Discussion

In India cultivation of tea started around 1830 when tea plants were raised from the seeds. With the huge demand of the planting materials for the plantation industry the economic method of vegetative propagation by clonal cuttings was established around 1938 but this type of propagation became essential after the release of Tocklai clones in 1949 (Bezbaruah and Singh, 1988). To get healthy tea plants, the importance of healthy seedlings is also essential. Presently several clones are available for raising tea plants. The clonal cuttings are raised in the nurseries. Proper disease management is required for raising healthy seedlings. Plant disease management is an important aspect of successful cultivation of any crop. The applications of broad-spectrum chemical fungicides are the common practice in most of the nurseries. However, this is hazardous to the environment. Hence, it is essential to adopt ecofriendly methods to control fungal diseases in the nurseries. With these observations the present work was undertaken and it is likely that the results will broaden the scientific base upon which total control of foliar fungal diseases of nursery tea plants may be established.

At the beginning, several fungi were isolated from the plant parts of young tea plants in the nurseries. These are *B. theobromae*, *C. eragrostidis*, *C. cammelliae*, *P. theae*, *C. theae*, *F. oxysporum* etc. After isolation their pathogenesis were tested through verification of Koch's postulations. It was found that six different fungi (*B. theobromae*, *C. eragrostidis*, *C. cammelliae*, *P. theae*, *C. theae*, *F. oxysporum*) were producing diseases in the nurseries in North Bengal. Out of six fungi, *B. theobromae* and *C. eragrostidis* were consistently found to be associated with the young seedlings. Out of the six organisms found, *C. cammelliae* and *P. theae* were mostly isolated from the mature maintenance leaves. This finding is very much in conformity with that of Sarmah (1960). Sarmah (1960) reported that *C. camelliae* and *P. theae* were extremely common in old tea leaves. After development of the new shoots upto 2 to 6 leaves stages *C. cammelliae* and *P. theae* were not found in the isolations made from the young leaves. In one isolation, *F. oxysporum* was also found associated with the mature leaves but the involvement of *F. oxysporum* was not found frequently. *F. oxysporum* was reported as a pathogen of tea by previous workers (Debnath and Barthakur, 1994; Pandey *et al.*, 2000). Another pathogen *C. theae* was found in cases of heavy rainfall followed by prevailing

humid weather for prolonged period generally in the month of July and August in North Bengal. But this was also found in few cases of isolations made from young tea plants raised from clonal cuttings. The fungi like *B. theobromae and C. eragrostidis* were very common in the nurseries of North Bengal and were found in the isolations frequently. Out of the two pathogens *C. eragrostidis* is a new report in tea (Saha *et al.*, 2001). *B. theobromae* may attack any part of the tea plant, young or old. In our study also, it was isolated from all the parts of young tea plants but the tender stem and leaves of nursery plants were more prone to attack by the fungi. *C. eragrostidis* (P. Hennings) Meyer. was originally isolated from naturally infected leaves of nursery tea plants of Matigara tea estate, Siliguri, West Bengal. After verification Koch's postulations the fungus was identified in the laboratory and was also sent to ITCC, IARI, New Delhi for identification. The two pathogens have major role for the failure of seedling growth in the tea nurseries.

Differential pathogenicity of a fungus to different varieties gives us information about the degree of susceptibility or resistance of a particular variety or pathogenicity of different fungi to a particular plant variety gives us information about differernt pathogen's different infecting ability. Pathogenicity of the isolated fungi, *B. theobromae* and *C. eragrostidis*, was tested following three different techniques, viz. detached leaf inoculation, cut shoot inoculation and intact nursery tea plant inoculation. Results obtained from studies following different techniques were in agreement with each other. Dickens and Cook (1989) also used all these three methods to detect resistance and susceptibility of *Camellia* plants against *Glomerella cingulata*. Brennan *et al.* (2003) examined the pathogenicity of five different fungal species (*Fusarium areenaceum*, *F. culmorum*, *F. graminearum*, *F. poae* and *Macrodochium nivale*) following the *in vitro* coleoptile growth rate of wheat seedlings (cv. Falstaff). Yanase and Takada (1987) followed only the cut shoot method for determining the resistance of tea plants to gray blight disease caused by *Pestalotia longiseta* in his laboratory.

The pathogenicity results distinctly showed that TV-11 was the most susceptible and TV-26 was the most resistant against *B. theobromae* among the varieties tested. TV-12 and TV-25 were found to be the most susceptible and resistant varieties respectively when tested against *C. eragrostitis*. Saha (1992)

observed that TV-26, TV-25 and TV-16 were resistant and TV-18, TV-9 and TV-17 were succeptable varieties when tested against *Bipolaris carbonum*, a foliar fungal pathogen of tea. Pathogenicity of other foliar tea pathogens like *Pestalotiopsis theae* and *Colletotrichum cammelliae* were studied by Chakraborty *et al.* (1995) who found that TV-18 was highly susceptible and TV-9 was moderately resistant among different clonal and seed varieties. Hu-Shu Xia (1996) studied the pathogenicity of *P. theae* in eighteen tea cultivers in Anhui province, China and found that two cultivers were highly resistant. The results of the present study is in conformity with that of the studies of earlier workers. Thus, the identity of a tea variety may inform us about its degree of susceptibility or resistance towards foliar fungal pathogens. Such information might be helpful in disease management specially during multiple pathogen attack.

Once a fungal pathogen is isolated and identified, a thorough understanding on the morphological and physiological features becomes necessarry. It forms the basis of further studies on understanding disease development, host-pathogen interaction and control of the disease caused by the pathogen. These basic knowledge becomes more essential when a fungus is newly recorded to be a pathogen. Since, one of the pathogens isolated during the present work is being reported as the tea leaf pathogen for the first time, any work on defense responses of the plant to pathogen attack or control strategies of the disease caused by the pathogen could not be worthwhile without basic knowledge on the morphological and physiological aspects of the fungal pathogen. Hence, a thorough microscopic observation of the morphological characters of mycelia and spores along with studies on growth conditions and nutritional requirements of both the pathogens were undertaken.

In the present study, six different solid media (PDA, MEA, CDA, OMA, PCA and RA) were used to study the growth of *B. theobromae* and *C. eragrostidis*. Among these, malt extract agar (MEA) was found to be the best growth medium for *B. theobromae* while *C. eragrostidis* showed maximum growth in potato carrot agar (PCA) although both the fungi showed satisfactory growth in all the solid media tested. However, differences in their radial growth pattern was observed when *C. eragrostidis* recorded maximum radial growth after 8 days of incubation while

B. theobromae showed the same after the second day. Rapid increase in growth was observed within the first 5 days after which the rate of growth decreased. After 15 days mycelial dry weight declined due to autolysis and depletion of media. There was no significant difference in the growth of the two fungi when mycelial dry weights were considered though the radial growth pattern differed. During growth, mycelia of *B. theobromae* adhered to the surface of the agar media and grew rapidly extending the radius while *C. eragrostidis* showed vertical extended fluffy growth and extended the radius at a much slower rate.

Studies on the nutritional requirements of the pathogens revealed that for *B. theobromae* glucose and sucrose were the best carbon sources for growth and sporulation respectively. For *C. eragrostidis* glucose was the best carbon source for both growth and sporulation. However, in sucrose, sporulation started earlier in case of both pathogens. Sucrose was also found to be the best source for growth and sporulation of *B. carbonum* by Saha (1992). Peptone was the best nitrogen source for both growth and sporulation of *C. eragrostidis*. For *B. theobromae*, peptone was best for growth but sporulation was recorded better when potassium nitrate and yeast extract were the nitrogen sources. Saha (1992) recorded that ammonium nitrate was the best nitrogen source for growth of *B. carbonum* and sodium nitrate was best for sporulation among the inorganic nitrogen sources tested while peptone was the best among the organic nitrogen sources tested for both growth and sporulation of the pathogen.

Wu and Wu (2003) found that *Alternaria protenta*, a pathogen of sunflower showed abundant sporulation on glucose peptone agar and leonien agar but not on dextrose nitrate agar.

The influence of various carbon and nitrogen sources of fungal metabolism has been studied by several workers (Jamaluddin, 1977; Roy, 1977; Devadath and Padmanabhan, 1977). In a study, potassium nitrate was found to be a satisfactory nitrogen source for the growth of *Aspergillus flavus* by Jamaluddin (1977).

Since spore germination is a determining factor at the onset of host colonization by a fungal pathogen, several studies were undertaken to evaluate the influence of environmental factors like pH, temperature, incubation periods etc. on

the germination of spores *in vitro*. *C. eragrostidis* showed slightly quicker and better germination than *B. theobromae* but germ tubes of *B. theobromae* were much longer than *C. eragrostidis* when their spores were allowed to germinate on microscopic slides. Germination started within 2 hours in case of *C. eragrostidis* and between 2-4 hours for *B. theobromae.* Saha and Chakraborty (1990) also observed that germination of *B. carbonum* begun between 2-4 hours *in vitro*. During the present study, the best temperature for germination was found to be 28 ^{0}C. Pretreatment of spores of both *B. theobromae* and *C. eragrostidis* at 45 ^{0}C and above totally inhibited their germination. Saha and Chakraborty (1990) observed that spore germination of *B. carbonum* was reduced to 27% when pretreated at 50 ^{0}C but pretreatment at 0 ^{0}C for even 12 hours had no effect on germination and germ tube elongation. Flett and Wehner (1989) observed that high temperature reduced the viability of spores. In another study, Achar (2000) observed that conidia from *Stenocarpella maydis* exposed at temperatures below 22 ^{0}C germinated only after 17 hours of incubation and rate of germination increased from 22-27 ^{0}C, afterwhich the germination rate declined.

The prevailing temperatures in areas covered during the present study and also in other tea growing areas do not reach above 40 ^{0}C. Hence, spores are never exposed to temperatures which may be as high to reduce germination. Both the pathogens under study may persist in the soil or leaves for extended time periods and germinate rapidly in the presence of a potential host. Studies of effect of pH on spore germination showed that pH 7.25 was the best pH for both germination and germ tube elongation. Saha and Chakraborty (1990) reported that pH 6.75 was best for germination of spores of *B. carbonum* while pH 7.2 was best for germ tube elongation. Callaghan (1974) reported that more than 97% of conidia in *Basidiobolus ranarum* germinated between pH range 7-9.

Common antigens generally show precipitin arc in the agarose gel. Clausen (1969) demonstrated immunodiffusion in semi-solid matrices (i.e., precipitin reactions of antigens and antibodies) as a major tool in serology. Three basic immunological techniques are radial immunodiffusion, immunoelectrophoresis and agar gel double diffusion. Several authors have successfully used these techniques while demonstrating cross-reactive antigens (Michael and James, 1981; Benhamou

et al., 1986), detection of plant pathogens (Burrell *et al.*, 1966; Ishizaki *et al*, 1981; Iannelli *et al.*, 1982) and common antigenic relationship (Burrell *et al.*, 1966; Chard *et al.*, 1985; Alba and DeVay, 1985; Purkayastha and Ghosal, 1987; Purkayastha and Banerjee, 1990; Chakraborty and Saha, 1994;Ghosh and Purkayastha, 2003). Enzyme-linked immunosorbent assay (ELISA) and direct and indirect fluorescent antibody staining were also reported as important techniques for detecting pathogen, cross-reactive antigens and establishment of common antigenic relationship (Warnock, 1973; Gendloff *et al.*, 1983; Geric *et al.*, 1987; Arie *et al.*, 1988; Fuhrmann *et al.*, 1989; Watabe, 1990). Chakraborty *et al.* (1997) demonstrated that the presence of cross-reactive antigens between *Exobasidium vexans* and the susceptible tea varieties were evident in immunodiffusion, indirect ELISA and indirect immunofluorescence tests. Using similar techniques, cross-reactive antigens were found between *Bipolaris carbonum* and susceptible tea varieties (Chakraborty and Saha, 1994) and also between *Pestalotiopsis theae* and susceptible tea varieties (Chakraborty *et al.*, 1995).

In the present study, the leaf antigens of susceptible and resistant varieties of tea were cross reacted separately with antisera of *B. theobromae* and *C. eragrostidis*. The mycelial antigens of *B. theobromae* and *C. eragrostidis* were also cross reacted with antisera of susceptible and resistant tea varieties. Serological comparisons were made in all the tests. Serological comparisons were also made by a non pathogen of tea viz. *Gliocladium virens* (isolate II). No common antigen could be detected in the agar gel double diffusion test when antigens of resistant varieties of tea were cross reacted with the antisera of the pathogens respectively. But the susceptible varieties of tea showed presence of common antigens cross reacted with the antisera of the pathogens. In the reciprocal reactions also common antigens were detected in the form of precipitin arcs. No precipitin arcs could be detected when antisera of tea varieties and antigen of *Gliocladium virens* (non pathogen to tea) was allowed to react.

Cross-reactive antigens has been detected in a number of host parasite combinations. Some of the combinations are as follows, soybean and *Macrophomina phaseolina* (Chakraborty and Purkayastha, 1983); Jute and *Colletotrichum corchori* (Bhattacharya and Purkayastha, 1985); soybean and

Colletotrichum dematium var. *truncata* (Purkayastha and Banerjee, 1990); groundnut and *Macrophomina phaseolina* (Purkayastha and Ghosal, 1987); soybean and *Myrothecium roridum* (Ghosh and Purkayastha, 1990).

Chakraborty and Saha (1994) in a study showed that two precipitin arcs were common when leaf antigens of susceptible tea variety reacted with antiserum of pathogen *Bipolaris carbonum*. No precipitin arc was detected in the agar gel double diffusion tests when resistant tea variety antigen reacted with the antiserum of *B. carbonum* and also in reciprocal reaction. Their finding suggests that common antigens play an important role in the compatibility of the host-pathogen interaction. Till date a number of host-pathogen/nonpathogen interactions have been studied by workers which include several plants, and their pathogen and nonpathogens.

Ghosh and Purkayastha (2003) done immunodiffusion tests using polyclonal antibodied and antigens of different ginger cvs, virulent (SR 2) and avirulent strains (IR) of *Pythium aphanidermatum* and a nonpathogen of ginger, *Colletotrichum capsici*. Cross reactive antigens (CRAS) were not detected between antigens of infected rhizome or nonpathogen and antiserum of avirulent strains of *P. aphanidermatum* SR 2, but CRA was easily detected when antigens of heavily infected ginger (cv. Mahima) were cross-reacted with antiserum of the pathogen, which was confirmed by immunoelectrophoretic and cross immunoelectrophoretic tests.

In all the cases the common antigens could be detected between susceptible varieties and pathogens. No common antigenic relationship was found between resistant varieties and pathogen. Similarly no antigenic relationship could be established between antigens of nonpathogens and antisera of the plants of interest. In the light of earlier studies the present finding is very much significant and in conformity with the earlier works.

The results of agar gel double diffusion were substantiated by immunoelectrophoretic studies. Antisera of *B. theobromae* when reacted of its own, it showed four precipitin arcs. Out of the four precipitin arcs only one precipitin arc was common with leaf antigen of susceptible variety (TV-11). No precipitin arc was found between antigen of resistant variety (TV-26), and antisera of *B. theobromae*.

In homologous reaction antiserum of TV-26 showed three precipitin arcs but in cross reaction with *B. theobromae* showed no precipitin arc. Similarly, when antiserum of *C. eragrostidis* reacted with antigen of its own, four precipitin arcs were found. Only one was common between the antiserum of *C. eragrostidis* and antigen of susceptible variety (TV-12). No precipitin arc could be detected between antigen of resistant variety (TV-25) and antisera of *C. eragrostidis.* No common relationship between host (*C. sinensis*) and nonpathogen was observed.

The results of the present study are in agreement with the works of the earlier workers. The results of the immunodiffusion and immunoelectrophoretic tests clearly indicate that there is no common antigenic relationship between hosts and nonpathogens and common antigenic relationship exists between pathogen and susceptible hosts. Absence of common antigenic relationship between pathogens and resistant hosts.

At a very low concentration, enzyme-linked immunosorbent assay (ELISA) is one of the most specific and rapid methods for detecting cross-reactive antigen (CRA) and identifying fungal diseases (Alba and DeVay, 1985; Mohan, 1988; Dewey and Brasier, 1988; Lyons and White, 1992; Linfield, 1993; Chakraborty and Saha, 1994; Chakraborty *et al.*, 1995, 1997). Croft (2002) used an enzyme-linked immunoassay for rating sugarcane cultivars for resistance to ratoon stunting disease caused by *Leifsonia* (*Clavibacter*) *xyli* subsp. *Xyli.* ELISA has also been used for early detection of pathogens by several workers (Chakraborty and Saha, 1994; Ghosh and Purkayastha, 2003).

In this study, CRA was detected in indirect ELISA using very low concentrations of antigens and antisera. The higher ELISA values in cross reactions indicated presence of more CRA and also indicated susceptibility and lower ELISA values indicated lower amount of CRA and also indicated resistance. It is to note that the degree of resistance and susceptibility results (by ELISA values) were in conformity with the results of the pathogenicity. The three concentrations of the antigens of *B. theobromae* showed higher absorbance values when tested with antisera of susceptible variety (TV-11) than when tested with antisera of resistant variety (TV-26). The reciprocal test of this combination also showed higher

absorbance values in case of the antigens of susceptible variety (TV-11) tested with antisera of *B. theobromae* than in case of the antigens of resistant variety (TV-26) tested with the antisera of *B. theobromae*. These clearly indicated the presence of maximum cross-reactivity between *B. theobromae* and susceptible variety (TV-11) than the other combinations of the antigens and antisera. In the similar way, all the three concentrations of the antigens of *C. eragrostidis* showed higher absorbance values when tested with two different dilutions of the antisera of susceptible variety (TV-12) than when tested with two different dilutions of the antisera of resistant variety (TV-25). The reciprocal test of this combination also showed higher absorbance values in case of the antigens of susceptible variety (TV-12) tested with antisera of *C. eragrostidis* than in case of the antigens of resistant variety (TV-25) tested with the antisera of *C. eragrostidis*. These clearly indicated the presence of cross-reactivity was maximum between *C. eragrostidis* and susceptible variety (TV-12) than the other combinations of antigens and antisera.

Chakraborty and Saha (1994) also detected presence of CRA between *Bipolaris carbonum* and susceptible tea varieties in indirect ELISA. They used polyclonal antibodies and goat-antirabbit IgG conjugate. In semipurified mycelial preparation of concentrations 5-25 µg/ml were cross reacted with antisera dilution of 1/125 and 1/250. Antigenic preparations of *B. carbonum* (isolate BC 1) exhibited higher absorbance value in cross reaction with antiserum of susceptible tea variety than the reaction with antiserum of resistant variety.

Alba and DeVay (1985) detected CRA in crude as well as in purified preparations of mycelia of *Phytophthora infestans* race 4 and race 12.3.47 with antisera of potatoes (cv. King Edward and cv. Peutland Dell) by using indirect ELISA. Mohan (1988) detected homologous soluble antigens at protein concentration of 2 µg/ml using antisera of *Phytophthora fragariae* in indirect ELISA.

Chakraborty *et al.* (1996) detected CRA in semipurified mycelial preparations at concentrations ranging from 5-25 µg/ml with antiserum dilution of 1/125. Antigenic preparations from Glomerella cingulata exhibited higher absorbance value (>2) when cross reacted with antiserum of susceptible variety than resistant one.

In the present study, polyclonal antibodies (raised in rabbits) indirectly labeled with fluorescein isothiocyanate conjugate (conjugated with antirabbbit globulin-specific goat antiserum) were used to locate CRA in the leaf sections of tea and mycelial cells of the fungal pathogens. When the leaf sections were treated with the antisera of respective leaf varieties i.e. with homologous antisera indirectly labeled with fluorescein isothiocyanate (FITC), bright fluorescence was observed. Fluorescence was observed in the epidermal regions, mesophyll tissues and xylem elements of the leaf. Leaf sections in heterologous treatments (i.e. when the leaf sections were treated with antisera of the two pathogens and then reacted with FITC) showed comparatively more fluorescence in susceptible varieties than the resistant one. The CRA observed in heterologous reactions, were mainly concentrated around the epidermal cells. In homologous treatments of the fungal mycelia, intense fluorescence was observed around the hyphae and conidia. In cross-reactions, when the fungal mycelia were treated with antisera of susceptible varieties indirectly labeled with FITC, fluorescence was observed in the hyphal tips and conidia but the fluorescence was not so strong when the mycelia were treated with the antisera of resistant varieties and FITC.

In indirect immunofluorescence test, strong fluorescence was observed in the hyphae and in the conidia of both the pathogens *B. theobromae* and *C. eragrostidis* in both homologous and heterologous treatments. Intense fluorescence was also found in the regions of epidermal cells and mesophyll tissues of the leaf sections of susceptible varieties treated with homologous and heterologous antisera indirectly labeled with FITC-antirabbit IgG conjugate indicating the tissue and cellular location of CRA. Similar tissue and cellular location of CRA in cross-sections of cotton roots (DeVay *et al.*, 1981a), potato leaves (DeVay *et al.*, 1981b) and tea leaves (Chakraborty and Saha, 1994; Chakraborty *et al.*, 1995, 1997) using fluorescein isothiocyanate (FITC) labeled antibodies have been reported.

Chakraborty and Saha (1994) showed locations of CRA in tea leaf sections using antibodies (raised against *B. carbonum*) indirectly labeled with FITC. CRA between TV-18 and fungal cells (*B. carbonum*) were mainly present in hyphal tips and in patch like areas on conidia, on mycelium and mainly around epidermal cells and mesophyll tissues of leaves. Cross reactions of young cotton (Acala-2) roots

inoculated with antiserum to *Fusarium oxysporum* f. sp. *vasinfectum* and stained with FITC-conjugated, antirabbit globulin-specific goat antiserum exhibited strong fluorescence at the epidermal and cortical cells and the endodermis and xylem tissues indicating a general distribution of the CRA determinants in roots (DeVay *et al.*, 1981a). DeVay *et al.* (1981b) also showed the interaction of *Phytophthora infestans* and potato plants.

The onset of systemic acquired resistance (SAR) correlated with the systemic induction of genes, especially genes encoding pathogenesis-related proteins (Lamb *et al.*, 1989; Ryals *et al.*, 1996). The basic concept of disease development requires a susceptible host plant, a virulent pathogen and a suitable environment (Agrios, 1997). It may be assumed that a change in any of these three factors can result in less or no disease. Changes in the physical environment can often have a profound effect on the physiology of the plants as it adapts to the change (Thomashow, 1999) Plant resistance is usually of the passive type; most plants are non-host for most pathogenic fungi. However, when plants are infected by fungal parasites, a number of situations are encountered ranging from very weak to no defense to very intense or active defense ultimately leading to resistance (Klarzynski and Fritig, 2001). Natural plant defence reactions gained high attention both in 'reasoned agriculture' and fundamental research. These studies were focused on plant-pathogen recognition, signal transduction and induction of resistance (SAR). It has been demonstrated that the activation of resistance in plants is generally initiated by host recognition of elicitors directly or indirectly released from the invading pathogen. Despite various chemical composition, many elicitors of plant defence reactions share a signaling pathway that coordinates the plant defences (Ebel and Cosio, 1994; Ebel and Mithöfer, 1998). Plant defense primarily depends on some need based dynamic responses to attempted infection, mostly an inducible phenomenon, its qualitative and quantitative aspects being regulated by signals from the invading pathogens, from phytohormones, salicylic acid and jasmonic acid (Cohen *et al.*, 1993; Durner *et al.*, 1997; Howe *et al.*, 1996; McConn *et al.*, 1997).

It was evident from the results that out of the nine different chemicals and one plant extract only three (nickel chloride, salicylic acid and jasmonic acid) were

found effective in controlling the disease caused by *B. theobromae* and *C. eragrostidis*. The mean disease indexes/shoot in the twigs treated with nickel chloride, salicylic acid and jasmonic acid were less in comparison to untreated twigs (control) after 24, 48 and 72 hours of inoculation. Similarly, nickel chloride (10^{-3} M), salicylic acid (10^{-3} M) and commercially available jasmonic acid (10^{-3} M) were sprayed on the twigs of both susceptible and resistant tea varieties and then were inoculated with *B. theobromae* and *C. eragrostidis*. It was observed from the results that the disease occurrence was much reduced following application of nickel chloride, salicylic acid and jasmonic acid.

Several previous workers have also reported that certain chemicals are capable of inducing resistance in plants. Chakraborty and Purkayastha (1987) induced resistance by using sodium azide (100 μgml^{-1}) in the susceptible soybean cultivar (Soymax) against *Mcrophomina phaseolina*, a pathogen of charcoal rot disease. Gibberelic acid (100 μgml^{-1}) was used by Ghosal and Purkayastha (1987) and showed increased resistance to sheath rot disease in susceptible rice cultivar (Jaya). Similarly, cloxacillin was used for induction of resistance in susceptible soybean variety against *Colletotrichum dematium* var. *truncata* (Purkayastha and Banerjee, 1990).

Systemic action of triazole compound was reported in clonal tea plants (TES-34) which are highly susceptible to *Exobasidium vexans*, a pathogen causing blister blight in tea (Premkumar *et al.*, 1998). SAR was induced by exogenous application of salicylic acid or synthetic compounds such as CGA-245704 (a benzothiadiazole derivative) and CGA-41396 (2,6-dichloroisonicotinic acid) were used by Kessmann *et al.* (1994); Lawton *et al.* (1996) and Lyon *et al.* (1997). Mohr and Cahill (2001) pretreated soybean hypocotyls with norflurazone and inoculated with a compatible race of *Phytophthora sojae*. The treatment displayed pathogen restriction in *planta*.

Taguchi *et al.* (1998) showed PAL A mRNA peaked up after 6-8 hours in tobacco cultured cells following treatment with methyl jasmonate. They showed methyl jasmonate activated defense through increased PAL (phenyl alanine ammonia lyase) activity. Cohen *et al.* (1993) used jasmonic acid and its methyl

esters as inducer of resistance in potato and tomato plants against *Phytophthora infestans*. Kato *et al.* (1984) used probenazole as a systemic compound to induce systemic protection against *Pyricularia oryzae*, a pathogen of rice.

A number of defined chemicals and extracts have been proposed (by different authors) with resistance inducing activity includes various inorganic salts, silicon, oxalate, phosphate, 2-thiouracil, polyacrylic acid, L-lysine but according to Kessmann *et al.* (1994) those compounds could not fulfill the criteria for SAR inducers. They explained possibly those compounds resulted in a local necrosis, which trigger as SA-dependent pathway like that induced by pathogen infection.

From the results of our study it may be concluded that nickel chloride, salicylic acid and jasmonic acid have some role in inducing defense reactions in tea plants. Our findings are also supporting the works of the earlier workers

Role of phenolics in the disease resistance in plants is proved beyond doubt (Daniel, 1995). Accumulation of phenolic compounds following infection with plant pathogens have been reported by several authors (Mahadevan, 1991, Mandavia *et al.*, 1997). Vidhyasekaran (1988) considered phenolic compounds to play an important role in disease resistance. Phenolic compounds are known to impart resistance to fungal disease. There are reports of an increase of total phenols in response to tikka disease in groundnut and of chlorogenic acid, caffeic acid and catechol in response to damping off in groundnut (Mandavia *et al.*, 1997).

In our study also the concentrations of orthodihydroxy phenols and total phenols extracted from the leaves of both susceptible and resistant twigs were measured. The concentrations of orthodihydroxy phenol were always maximum in the leaves of resistant twigs in comparison to susceptible one in both inoculated (inoculated with *B. theobromae* or *C. eragrostidis*) and uninoculated plants when they were treated with nickel chloride, salicylic acid and jsmonic acid. But in comparison to untreated-inoculated twigs, the orthodihydroxy phenol contents were found more in the treated leaves of both resistant and susceptible twigs (both inoculated and uninoculated).

When total phenol contents were measured, the concentrations of total phenol were always maximum in the leaves of resistant twigs in comparison to

susceptible one in both inoculated (inoculated with *B. theobromae* or *C. eragrostidis*) and uninoculated plants when they were treated with nickel chloride, salicylic acid and jsmonic acid. But in comparison to untreated-inoculated twigs, the total phenol contents were found more in the treated leaves of both resistant and susceptible twigs (both inoculated and uninoculated).

From the above discussions, it may be concluded that jasmonic acid, salicylic acid and nickel chloride have the ability to induce systemic resistance in tea plants probably through phenyl propanoid pathway as evidenced by the significant changes in the phenolic compounds.

Chemical control continues to play an important role in the integrated control of tea diseases. In the present study, minimum inhibitory concentration (MIC) of bavistin was maximum among the six fungicides tested against *B. theobromae*. The MIC value of calixin was minimum among the six fungicides when tested against *C. eragrostidis*.

Introduction of systemic fungicides like pyracarbolid and tridemorph showed good performance against blister blight (Venkata Ram, 1974, 1975; Venkata Ram and Chandra Mouli, 1976). Presently, ergosterol biosynthesis inhibiting fungicides such as cyproconazole, bitertanol and hexaconazole were found effective against *Exobasidium vexans* causing blister blight of tea, even at very low concentration (Agnihothrudu and Chandra Mouli, 1990). Combination of chlorothalonil and benomyl was used to control anthracnose of tea in Japan (Horikawa, 1988). Benomyl and thiophanate were combined and used to control *Colletotrichum cammalliae* causing brown blight of tea. Benomyl and thiophanate also controlled white scab in China (Chen and Chen, 1990). Fungicides with diverse chemical nature were evaluated against blister blight disease and a few fungicides belonging to morpholine (tridemorph) and triazole (bitertanol, hexaconazole and propiconazole) groups were found promising. Recommendations were made to tea industry on the usage of tridemorph (Venkata Ram, 1974), bitertanol (Chandra Mouli, 1993), hexaconazole (Chandra Mouli, 1993; Premkumar and Muraleedharan, 1997) and propiconazole (Premkumar, 1997). The cost effectiveness of various schedules has also been reported (Premkumar and Baby, 1996).

Indiscriminate use of fungicides is not only harmful to human beings but adversely affect the microbial population present in the ecosystem. A number of plant species have been reported to possess some natural substances in their leaves and bulb which were toxic to many fungi causing plant diseases (Biswas *et al.*, 1995).

In the present study, among the 21 plant extracts (both aqueous and ethanol) tested against *B. theobromae*, aqueous extract of *Melia dubia* showed significant antifungal activity by inhibiting spore germination of *B. theobromae*. Aqueous bulb extracts of *Allium cepa* and aqueous leaf extract of *Clerodendrum viscosum* also inhibited germination of spores of *B. theobromae*. Ethanol extract of M. dubia leaves and *Azadirachta indica* were also effective in controlling germination of *B. theobromae* significantly. Spore germination of the other pathogen *C. eragrostidis* was also inhibited by the aqueous bulb extract of *A. sativum*, *Cascabela thevatea*, *Dryopteris filix-mas* and *Embelica officinalis*. Ethanol extract of *A. sativum* bulb and *Polyalthia longifolia* leaves were also effective significantly in controlling germination of spores of *C. eragrostidis*. On the basis of the antifungal activity *M. dubia* leaf extracts and *A. sativum* bulb extract were selected for on the 'chromatogram inhibition assay' (TLC plate bioassay) respectively against *B. theobromae* and *C. eragrostidis*. The inhibition zone on the chromatogram was found at R_f 0.8 against *B. theobromae* when *M. dubia* leaf extract was used for antifungal activity. Similarly, at R_f 0.98 inhibition zone was formed against *C. eragrostidis* when aqueous bulb extract of *A. sativum* was used for antifungal activity. Our results are in conformity with the previous workers.

Several investigators evaluated crude extracts prepared from the plants collected either randomly or based on known ethnomedical use. Such studies have focused on screening plant materials for antimicrobial (Al-Shamma and Mitscher, 1979; Khatibi *et al.*, 1989; Navarro *et al.*, 1996; Rao, 1996), anthelmitic (Naqvi *et al.*, 1991), antiviral (Vlietinck *et al.*, 1995), cytotoxicity and muagenicity (Alkofahi *et al.*, 1996, 1997), molluscidal activity (Nick *et al.*, 1995), as well as for general pharmacological effects (Nick *et al.*, 1995). Biological evaluations have also been conducted on plants from different regions including India (Naqvi *et al.*, 1991), Jordan (Alkofahi *et al.*, 1996, 1997), Kenya (Githinji and Kokwaro, 1993), New Zealand (Bloor,

1995), Pakistan (Rizvi *et al.*, 1987), Papua New Guinea (Rao, 1996), Saudi Arabia (Khatibi *et al.*, 1989) and Somalia (Samuelsson *et al.*, 1992). The essential oils of *Origanum syriacum* exhibited a strong antifungal actions against *Fusarium oxysporum*, *Aspergillus niger* and *Penicillium* spp (Daouk *et al.*, 1995).

Chouksey and Srivastava (2001) screened the compound I from *Terminalia arjuna* which has antifungal activity against the fungi *Aspergillus niger*, *Candida albicans* and *Bacillus oryzae* at 25 and 50 ppm. The extract of mimosa bark and gullnat powder inhibited the development of *Alternaria alternata*, having inhibition zones of 21 and 15 mm, respectively. However, they were effective against *Penicillium italicum*, *Fusarium equiseti* and *Candida albicans* as reported by Digrak *et al.* (1999). Among the 11 plants of Mimoaceae from Pakistan were tested for their antimicrobial activity, the methanol extract of *Acacia nilotica* was found active against fungi (Ali *et al.*, 2001). The leaf extract of *Clerodendrum viscossum* completely checked the radial growth of the test fungus, *Curvularia lunata*. The leaf extract (1:10 dilution was the most successful for the inhibition of the test fungus in terms of its growth (Parimelazhagan and Francis, 1999).

Several authors have reported antifungal activity in different crops (Mittal *et al.*, 2002; Yasmin and Saxena, 1990; Sharma *et al.*, 2002; Appleton and Tansey, 1975; Barone and Tansey, 1977; Singh *et al.*, 1979; Yoshida *et al.*, 1987; Singh *et al.*, 1990; Reimers *et al.*, 1993; Singh *et al.*, 1995; Parimelazhagan and Francis, 1999; Ali *et al.*, 2001).

Biological control of plant diseases involves the use of one organism to control or eliminate a pathogenic organism. Biological control has attracted a great interest in plant pathology because the unnecessarily frequent use of pesticides and fungicides is increasingly causing concern in modern society in terms of human toxicity and hazardous effects on natural environments (Goto, 1990). Search for effective biocontrol agents for the management of plant diseases has been intensified in recent years to reduce the dependence on ecologically hazardous chemicals (Sharma *et al.*, 2001). Several microorganisms with high activity have been identified (Tronsmo and Raa, 1977; Janisiewicz, 1988; Peng and Sutton, 1991; Droby *et al.*, 1992). Biological control has been reported only in very few cases of tea diseases,

in comparison to pests. Application of beneficial microorganisms has been attempted in the control of tea root diseases. *Trichoderma viride* Pers. & Fr. and *T harzianum* Pers. & Fr. showed inhibitory activity against *Poria hypobrunnea* (Barua *et al.*, 1989). Horikawa (1988) isolated *Streptomyces roseosporus* from the gray blight spots on tea leaves which parasitized *P. longiseta* spores. After three days of inoculation, the conidia of *P. longiseta* and *P. theae* were destroyed by *S. roseosporus*.

Antagonistic nature of several species of the genus *Trichoderma* have been studied (Papavizas, 1985; Chet, 1987). Researches on *T. harzianum* as a biocontrol agent also showed differential antagonistic potential among isolates (Maity and Sen, 1985; Biswas, 1999). Eight isolates of *T. harzianum* Rifai isolated from soils of different betelvine plantations of West Bengal were investigated against four major fungal pathogens (*Phytophthora parasitica*, *Sclerotium rolfsii*, *Rhizoctonia soiani* and *Colletotrichum capsici*) of betelvine. (D'Souza *et al.*, 2001).

In the present study, *Gliocladium virens* (isolate II) inhibited *B. theobromae* significantly in dual culture technique and poisoned food technique. In dual culture technique *Gliocladium virens* (isolate II) directly inhibited the pathogen but in case of poisoned food technique cell-free culture filtrate showed the inhibition. It clearly showed that the inhibiting activity lies in the extracellular fluid. Similarly, *Trichoderma harzianum* showed significant inhibition of *C. eragrostidis* in both the tests (Dual culture technique and Poisoned food technique) performed. Cell free culture filtrates have been used to demonstrate the rate of antibiosism a mechanism of biological control (Khara and Hadwan, 1990; Tu, 1992; Naik and Sen, 1992). Antibiotic substances such as cell free culture filtrates have been emphasized (Robinson, 1969; Fravel, 1988). As going for biocontrol methods is normally cost effective compared to chemical control of plant diseases, Shanmugam and Sukunara Varma (1999) clearly established the efficacy of the antagonists *Aspergillus niger*, *A. fumigatus*, *A. flavus* and *Trichoderma viride* in inhibiting the rhizome rot pathogen.

Despite the progress in biocontrol research biocontrol products is often less consistent than chemical control. However a greater understanding of the ecology of the organisms involved, as well as the epidemiology of the system, will

help us to develop ecologically rational approaches to disease management. More efficient and effective ways of growing and formulating biocontrol organisms are needed in many cases in order to make biocontrol economically viable (Fravel, 1999).

Potential plant extracts (*M. dubia* and *A. sativum*), fungicide (Calixin) and biocontrol fungi (*T. harzianum* and *G. virens* – isolate II) reduced disease significantly in the nursery tea plants of the susceptible varieties (TV-11 and TV-12) against *B. theobromae* and *C. eragrostidis*

Pretorius *et al.* (2002) reported the *In vivo* control of *Mycosphaerella pinodes* on pea leaves by a crude bulb extract of *Eucomis autumnalis*. The crude extract prevented *M. pinodes* spore infection of the leaves when the leaves were inoculated with spores both before or after treatment with the extract, confirming complete inhibition of spore germination. The crude *E. autumnalis* extract showed no phytotoxic reaction on the leaves even at the highest concentration applied. Premkumar *et al.* (1998) studied the systemic activity and field performance of triazole fungicides against blister blight pathogen of tea and established the antisporulant actions of the triazole fungicides.

The present study has confirmed and also extended some of the findings of early workers. *C. eragrostidis* have been reported as a new pathogen of tea. This study also reveals certain new facts of fundamental importance. The significance of antigenic relationship with regard to compatible interaction between the hosts and the pathogens (*C.sinensis-B.theobromae* and *C.sinensis-C.eragrostidis*) has been demonstrated by various serological techniques. The pathogenicity of different tea varieties have been done in three different ways and also correlated with that of indirect ELISA. Major cross-reactive antigens were located in the cells of tea and pathogens by staining with fluorescence isothiocyanate conjugated with antibodies. Resistance was induced in susceptible tea plants by the application of some chemicals. However, more works need to be done before formulating a definite defense inducers although this investigation has provided an insight into the control of diseases caused by pathogens. Suitable control measures may be designed from the present study at least for the nursery tea plants.