Discussion

Tea is subjected to varying environmental conditions throughout its life, as well as to numerous attacks by pests and fungal pathogens, which in turn are influenced by various environmental conditions. If the microorganism is successful, disease is the end result; but more often than not, the host emerges the winner as the invader is successfully warded off. Plants have to make necessary metabolic and structural adjustments to cope with stress conditions (Ho and Sachs, 2000). The aerial surfaces of tea plants are usually inhabited by a variety of microorganisms, many of which are capable of influencing the growth of foliar pathogens (Chakraborty and Chakraborty, 1997). The interactions between these microorganisms might result in the suppression of pathogen activity. Besides, it is likely that the tea plant, in the course of its adjustment to varying environments, has evolved a very effective defense mechanism, which successfully wards off most of the fungal pathogens. The common plant pathogens induce some type of resistance in plants to subsequent challenges, both to the original and as well as to other biotic agents. In general, the defenses of higher plants against any form of stress, whether biotic or abiotic, fall under two categories: preformed and induced.

In the present investigation, 18 tea varieties were screened against *S. rolfsii*. Among all the tested varieties, UP-8 and T-17/1/54 were found to be most susceptible while K1/1 and HV-39 were found to be most resistant. Although less in known with certainty about the specific recognition events that predict incompatible host-pathogen interaction, considerable genetic and biochemical evidence indicates that constitutive specificity imparting molecules must exist in the incompatible pathogen and the resistant host plants that dictate the ultimate accumulation of antifungal compounds at the infection site. Cell recognition has been defined as the initial event of cell-cell communication which elicits morphological, physiological and biochemical response. Surface molecules of eukaryote cells have been involved in cell-cell recognition and/or adhesion and as receptors for various effects. Many of these specificity imparting molecules are glycoproteins, and fungi are known to possess them on their cell-walls and plasma membranes (Keen and Legrand, 1980; Ransom *et al.*, 1992).

In the initial stage of infection at the cellular level the exchange of molecular signals between host and parasite is considered to be one of the mechanisms resulting in the specificity of such interactions. The genetic information contained in nucleic acid is expressed in the cell via protein synthesis. Several proteins function as enzymes in the metabolic pathways, which synthesize or breakdown cellular components. When plants containing various kinds of proteins are infected by pathogens, the proteins in the penetrated plant cells are changed chemically and physically. Thus qualitative and quantitative changes in proteins are related to both plant and pathogen. A protein competition model was proposed by Jones and Hartley (1999) for predicting total phenolic allocations and concentration in leaves of terrestrial higher plants. They suggested that protein and phenol synthesis compete for the common limiting resource-phenylalanine and hence protein and phenolic allocations are inversely correlated.

In the present investigation changes in the protein content was noted in the *S. rolfsii* inoculated leaves of susceptible varieties in relation to their healthy control. Increased protein level was also detected after infection of susceptible bean leaves by *Uromyces phaseoli*. The greater accumulation of protein in susceptible host after inoculation may also be attributed to the total proteins of both host and parasite. However, it is difficult to separate the relative contribution of host and parasite to the total protein content. It is evident from the above statement that some changes occur in proteins of infected plants. However, these changes are not always significant. Sometimes protein content of the host remains more or less similar even after inoculation but isozyme pattern may change (Sako and Stahman, 1972).

Advances made in the formulation of concepts and techniques of modern, quantitative cell biology in recent years have paved the way for a basic understanding of the physiology and biochemistry of plant host pathogen interactions. The success or failure of infection is determined by the dynamic competition and the final out come is determined by the sum of favourable and unfavourable conditions for both the pathogen and host cells. At the same time the potential host may be able to detect or recognize a fungal pathogen and use the initial act of recognition to trigger a range of induced resistance (Purkayastha, 1994). The initial cellular recognition is followed by communication between its components.

This exchange of information is generally mediated by soluble antigens located on or near the cell surface (Chakraborty, 1988). The significance of antigenic relationship between plant hosts and pathogenic organisms with regard to disease susceptibility has been recognized by many investigators. Whenever an intimate and continuing association of cells of host and pathogen occur it has been observed that partners of this association have unique serological resemblance to one another involving one or more antigenic determinants (Chakraborty *et al.*, 2002d).

Enzyme linked immunosorbent assay (ELISA) is probably one of the most sensitive serological techniques for the detection of pathogen in host tissues (Chakraborty and Chakraborty, 2003). In the present study polyclonal antibody was raised against mycelia of *S. rolfsii*. The antisera obtained were purified to minimize non specific binding. At the beginning, the sensitivity of the assay was optimized. Root antigens of 18 tea varieties, one non host (*O. sativa*) and one non pathogen of tea (*F. graminearum*) were cross reacted separately with PAb of *S. rolfsii*. Presence of cross reactive antigens (CRA) between *S. rolfsii* and tea varieties (T-17/1/54, TV-30, UP-3, UP-8, UP-9, UP-26, T-135 and B-157) was evident in immunodiffusion test. However, weak precipitation reaction was observed with antigens of tea varieties (TV-18, TV-22, TV-25, TV-26, UP-2, T-78, AV-2 and B-157). No common antigenic substance was found between *S. rolfsii* and other varieties. The presence of CRA and their involvement in various host parasite combinations have been observed. These are cotton and *Fusarium oxysporum* f. sp. *vasinfectum* (Charudattan and De Vay, 1970); cotton and *Verticillium alboatrum* (Charudattan and De Vay, 1972); sweet potato and *Ceratocystis fimbriatae* (De Vay *et al.*, 1972); potato and *Phytophthora infestans* (Palmerly and Callow, 1978, Alba and De Vay, 1985); coffee and *Hemilea vastatrix* (Alba *et al.*, 1983); soybean and *Macrophomina phaseolina* (Chakraborty and Purkaystha, 1983); soybean and *Colletotrichum dematum* var. *truncata* (Purkaystha and Banerjee, 1986), jute and *Colletotrichum corchori* (Bhattacharya and Purkaystha, 1985); soybean and *Myrothecium roridun* (Ghose and Purkayastha, 1990); groundnut and *S. rolfsii* (Purkayastha and Pradhan, 1994); tea and *Bipolaris carbonum* (Chakraborty and Saha, 1994a); tea and *Pestalotiopsis theae* (Chakraborty *et al.*, 1995a); tea and *Glomerella cingulata* (Chakraborty *et al.*, 2002d), soybean and *Fusarium oxysporum*

(Chakraborty *et al.*, 1997b). In the present study PTA-ELISA readily detected CRA between tea root antigens and *S. rolfsii* at a concentration of 1:250 antiserum dilution. Alba and De Vay (1985) also detected CRA in crude preparations and in purified preparations from mycelia of *Phytophthora infestans* (races 4 and 1.2.3.4.7) using antisera of two potato cultivars (King Edward and Petland Dell) at concentrations lower than 50 µg/ml protein in indirect ELISA. Among the 18 tea varieties tested with PAb of *S. rolfsii*, very high absorbance values were obtained in case of UP-8, UP-9, T-17/1/54, TV-30, T-135, B-157 and UP-2, BSS-2, TV-26, HV-39 and K1/1 showed very low absorbance values.

Visible outcome of a compatible host pathogen interaction may be obtained in many cases only after few days of infection, by which time the pathogen would be well established in the host tissues. Recent trends have developed highly specific techniques for the detection of pathogen at a very early stage. Various formats of ELISA using polyclonal antiserum have found widespread application in plant pathology and are routinely used for detection and identification purposes (Lyons and White, 1992; Hansen and Wick, 1993, Chakraborty *et al.*, 1995a; 1996b; Kennedy *et al.*, 1999; 2000; Chakraborty *et al.*, 2002d).

It is also important in the studies on host parasite relationship to determine the cellular location of the pathogen. For this purpose in this study, indirect immunofluorescence tests were conducted with cross sections of healthy and artificially inoculated (with *S. rolfsii)* tea roots, and mycelia. Transverse sections from infected roots were made and PAb raised against mycelial antigens of *S. rolfsii* were used for probing the fungal hyphae which penetrate the root tissues. Bright fluorescence was observed in the cross sections of tea roots. De Vay *et al.* (1981) determined the tissue and cellular location of major cross reactive antigens (CRA) shared by cotton *and F. oxysporum* f. sp. *vasinfectum*. Cellular location of CRA in tea leaf tissues shared by three foliar fungal pathogens such as *Bipolaris carbonum* (Chakraborty and Saha, 1994a); *Pestalotiopsis theae* (Chakraborty *et al.* 1995a) and *Exobasidium vexans* (Sharma and Chakraborty, 2004) have been demonstrated. Besides detection of pathogen in host tissues using antibody based immunofluorescent technique has been reported by several previous authors

(Warnock, 1973; Reddy and Ananthanarayan, 1984). On the basis of immunofluorescence studies, Dewey *et al.* (1984) suggested that chlamydospores, basidiospores and mycelia of *Phaseolus schweinitzii* contained molecules antigenically related to species specific antigens secreted by mycelia grown in liquid culture. They also demonstrated the presence of mycelium and chalmydospores in naturally and artificially infested soil samples, using this technique. Different test formats including indirect ELISA, western blotting, dot blot and indirect immunofluorescence was assessed for their potential to detect resting spores of *Plasmodiophora brassica* (Wakeham and White 1996) as well as *Fomes lamaoensis* (Chakraborty *et al.*, 2002a) in soil.

The dot immunobinding technique has also been found to be a rapid and sensitive method for detection of fungal pathogens. In the present study, antigens were prepared from sclerotial blight infected tea plants. Healthy and artificially inoculated (with *S. rolfsii*) tea roots were tested on nitrocellulose paper. Infected and artificially inoculated root antigens gave intense dots when compared to the healthy control confirming the presence of fungal pathogens. Blake *et al.* (1984) has described a method using the alkaline phosphates substrate 5-bromo-4-chloroindolyl phosphate (BCIP) and nitro blue tetrazolium chloride (NBT) to detect the precipitated indoxyl group. When the substrate 5-bromo 4-chloroindolyl phosphate is used, the phosphate is cleaved by the enzyme and the indolyl group precipitates. The hydroxyl group of the indigo then tautomerizes forming a ketone, and under alkaline conditions dimerization occurs, forming a dehydroindigo. In the process of dimerizing, it releases hydrogen ions and reduces the nitroblue tetrazolium which precipitates, forming an intense blue deposition of diformazan. So, early detection of disease is an important requisite for development of management strategies. A microtitre immunospore trapping device, which uses a suction system to trap air particulates directly by impaction into microtitre wells, has been used successfully for the rapid immunodetection and quantification of ascospores of *Mycosphaerella brassicicola* and conidia of *Botrytis cinerea* (Kennedy *et al.*, 2000, Kennedy and Wakeham, 2006).

Plants have well developed defense mechanisms which enable them to defend themselves against parasites in their tissues. The biochemical basis for this

resistance against microbial attack consists of both preformed and post-infectional ones. Preformed defenses are often regarded as general or unspecific as compared to inducible defense systems which are highly specific. Though the versatile multicomponent defense is adequate to provide them protection against most of their potential pathogens, only a few of them can overcome this defense and cause disease. Varieties within the host species are resistant when they possess one or more resistant gene(s) and susceptible when they lack any such gene. To account for the observed specificity and degree of variability of host parasite system, the fungal receptor must have high information content which involves recognition between the host and pathogen both at the cellular and subcellular level. A cell reacts in a special way as a consequence of an association with another cell because it acquires information, which is conveyed through chemical or physical signals in the process or recognition. The spatial and temporal deployment of plant defense responses involves the complex interplay of signal events, often resulting in superimposition of signaling processes. In spite of lacking immune responses like animals, plants have nevertheless evolved immune mechanisms of various types by which they can account for the advance of foreign organisms. The result is that disease tends to be specific, a given pathogen usually infecting a distinct range of host plants.

Environmental effects in phenolics are all the more long-lasting, as they have to cope of such conditions year after year. In a similar study on tea with the fungus *Glomerella cingulata* which causes brown blight of tea, it was reported that high humidity and rainfall were the most important factors predisposing the plants to infection (Chakraborty *et al.*, 2002e). Phenols are also known to play definite roles in a plant defense. Considering this in the present study phenol contents of the healthy and artificially inoculated (with *S. rolfsii*) plants were determined. It has been reported previously that quinones in plant tissues react with proteins to form melanin and other tannins leading to the discoloration of damaged tea leaves (Sudhakran *et al.*, 2000). Many studies have demonstrated the importance of phenolic compounds in plant defense. In general, plant phenolics have a diverse range of biological activity, depending on their structure, degree of polymerization, stereoisomeric differences etc. interaction between phenolic compounds and environmental conditions determines their action. Polyphenols have a distinctive

ability to engage in molecular recognition, or formation of intermolecular complexes with each other and with other molecules (Haslam, 1999). In the present study, total phenol content decreased with pathogen infection. The decrease was most significant in UPASI varieties. However, in these varieties, PAL activity increased significantly. In the present study, the level of antifungal phenolics (pyrocatechol) in healthy and *S. rolfsii* infected tea varieties were estimated. In resistant varieties accumulation of pyrocatechol increased sharply following inoculation with *S. rolfsii* in relation to healthy plants. Accumulation of pyrocatechol in susceptible varieties were not greater than the resistant one. Increased level of pyrocatechol may be associated with the host response to resistant reaction. One of the reasons for the observed tolerance of certain varieties to fungal attack could be their ability to maintain higher levels of phenolics in the face of attack.

Accumulation of defense enzymes such as phenylalanine ammonia lyase (PAL), polyphenol oxidase (PPO), peroxidase (PO), in tea varieties following inoculation with *S. rolfsii* were determined. PPO usually accumulated upon wounding in plants. PPO transcript levels systemically increased in tomato when mature leaflets were injured (Thipyapong and Steffens, 1997). Increased activity of PPO and PO was demonstrated in the cucumber leaf in the vicinity of the lesions caused by some foliar pathogens or by phosphate application (Avdiushko *et al.*, 1993). Moreover PPO could be induced by jasmonic acid (Constabel and Ryan, 1998). Among all the stress related enzymes, the role of peroxidase has been most thoroughly worked out. PO is a metallo-enzyme containing porphyrin bound iron. The enzyme acts on a wide range of substrates including phenols, aromatic amines, amino acids and inorganic compounds (Balasimaha, 1982). These are ubiquitous to plants and are characterized by a large number of isozymes. Various naturally occurring and synthetic substances, growth regulator and environmental factors markedly influence the activity of these peroxidases. Akhtar and Garraway (1990) observed increased PO activity in susceptible cultivars compared with the resistant one when both were treated with sodium bisulphate prior to inoculation with *Botrytis maydis*. On the other hand there are also reports of increased PO activity due to induction of resistance (Chen *et al.*, 2000; Chakraborty *et al.*, 2005d). Curtis *et al.* (1997) also reported the induction of PO activity by pathogens and methyl

jasmonate. The existence of multiple molecular forms of peroxidase in tea has also been reported (Sharma and Chakraborty, 2004).

Previous reports indicate that oxidative enzymes such as PPO and PO as well as those involved in phenolic biosynthesis such as PAL are involved in defense reactions in plants (Chen *et al.*, 2000). Considering the importance of phenol metabolism in tea plants, those three enzymes were selected for studies. Results showed that the constitutive enzyme activities under no stress conditions of the different clones varied. Matsumato *et al.*, (1994) reported that Japanese green tea cultivars belonging to the variety 'sinensis' could be divided into three groups on the basis of their PAL cDNA cloning. Assam hybrids could not be placed into any specific groups because complex patterns were produced. They confirmed the existence of many kinds of PAL genes, expressing of which varied depending on the varieties. An elevation in the level of activity of PAL has been frequently demonstrated to be one of the earliest responses of plants to biotic (Chakraborty *et al.*, 1993; Shiraishi *et al.*, 1995) or to other environmental stresses (Eckey-Kaltenbach *et al.*, 1997). It was reported by Orczyk *et al.*, (1996) that in sorghum, naturally occurring high levels of PAL activity induced by light should be differentiated from the activity induced as a response of attempted fungal infection. Bhattacharya and Ward (1987) reported that PAL activity in soybean was enhanced in the resistance response of soybean hypocotyls to *Phytophthora megasperma* f.sp. *glycinea*. Considering that PAL is a key enzyme in the biosynthesis, not only of phytoalexins, but also of phenolic compounds in general, and melanins, all of which have been associated with resistance responses in various host plants, it may be suggested that activity of PAL could be useful indicators of the activation of defense related enzymes.

Polyphenolics are major constituents of tea leaves and it is expected that they would be affected by the different abiotic and biotic stresses (Chakraborty *et al.*, 2005a; 2005e). In case of temperature stress it was observed that there was a correlation between the inherent phenol contents in the tea variety and its increase following exposure to elevated temperatures. In general, in those varieties with high inherent phenol content, accumulation of phenols kept increasing till 50^0C. A wide variation in the phenol contents in the different tea varieties was also evident

(Chakraborty *et al.*, 2001a). The observed trend could be explained by the fact that phenols are considered to be involved in plant's defense to various stresses. When subjected to temperature stress, varieties with low inherent phenol content increased its accumulation while those that already had a higher content did not have to increase synthesis (Chakraborty *et al.*, 2005e). In case of tea, polyphenols are also known to vary seasonally (Zakoskiva *et al.*, 1991). Thus phenol biosynthesis seems to be well regulated to help the tea plant to overcome various stresses. Similarly, in case of drought too, phenol content increased initially up to 8 days of stress after which there was a decline (Chakraborty *et al.*, 2001b).

Alteration of phenol metabolism following fungal infection has been observed in many diseases and phenolics have been implicated in the defense reaction in several instances (Mahadevan, 1991). There is often a greater increase in phenolic biosynthesis in resistant host species than in susceptible hosts and it is sometimes postulated that the increase in phenolic compounds is part of the resistance mechanism. Some of these compounds are toxic to pathogenic and non-pathogenic fungi and have been considered to play an important role in disease resistance. The involvement of phenol in the defence strategies of tea plants against foliar fungal pathogens e.g *Bipolaris carbonum*, *Pestalotiopsis theae*, *Glomerella cingulata* has been described by Chakraborty *et al.*, (1995b) and Chakraborty (1996). Biochemical responses to tea plants exposed to biotic stress due to blister blight infection caused by *Exobasidium vexans* in the levels of phenols and enzyme activities were studied (Chakraborty *et al.*, 2002c; Sharma and Chakraborty, 2004).

In the present study, the levels of phenolics in leaves of resistant and susceptible tea varieties were estimated following inoculation with *S. rolfsii*. Host responses could be differentiated by changes in content of phenolic compounds. In both the cases total phenol and orthodihydroxy phenol content increased in resistant varieties but decreased in susceptible varieties in comparison to their healthy controls. Hammerschmidt and Nicholson (1977) demostrated a clear difference between resistant and susceptible interaction of maize to *Colletotrichum graminicola* based on accumulation of phenols. Sridhar and Ou (1974) reported diffences in total phenolics accumulation in the interaction of *Pyricularia oryzae* with rice. However, no differences were found in the phenolic content in the interaction *of*

Helminthosporium maydis race T (Macri *et al.*, 1974). On the other hand, a resistant cotton cultivar contained fairly high amount of total as well as orthodihydroxy phenol than susceptible cultivar. In the present study, greater accumulation of orthodihydroxy phenol in resistant interaction of *S. rolfsii* and tea varieties indicated that this might play a role in disease resistance mechanism. Orthodihydroxy phenols play a major role in disease resistance and disease development. They are easily oxidized to highly reactive quinones which are effective inhibitors of sulphydryl enzymes, thereby preventing the metabolic activities of host and parasite cells (Kalaichelvan and Mahadevan, 1988). There are ample evidences that an increased production of phenolic compounds is involved in phytoalexin accumulation (Mansfield 2000). The UV spectra from both the healthy and *S. rolfsii* inoculated tea roots were analysed at 290 nm. A sharp peak at retention time 2.6 was present in both the compounds but in the healthy extracts the peak height was much smaller than the inoculated one. Other small humps and shoulders were also evident in both the cases.

It is known that catechin is oxidatively cleaved to some simpler phenols and phenolic acids like catechol, phloroglucinol and protocatechuic acid. Sambandam *et al.*, (1982) isolated an enzyme (catechin 2-3 dioxygensis) from *Chaetomium cupreum* which cleaved catechin into simpler phenols. It is not unreasonable to speculate that the antifungal compound cleaved to some simpler phenols in the present study. In the susceptible variety, the breakdown of catechin was almost complete while traces were evident in the resistant variety even after 48 h of inoculation. Accumulation of pyrocatechol in resistant varieties increased after 48 h of inoculation with *S. rolfsii*. Increased level of pyrocatechol may be associated with the differential host responses to disease production. Nagahulla *et al.* (1996) reported the production of antifungal compounds in tea leaves following infection with blister pathogen (*Exobasidium vexans*). HPLC analysis of the catechins from healthy and blister infected tea leaves showed marked differences and some quantitative changes (Chakraborty *et al.*, 2002c; 2004).

Research in biological control has progressed from the initial discovery and evaluation phase to the development of practical application techniques. With the emergence of numerous strains or isolates of bacterial and fungal agents, researchers

have investigated methods that will lead to the practical implementation of biocontrol agents. With increased understanding into the mechanisms by which these agents control plant diseases, selection and screening criteria as well as new technology in fermentation and formulation can be evaluated. Several fungi have exhibited biological control activity against plant pathogenic fungi (*Sclerotinia sclerotiorum*, *Rhizoctonia solani* and *Alternaria brassicae*) of rapeseed and canola. These include *Trichoderma* spp., *Gliocladium virens*, *Myrothecium verrucaria*, *Talaromyces flavus*. Strategies used for control of these diseases included reducing initial inoculum, reducing secondary inoculum and spread and controlling infection in the rhizosphere and phyllosphere (Boyetchko, 1999). One of the most successful media for sporulation and growth of *T. harzianum* was shown to be wheat bran which acted as both a food base and carrier. Conidia viability and shelf life were significantly improved. By adding peat to the wheat bran at a 1:1 (v/v) ratio, the pH of the medium was more effectively controlled and was found to be a better carrier than wheat bran alone (Sivan *et al.*, 1984). Several *Trichoderma* spp. have been used to reduce the number of sclerotia in soil. *T. viride* and *T. harzianum* parasitized sclerotia and mycelium of *S. sclerotiorum* by penetrating into host mycelia and prodcing lytic enzymes such as β-1,3-glucanases (Jones *et al.*, 1974).

In the present investigation, experiments were conducted using *T. harzianum* and *T. viride* as a biocontrol agents *in vitro*. These were also evaluated against *S. rolfsii* in laboratory by dual culture filtrate test. Consequent to the study, experiments were conducted *in vivo* for the management of the disease. Both antagonists over grew the pathogen and restricted the growth of *S. rolfsii in vitro* but *T. harzianum* was the most effective. Similar observations were reported on wilt of potato caused by *S. rolfsii* (Rao *et al.*, 2004). Studies were made by them to understand the antagonistic activity of microorganisms on mycelial growth, sclerotial production, and inhibition zone against *S. rolfsii*. This result led to their application in integrated disease management practices.

There are several reports on the ability of *T. harzianum* and *T. viride* to inhibit the growth of pathogen in *in vitro* condition. Patel and Anahosur (2001) tested antagonistic potential of *T. harzianum* against four soil borne pathogens

isolated from chickpea plants viz. *Fusarium oxysporum, F. solani, Macrophomina phaseolina* and *S. rolfsii in vitro*. In dual culture, the mycoparasite over grew the pathogen and inhibited their growth by producing antibiosis through the production of some antifungal substances. Sharma and Sharma (2001) reported that among the antagonists tested by them *T. harzianum* and *T. viride* were found most effective in inhibiting mycelial growth of *Dematophora netrix* in dual culture. Prasad *et al.*, (1999) obtained three *T. harzianum* isolates that were highly effective in controlling root / collar rot of sunflower caused by *S. rolfsii* under green house conditions.

In the present investigation, the efficacy of systemic fungicides and plant extracts were also investigated *in vitro*. A selective one of each was tested *in vivo* for the management of sclerotial blight diseases. Results of *in vitro* tests revealed that the systemic fungicides (thiodan, calixin, captan, carbendazim and idofil M-45) were significantly superior over control in checking the mycelial growth of *S. rolfsii*. However, thiodan and calixin completely arrested the growth of pathogen at concentration as low as 0.0125% in *vitro*. Venkata Ram (1974) also reported calixin, a systemic fungicide against blister blight (*Exobasidium vexans*) on tea plants. There are several reports on the efficacy of fungicides on pathogen to inhibit the growth of pathogen *in vitro* condition. The efficacy of different fungicides was studied by Tiwari and Sing (2004) against *Rhizoctonia solani, S. rolfsii,* seed mycoflora and their non-target effect on *T. harzianum* and *Rhizobium leguminosarum*. It was found by them that fungicides *viz.* carboxin, epoxiconasole, hexaconazole, propicanazole and triadimetox which were found highly effective against *R. solani* and *S. rolfsii* can be formulated as seed dresser either with thiram or mancozeb to control both collar rot or rot root as well as seed mycoflora effectively. They also advised to integrate *T. harzianum* and *R. leguminosarum* with these fungicides for seed and seedling protection. Similarly *in vitro* evaluation of fungicides against *S. rolfsii* was studied by Gupta and Sharma (2004). It was observed by them that penconazole, hexaconazole, propineb and mancozeb inhibited mycelial growth of *S. rolfsii*. Propineb was found to be the most effective in reducing disease incidence on crown and pods. Among the biocontrol agents, *G. virens* and *T. viride* were also found to be the most effective against the pathogen. Evaluation of fungicides and plant extracts against *Fusarium solani* leaf blight in *Terminalia catappa* was reported by Mamatha

and Rai (2004). Leaf extracts of *Lantana camara* followed by *Azadirachta indica* and *Acalypha indica* were found to be equally effective in inhibiting the growth of *F. solani in vitro*.

In this investigation, the efficacy of antifungal effect of *Azadirachta indica* and *Catharanthus roseus* were tested *in vitro*. Results revealed that both plant extracts were inhibitory to the mycelial growth of *S. rolfsii* however extract of *A. indica* showed superior upon *C. roseus* extract *in vitro*. It was also observed that as the concentration of extracts increased in the medium the effectiveness of extracts also increased and maximum growth inhibition was recorded at 100% concentration. Similar observation was observed by Sharma and Bohra (2003) in the investigation carried in laboratory and in the field to study the effect of extracts of three medicinal plants species for their antifungal activity against cumin wilt pathogen. Evaluation of plant extracts against *Rhizoctonia solani* incitant of black scurf disease in potato was investigated by Shinde and Patel (2004). It was obtained that garlic extract at 10% concentration showed a complete inhibition of growth (100%). Investigation on the effects of aqueous leaf extracts on neem in inducing resistance against the leaf stripe pathogen by barley, *Drechslera graminis* (Paul and Sharma, 2002) had also been reported.

In another set of experiments different organic amendments were treated in different tea seedlings to observe growth promotion and percentage increase in shoot length in healthy and treated tea seedling varieties. Results revealed that the growth promotion and percentage increase in shoot length in tea seedlings treated with neem cake and oil cake were more in seedlings inoculated with *S. rolfsii* (after treatment) in comparison to the treated uninoculated tea seedlings. Total phenol and orthodihydroxy phenol contents were also increased in treated inoculated tea varieties with neem cake and oil cake. This is due to the decomposition of organic matter that helps in alteration of the physical, chemical and biological conditions of the soil and the altered conditions probably reduce the inoculum potential of soil-borne pathogens (Singh, 1983). In addition, the practice also improves soil structure, which promotes root growth of the host. Various antibiotics and phenols are released during decomposition, which induces resistance in the root system and increases over all growth of the plant. In case of organic amendments, *i.e.*, cowdung, rabbit

manure and chicken manure, the growth promotion as well as percentage increase in shoot length in different tea seedlings varieties were higher in uninoculated tea seedlings than the treated inoculated ones. But total phenol and orthodihydroxy phenol contents were higher in treated inoculated tea seedling varieties as in oil cake and neem cake treated tea seedling. This observation may correspond to the fact that microorganisms being present in soil enhance the decomposition processes releasing phenols which increases total and orthodihydroxy phenol contents in treated inoculated tea seedling varieties with cowdung, rabbit manure and chicken manure.

Further in this investigation, effective integrated management practices against *S. rolfsii* were tested *in vivo*. In this experiment, under pot culture conditions *T. harzianum* alone and in combination with neem cake, oil cake, aquous extract of *A. indica* and calixin (0.1%) provided a total control of seedling blight in all the three modes of application *viz*, simultaneous, repeated and post infection. Similar results were obtained by Sonali and Gupta (2004) when *T. viride* alone and in combination with neem oil, neem cake and deodar needles used in radial growth of *S. rolfsii* resulted in a total control of the disease. But repeated application of neem cake, oil cake with various combinations of cowdung, rabbit manure and chicken manure were found to be less significant. Finally it was observed that *T. harzianum* and in combination with neem cake, oil cake, neem extract and calixin (0.1%) were found most effective in reducing disease incidence on tea seedling plants *in vivo*.

There are several reports on the management of disease by Integrated Disease Management (IDM). Management of chickpea root rot and collar rot against *S. rolfsii* by integration of biological and chemical seed treatment was reported by Tiwari and Mukhopadhyay (2003). They observed that application of carboxymethyl cellulose (CMC) with *G. virens* powder (10^9 spores per g) in combination with vitavax provided maximum protection (81.9%) to the crop against chickpea root rot and collar rot pathogens in glasshouse. Chickpea seeds treated with GV powder + CMC + vitavax significantly increased seedling emergence (47.9%); final plant stand (85.8%) and grain yield (79.7%) which was statistically at par with the treatment GV powder + vitavax and GV suspension + vitavax in a sick plot. Upamanyu *et al.*, (2002) reported the management of root rot and web blight caused by *Rhizoctonia solani*. They obtained that *T. viride* showed the maximum tolerance to carboxin,

tebuconazole and carbendazim followed by *T. virens, T. harzianum* and *A. niger* when used in integrated disease management along with fungicides and oil cakes both under glass house and field conditions. Soil amendment (cotton cake) + *T. virens* and carboxin (ST), mustard cake + *T. virens* + tebuconazole and soil amendement (mustard cake) + carbendazim (ST) were found effective in containing the root rot under glass house conditions while soil amendment (mustard cake) + carbendazim (ST) + carbendazim (FS) were found highly effective in reducing pre- and post- emergence root rot and web blight. Severity was best contained by soil amendement (mustard cake) + carbendazim (ST+FS) followed by tebuconazole + *T. virens* (ST) + carbendazim (FS).

In the present investigation, using PTA-ELISA formats and PAb of *S. rolfsii*, treated and untreated plants exposed to natural inoculum after 15 and 30 days of soil ammendments were compared. The absorbance (A_{405}) values were always reduced in treated root tissues than untreated ones. It indicates clearly that in the treated root tissues the establishment of the pathogen (*S. rolfsii*) was not successful due to the application of bioresources. Detection of *S. rolfsii* in tea root tissues and rhizosphere soil of different treatment with pathogen and biocontrol agents was also determined immunologically in both root tissues and soil. For this purpose, PTA-ELISA format was carried out. Results showed that ELISA values of root tissues treated with *T. harzianum* and *T. viride* were significantly lesser than with *S. rolfsii* alone. The same trend of results was obtained in infested rhizosphere soil through PTA-ELISA 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. However they observed that the reduction of mortality of plant increased to 62.2% when *T. harzianum* were applied soil to soil drench. Significant control of charcoal stump rot of tea with antagonistic microflora obtained previously by Borthakur and Dutta (1992). The role of *T. harzianum* and *T. viride* as biocontrol crop is well established. In the present study, antigens prepared from mycelia of *S. rolfsii*, amendment soils and 4 different soil fungi were prepared and tested on nitrocellulose paper PAbs raised against mycelia of *S. rolfsii* using NBT/BCIP as substrate. Antigens of homologous source showed deep coloured dot when compared with soil antigens prepared from treated

organic amendments. Other tea root pathogens responded slightly reactivity with *S. rolfsii* walsh *et al.* (1996) also performed western blotting using the raw serum of *Spongospora subterranean* spore balls. Watabe (1990) demonstrated the presence of mycelium and chlamydospores in naturally and artificially infested soil samples, using this technique. Different test formats including indirect ELISA, western blotting, dot blot and indirect immunofluorescence was assayed by Wakeham and White (1996) for their potential to direct resting spores of *Plasmodiophora brasica* in soil. In conclusion, it can be stated that sclerotial blight can cause severe damage to tea plants, particularly to those growing sandy soil. Such detection techniques makes it possible to detect microquantities of the pathogen within root tissue and rhizosphere soil before much damage cause by the pathogen. Therefore, an accurate, rapid and cost-effective diagnosis is the cornerstone of efficient field disease management. Rapid detection of the pathogen is important to take preventive steps.

A possible long-term benefit of increased implementation of microbial control would be reduced input into agriculture, particularly if seasonal colonization and introduction-establishment come into widespread use. Initially, inputs due to implementation of microbial control are more likely to increase than decrease. There is potential for yield increase in the near future. Microbial control is simply one of the best potential alternatives for disease control that could be made available in a relatively short time period. A successful disease control program depends on a crop production system which is closely aligned with the goals of disease management. Integrated Disease Management (IDM) as applied to disease means using all the tactics available to the grower (cultural, biological, host plant resistance and chemical) that provides acceptable yield and quality at the least cost and is compatible with tenets of environmental stewardship.