragariae* because it was raised to components shown to be antigenically similar to all *Phytophthora* sp. tested. Similar results with *P. frageriae* were also reported by Amouzon-alladaye *et.al.* (1988). In their studies, antibodies obtained with a strain of *P. fragariae* detected two different strains of this pathogen in pure culture by DAS and DAC-ELISA. Non-specific cross reactions with most fungi of the root flora of strawberry represented an advantage. Harrison *et. al.* (1990) further reported that anti-*P. infestans* g globulin reacted strongly withextract of *P. erythroseptica* in DAC-ELISA but not with extracts of nine unrelated fungi or a culture of bacterium *Erwinia carotovora*, all of which were saprophytes of pathogens of potato.

Visible outcome of a compatible host pathogen interaction may be obtained in

many cases only after several days of infection, by which time the pathogen would be well and truly established in the host tissues. Recent trends in detection of plant pathogenic fungi include the development of more rapid diagnostic techniques with high specificity for the target organism. These techniques can be used to detect fungi present in law amount in or on plant tissue and therefore in many cases the pathogen can be detected in much earlier stage of disease development than that was previously possible (Hansen and Wick, 1993) and also when visible symptoms have not yet developed. This offers a definite advantage over classical techniques and is thus gaining an importance for pathogen detection purposes. Various formats of ELISA using polyclonal antisera has found wide spread application in plant pathology and are routinely used for detection and identification purposes (Clark, 1981; Lommel *et.al.* 1982; Sundaram *et.al.* 1991; Lyons &White 1992 Chakraborty *et. al.* 1996 and Viswanathan *et.al* 2000). In the present study antisera raised against mycelia or cell wall antigen preparations of *F. lamaoensis* were used for the detection of the pathogen in tea roots and the responses were in general slightly higher (about 0.1) in case of cell wall PAb than mycelial PAb. Infected tea root extracts generally had higher ELISA values than the healthy ones. In case of two years old plants the difference of absorbance values between infected and healthy root extracts was not significantly higher except in susceptible varieties. Again the difference increased with the age of the plants as evident in present investigation using plants of different ages, from 1 yr. to 5 yr ld. Results from data also showed that the degree of detection of *F. lamaoensis* significantly higher from 3 yr. upward in susceptible varieties (TV-18, UP-26 and T-78) but resistant varieties (eg. TV-26) did not show high A405 values even in case of 5 yr plants. Holtz *et.al.* (1994) raised polyclonal antisera to both the cell wall fraction and the soluble cytosol fraction of *Thielaviopsis basicola* . Both indirect ELISA and fungal capture sandwich ELISA revealed no difference in specificity of the cell wall and the cytosol antisera and the antibodies to each immunizing fraction reacted with the other fraction. Viswanathan *et.al.* (2000) Reported that presence of *Colletotrichum falcatum* in sugarcane tissues could be detected by ELISA. They reported that when twenty different sugarcane varieties were subjected to ELISA test after pathogen inoculation

a clear variation in disease resistance was seen. They suggested that this technique could be reliably used to screen sugarcane genotypes for red rot resistance at an early stage. In the present study presence of *F. lamaoensis* in tea root tissues could be detected by both DAC and DAS- ELISA using PAb raised against either mycelial or cell wall antigens . It was observed that PAb *F. lamaoensis* also weakly reacted with antigens from tea roots infected with other pathogens showing certain degree of cross reactivity. Pathogen detection in the host tissue by ELISA have also been reported by a number of previous workers . These include the detection of *Pythium* species, *Rhizoctonia solani* and *Sclerotinia homeocarpa* in turfgrass (Miller *et.al.*,1986; *Humicola laniginusa* in rice (Dewey *et.al.* ,1989), *Leptosphaeria korrae* in turfgrass (Nameth *et.al.*,1990), *Phytophthora* species in a variety of woody ornamentals (MacDonald *et.al.*,1990;Benson ,1991), *Septoria nodorum* and *S. tricity* in wheat (Mittermier *et.al.*,1990;Peterson *et. al.* 1990) and *Sclerotinia sclerotiorum* in rapeseed (Jamaux and Spire, 1994). Mohan (1998) reported the ability of anti *P. fragariae* antiserum to detect infection in strawberry cultivars. *P. fragariae* infections were also detected readily in field infected samples of strawberry cultivars . Amouzou-Alladaye *et.al.*(1998) also reported the use of specific IgG of *Phytophthora fragariae* in DAS ELISA which constituted a method of early detection of the fungus in roots of inoculated plants. For the 5 varieties studied *P. fragariae* could be detected between 15 and 25 days after inoculation. In the present study natural brown blight infections could also be detected easily in ELISA.

Using PAb prepared against mycelial and cell wall antigenic determinants the dot detection of infection in root tissue and rhizosphere soil was done. The dot immunobinding technique has been found to be rapid and sensitive method for detection of virus and plant pathogenic bacteria. This procedure was tested for the specificity of antiserum for possible cross reaction with other root inhabiting fungi by Lange *et.al.*(1989). The antiserum against *Plasmodium brassicae* used in their study showed no cross reaction with other common pathogen (*Pythium ultimum Rhizoctonia solani* and *F.oxysporum*) and did not cross react with resting spores of *Polymyxa betae*, which is also member of the Plasmodiophoraceae . In this study, PAb raised against antigen of mycelia, cell wall of *F. lamaoensis* using was tested fast red or NBT/BCIP

as substrate. Antigens of homologous source and infested soil showed deep coloured dot. Infected tea root antigens also showed deep coloured dot when compared to healthy confirming the presence of fungal pathogen. Other tea root pathogens responded slightly or not at all with *F. lamaoensis*.

Complex mixture of antigens can be separated by high resolution techniques such as sodium dodecyl acrylamide gel electrophoresis using discontinuous buffer systems and two dimensional techniques. However once separated in this way, it has been difficult to determine which of the separated species reacted with a given antiserum. Towbin (1979) et.al. overcame these problems by electrophoretically transferring the separated mixture onto nitrocellulose. Once attached to the nitrocellulose, the antigenicity of each of the separated species could be tested by treating the blot with antiserum and the bound antibody detected with radio labaled staphylococeal protein A or corresponding anti-antibody. Blake *et.al.* (1984) have described a method of using alkaline phosphatase substrate 5-bromo 4-chloroindoxyl phosphate (BCIP) and nitroblue tetrazolium (NBT) to detect the precipitated indoxyl group. When the substrate BCIP is used, the phosphate is cleared by the enzyme and indoxyl group precipitates. The hydroxyl group of the indigo then tautomerizes forming a ketone, and under alkaline conditions dimerization occurs, forming a dehydro indigo. In the process of dimerization it release hydrogen ion and reduces the NBT which precipitates, forming an intense blue / violet deposition of dimerization. For Western blot analysis, electrophoresis of the soluble mycelia proteins from *V.dahliae* mycelium extract reacted with the purified protein of *V.dahliae* antigens, but not with the *F.oxysporum* protein. In the present study, antigens prepared form mycelial, cell wall, culture filtrate of *F. lamaoensis* healthy and artificially *F. lamaoensis* inoculated root and infested soil were prepared and probed with *F. lamaoensis* PAb.

Since Pabs raised against *F. lamaoensis* could detect the presence of the pathogen in root tissues it was decided to determine the efficacy of the PAb in detecting the specific pathogen in the soil. Detection of specific soil -borne pathogen in soil is equally or more important than detecting the pathogen in the root tissues. Detection of specific pathogens in soil requires very sensitive techniques, which would make it

possible to differentiate between the various microorganisms. Use of serological techniques, most specifically ELISA are gaining importance in such studies. In the present study, initially antigens prepared from soil collected from various tea estates were tested against PAb of *F. lamaoensis* by DAC-ELISA. Of the 38-40 samples tested 7 samples from Matigara, Bijoynagar and Trihana Tea Estate gave marginally high A405 values while all the others had relatively weakly reacted with PAb of *F. lamaoensis*. In case of amended soil sample ELISA values decreased with the increase in period of inoculation after an optimum period as data showed that after 70 days of amendment the values had decreased greatly. This was probably because the pathogen could not survive in the soil for such a long period. The ability of polyclonal antisera of *Plasmodiofora brassicae* to detect the presence of the pathogen in soil was reported by Wakeham and White (1996). In another study Walsh *et.al.* (1996) Reported that the antiserum could detect about 100 spore ball; levels appear to be better for concentration greater than 2000/gm soil. There was a quantifiable relationship between concentration of spore balls and ELISA values . Thus ELISA showed potential for detection of *F. lamaoensis* in soil.

Dot Blot analysis of rhizosphere soil samples collected from different tea fields and amended soil showed same trend of result obtained in DAC-ELISA. Wakeham and White (1996) got positive detection of soluble components of the spore wall and whole resting spores of *P. brassicae* in PBST as seen at concentrations at or above $1x10^7$ and $1x10^6$ resting spores ml^{-1} resting spores ml^{-1} respectively. When the sonicated fraction of the resting spores was assayed, the limit of detection was $1x10^8$ resting spores ml^{-1}. Identification of resting spores in artificially infested soil required a minimum concentration of $1x10^8$ spores gm^{-1} soil. The PAb of *F. lamaoensis* was found to be very specific for detection of the pathogen in the soil, infected root tissues and in different isolates of fungi. Walsh *et.al.* (1996) also performed Western blotting using the raw serum of *Spongospora subterranea* spore balls. Earlier workers have used immunofluorescent assays for diagnostic tests of infection on seeds, plant parts and soil. Thornton et.al.(1993) had previously reported that dip-stick, ELISA and immunofluorescence gave greater sensitivity for detection of live propagules of *Rhizoctonia solani* in soil. These assays could also prove useful in the rapid

determination of *P.brassiceae* infestation in soils (Wakeham and White, 1996). Fluorescence of hyphal structure was observed in immunofluorescence test of infested rhizosphere soil. So, propagules of *F. lamaoensis* could also be detected in soil through this study. Results of study have led to substantially similar conclusions using ELISA, dot-blot, immunofluorescence tests.

As detection of cross reactive antigens between *F. lamaoensis* and tea roots as well as detection of the fungus within infected tissues was possible by the antiserum raised against whole mycelial antigen *F. lamaoensis*; it was decided to purify the antigenic proteins from the crude preparation. This was carried out by purification procedures involving ammonium sulphate saturation, analysis by SDS-PAGE. In order to determine the fractions, which contained the antigenic proteins, immunodiffusion, DAC-ELISA and western blot were performed with PAb of *F. lamaoensis*. Results revealed maximum cross reactivity through immunodiffusion and protein bands through Western Blot analysis in the 60-80%SAS fraction. Alba and DeVay (1985) also purified cross reactive antigens from *Phytophthora infestans* by ammonium sulphate fractionation which was followed by detection in ELISA. They reported that most of the cross-reactive antigens were precipitated at 40% SAS.

Since, results of immunodiffusion and Western blot pointed to the 60-80% SAS fraction to be the major antigenic proteins the ability of this fraction to raise antibody was further tested. In this study evaluation of the antiserum raised against 60-80% SAS fraction of mycelial antigen of *F. lamaoensis* was also done by DAC-ELISA, Dot blot, Western blot analysis. CRAs between *F. lamaoensis* and tea varieties was detected in DAC-ELISA. This PAb behaved in a similar manner as that raised against whole mycelial preparation and hence presence of the antigenic proteins was confirmed in the 60-80% SAS fraction. Chard et.al.(1985) also raised antiserum against purified mycelial preparation of *Mycena galopus* and compared with the antiserum against whole mycelia . Both the antisera were shown to be specific to *M. galopus* by immunodiffusion tests. This PAb could also detected *F. lamaoensis* infection in inoculated tea root tissue by DAC-ELISA and showed highly significant differences in A 405 values between healthy and infected roots in susceptible varieties. In dot

blot analysis the PAb also detect *F. lamaoensis* showing violet dot with infected root antigen while faint colour dots with healthy root antigens; Western blot analysis of this PAb with infected root antigens of susceptible variety (UP-26) showed four bands while no band in case of healthy was observed. Results of all the above therefore, confirmed the presence of the antigenic proteins in 60-80% SAS fraction, since, this fraction was effective in raising specific antiserum against *F. lamaoensis.*

The importance of cross reactive antigens between host and pathogen in determining the response of the host to pathogen has been established in this study and also by previous workers (DeVay and Alder, 1976 ; Chakraborty and Purkayasatha , 1983; Chakraborty and Saha, 1994; Chakraborty *et.al.*, 1995). For this purpose in this study fluorescence tests were conducted with cross section of tea roots as well as mycelia *F. lamaoensis.* Cross section of tea roots were treated with *F. lamaoensis* PAb followed by staining with FITC conjugated anti rabbit globule specific goat antiserum. Bright fluorescence was observed throughout the sections, extending upto the vascular tissues as well as outer surface. Treatment of mycelia of *F. lamaoensis* with homologous antiserum and FITC showed a general fluorescence that was more intense on young hyphae. DeVay *et.al.*(1981a) determined the tissue and cellular location of major CRA shared by cotton and *F. oxysporum f.sp.vasinfectam.* On the basis of strong fluorescence obtained at the epidermis, cortex, endodermis and xylem tissues, they suggested that the CRA determinants in roots have a general distribution. DeVay *et.al.* (1981b) also used FITC labelled antibodies for races of *P. infestans*, to detect the CRA of potato leaf section .It was also reported by Chakraborty and Saha (1994) that CRA between tea and *B. carbonum* were mainly present in the hyphal tips and in patch like areas on the conidia, mycelium and mainly around epidermal cells and mesophyll tissues of the leaf. The cellular location of CRA in tea leaves shared by *P. theae* (Chakraborty *et.al.*1995), *E. vexans* (Chakraborty *et.al.* 1995) and *G. cingulata* (Chakraborty *et.al.* 2002c) have also been previously determined.

Present study reports the use of indirect immunofluorescence test using polyclonal antibodies of *F. lamaoensis* as suitable technique for localization of fungal mycelium and could be employed immunodetection of *F. lamaoensis* present in tea root tissue.

Presence of fungal mycelium was evident with strong fluorescence in infected root tissue of susceptible varieties (TV-18, UP-26& T-78) after immunofluorescent treatment. Detection of pathogen in host tissues using antibody based immunoflurescent technique has been reported by several previous authors (Warnock, 1973; Hornok and Jagicza, 1973; Reddy and Anantanarayanan, 1984). Dewey *et.al.*(1984) suggested, on the basis of immunofluorescene studies that chlamydospores, basidiospores and mycelia of *Phaeolus schweinitzii* contained molecules antigenically related to species specific antigens secreted by mycelia grown in liquid culture. They also demonstrated the presence of mycelium and chlamydospores in naturally and artificially infested soil samples, using this technique. *Phytophthora* could be detected in soil by immunofluorescence antibody technique (Watabe, 1990).

Fungal hyphae and spores have been visiualized successfully in natural substrata with specific immunofluorescent (Deway *et.al.*, 1984) or nonfluorescent immunocytochemical stains (Gerik *et.al.*, 1987) based on polyclonal antibodies. The immucoenzymatic assay has several advantages over conventional bioassays in determining the colonization pattern of fungi in roots. It allows direct detection of the hyphae present on the root; the specific of the staining of hyphae is dependent on the specificity of the serum. A specificity fungal antiserum employed with an enzymatic staining technique was developed and proved to be a valuable tool in the study of root infecting fungi, for it permits their direct, selective observations in host tissue. Moreover this staining assay offers many advantages over fluorescence antibody techniques. It does not require the use of fluorescence microscope, and specimens can be viewed with a dissecting microscope. The increased field of view of dissecting microscope allows for the screening of more specimens in less time than is required with fluorescent techniques. It may be possible to sequentially stain different fungi in the same root tissue by using different enzymes linked to the antibodies and / or different diazo dyes. Geric and Huisman (1988) used this specific serological staining procedure to detect *Verticillium dahlae* on and in the root cortex of host with an alkaline phosphatase antirabbit IgG conjugant that hydrolyzed the substrate napthol-As-phosphate to a product that reacted with a diazonium salt. In 1988, Geric and Huisman

again used this technique to determine the dynamic colonization of cotton roots by *V. dahlae* under field condition. They observed that the colony density of *V.dahlae* on roots varied with the distance of tissue from the root tip. Young and Andrews (1990) also used this immunocytochemical staining for *Athelia bombacina* for detection of hyphae of the fungus within apple leaf tissue, and used the stain to examine the effect of the antagonists on pseudothecial development of *Venturia inaequalis*. In the present study detection of *F. lamaoensis* in tea root tissues was done using napthol-AS-phosphate and fast blue BB salt as substrate .The cross sections of infected tea root tissues were treated with PAb diluted with 1:100 in PBS-BSA followed by treatment with Goat antirabbit IgG alkaline phosphatase conjugate and substrate reaction was carried out at dark. Intense fungal colony as shown by blue colour were visible clearly along the epidermis and hyphal penetration throughout root tissue was evident. Deep blue coloured thick layer on outer surface, was an evidence of brown or blackish sheet on the root surface formed by the fungal mycelia.

Consequent to the study on the detection of *F. lamaoensis* in root tissues and soil, experiments were conducted both *in vitro* and *in vivo* for the management of the disease. Since it is necessary to reduce the use of fungicide / pesticide in tea plantation it was decided to test the efficacy of biocontrol agents in disease management. For this purpose *Trichoderma harzianum* and *T.viride* were selected and experiments were conducted using these as biocontrol agents. Both *T.harzianum* and *T.viride* inhibited the growth of *F.lamaoensis in vitro*. There are several reports on the ability of *T.harzianum* and *T.viride* to inhibit the growth of pathogen under in vitro condition. Ten isolates of *Trichoderma* species were screened by Padmodaya and Reddy (1996) in in vitro for their efficacy in suppressing the growth of *Fusarium oxysporium f. sp. lycopersici. Trichoderma viride* (H) was found to be highly inhibitory to *F. oxysporum f. sp. lycopersici* in dual culture followed by *T.harzianum* sp. (A.P). Studies on production of volatile compounds by *Trichoderma* species revealed that *T.viride* (H), *T.viride* (A.P.) and *Trichoderma* sp. (D) as effective in reducing radial growth of *F.oxysporum f. sp. lycopersici* in a study on production of non-volatile compounds by

Trichoderma sp. Baby and Chandra Mouli (1996) tested antagonistic potential of *Trichoderma* sp. and *Gliocladium virens* against primary root pathogens of tea viz. *Fomes noxius, P.hypolaterita, Rosellinia arcuata* and *Armillaria mellea in vitro.* In dual culture, the mycoparasites invariably invaded the pathogen, though there was variation in the rate and quantity of invasion. *T.harzianum* was found to be the best colonizer of *Poria* and *Armillaria* and *T.viride* colonized with *Rosellinia. G.virens* colonized all the pathogens fairly well. The antagonists showed moderate to high antibiosis to *Rosellinia.* Production of toxic metabolite(s) was more in *G.virens* than *Trichoderma.* Hazarika et. al., (2000) also tested the antagonistic effect of *Trichoderma harzianum* against *U.zonata,* causing charcoal stump rot of tea in dual culture method. Both antagonists were most effective in inhibiting the mycelial growth of *U.zonata.* Assam and Tamil Nadu isolates of *T.harzianum, T.viride* and *T.virens* were tested by Hazarika and Das (1998) for the potential to suppress *Rhizoctonia solani.* Culture filtrate of *T.harzianum* and *T.viride* inhibited mycelial growth and sclerotial germination. Wheat bran substrate supported maximum growth of all isolates followed by firm yard manure and tea waste. Both *T.harzianum* and *T.viride* effectively controlled the bean rot disease when they were applied as seed and soil treatment. In dual culture of 11 isolates of *T.harzianum,* three isolates, viz. T8, T10 and T12 was effective against *Sclerotium rolfsii,* the causal agent of stem rot of groundnut and they overgrew the pathogen up to 92%, 85% and 79% respectively, in vitro. Phookan and Chaliha (2000) reported that growth of *Sclerotinia sclerotiorum* was significantly suppressed by *Gliocladium virens* and *T.viride* significantly. Amongst fungal antagonists tested by Sharma and Sharma (2001). *Trichoderma harzianum* and *T.viride* were found most effective in inhibiting mycelial growth of *Dematophora nectrix* in dual culture.

T.harzianum and *T.viride* were tested *in vivo* for the ability to reduce brown root rot intensity. Of various delivery systems tested for this biocontrol agent, tea waste formulations were found to be most effective. Disease intensity was reduced by both *T.harzianum* and *T.viride* when tested under potted condition as well as in the field condition. This was observed in all tested varieties. Detection of *F.lamaoensis*

in tea root tissues and rhizosphere soil of different treatments with pathogen and biocontrol agents was also investigated. Reduction of intensity of disease was also determined immunologically in both root tissues and soil. For this purpose DAC-ELISA, competition-ELISA, Dot-Blot and Western blot were carried out. Results showed that ELISA values of root tissues treated with *T. harzianum* and *T. viride* were significantly lesser than with *F. lamaoensis* alone. The same trend of result was obtained in infested rhizosphere soil through DAC and Competition ELISA, Dot Blot and Western blot analysis. This result is in conformity with that of Hazarika et. al., (2000) who reported that planting of tea seedlings after dipping roots in spore suspension of *T. harzianum* reduced 56.6% mortality of plant due to *U. zonata* infection. This was also obtained with *T. viride* and *G. virens*. However they observed that the reduction of mortality of plant increased to 62.2% when *T. harzianum* were applied to soil to soil drench. Significant control of charcoal stump rot of tea with antagonistic microflora obtained previously by Borthakur and Dutta (1992) and Hazarika et. al., (1999). The role of *T.harzianum* and *T.viride* as biocontrol crops is well established. Sarker and Jayarajan (1996) reported that root rot of sesamum caused by *Macrophimina phaseolina* was significantly reduced to 12% by seed treatment with antagonist (*T.harzianum*) in comparison to untreated inoculated control plants where 62% disease incidence was noticed. Prasad *et. al.*, (1999) found three *T.harzianum* isolates (PDBCTH-2, 7 and 8) and the *T.viride* isolates (PDBCTV4) highly efficient in controlling root/ collar rot of sunflower caused by *Sclerotium rolfsii*. Under green house condition PDBCTH-8 showed maximum disease control of 66.8% followed by PDBCTH-7 (66%), PDBCTV-4 (65.4%). PDBCTH-2 (61.6%) and were even superior to systemic fungicide.

In conclusion, it can be stated that brown root rot can cause severe damage to tea plants, particularly to those growing on sandy soil. Such detection techniques makes it possible to detect microquantities of the pathogen within root tissue and rhizosphere soil, which is more advantageous than the conventional techniques involving pathogen inoculation, visible symptoms and microscopy. Being perennials, plants harbor the pathogen in root tissues and rhizosphere over a long time and the

depletion of pathogen by the conventional techniques is difficult and by the time the above ground symptoms appear it is too late. So, an important area of immunological studies of diseases involves the use of pathogen antiserum for detection of infection in host at an initial stage which can lead to formulation of control measures before much harm has been done. In this study, effective formulations of *Trichoderma harzianum* and *Trichoderma viride* have been prepared, by which brown root rot can control.