5. DISCUSSION

Host-parasite interaction is one of the most complex biological processes occurring in nature, both at the cellular and sub-cellular level. This interaction basically is antagonistic. 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 therefore have highly well-developed defense mechanisms which enable them to defend themselves against penetration, intracellular growth and the development of parasites in their tissues. The basis for this resistance against microbial attack is a plants' chemical defense consisting 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.

In the present investigation, at the onset, pathogenicity test of P. theae was carried out on detached leaves, cut shoots and whole plants of ten tea varieties released by Tocklai Experimental Station, Jorhat, Assam. Detached leaves as well as cut shoots gave satisfactory results which were in good agreement with the whole plant tests. P. theae was most virulent on TV-23 and least on CP-1. The results of pathogenicity tests performed on detached leaves of tea varieties corresponded to the same degree of resistance as determined by cut shoot methods. In order to detect the resistance of tea plant to grey blight disease caused by Pestalotia longiseta Yanase and Takeda (1987) also used cut shoot method. The texture and physical structure of the tea leaves no doubt play an important role in the initial stage of disease production. Kabir et al. (1991) studied the epicuticular wax content and its distribution on the tea leaf surface by electron microscopy. They correlated the epicuticular wax content on the leaf surface of the clones with their tolerance to drought and other stress conditions. This external layer provides a micro-habitat for a variety of parasitic and saprophytic organisms and act as a barrier to fungal pathogens. Ando and Hamaya (1986) reported that the anthracnose

fungus, Gloeosporium theae-sinensis was able to infect the plant through trichomes of young leaves. They also reported that in many cases, the pathogen was inhibited from gaining entrance by a callosity which was produced by swelling of the trichome cell wall inward in a way that enveloped and preceded the invading hyphae.

Fungal plant pathogens invade host plant cells with variety of specialized infection structures of which germination of spore is one of the most important steps in diease establishment. In view of this effect, some important factors on spore germination of P. theae has been investigated. Low concentration of spores (4.8×10^4 spores/ml), 24h incubation period and pH 6.5 from 15-20 day old culture of P. theae were optimum for spore germination. Maximum mycelial growth occurred at an incubation period of 20 days, pH 6.5 and with sucrose, ammonium nitrate and casein as carbon, inorganic nitrogen and organic nitrogen sources, respectively.

In 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. Some enzymatic proteins are also produced in penetrated cells by the pathogens themselves. Thus qualitative and quantitative changes in proteins are related to both plant and pathogen (Uritani, 1971). In the present investigation change in the protein content was noted in the P. theae 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 (Staples and Stalmann, 1964). Similar findings were reported by other workers (Tomiyama,

1966; Daly, 1972; Ouchi et al., 1974). They suggested that in case of compatible combination, changes in protein configuration in the host may induce the host's accessibility to the pathogen which is related to susceptibility. The greater accumulation of protein in susceptible host after inoculation could 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. Changes in protein patterns in barley leaves after infection with Erysiphe graminis f. sp. hordei was detected by polyacrylamide gel electrophoretic study, but no change could be detected in total buffer soluble protein content of mildew infected barley leaves in comparison with healthy control (Johnson et al., 1966). In the present investigation protein pattern of healthy and P. theae inoculated leaves of resistant (CP-1) and susceptible (TV-23 and TV-18) varieties were evaluated by SDS-Polyacrylamide gel electrophoresis. Inoculated leaves of CP-1 exhibited one additional high molecular weight (173.9 kD) protein band, whereas two additional protein bands (at 82.4 and 48.2 kD) and three additional bands (at 173.9, 114.1 and 97 kD) were noticed in case of inoculated leaves of TV-18 and TV-23 respectively.

This is in conformity with the results of other workers. Uritani and Stahman (1961) reported that sweet potato infected by Ceratocystis fimbriata produced new proteins in both resistant and susceptible varieties. Similarly, Sako and Stahman (1972) also detected 5 new isozyme bands viz., acetyl esterase, acid phosphatase, malate dehydrogenase, succinate dehydrogenase and peroxidase in the susceptible line of barley after infection by Erysiphe graminis f. sp. hordei.

The interaction between Cladosporium fulvum and tomato has been used as a model system by Joosten and Dewit (1988) to study the accumulation of host-, pathogen-, and interaction-specific proteins in leaf apoplastic fluids from compatible and incompatible combination. Electrophoresis of apoplastic fluids under low pH and non-denaturing conditions revealed one protein which was present in all compatible interactions studied, but not in incompatible interactions nor in uninoculated controls. Purification of this protein from the apoplastic fluids from several compatible interactions was achieved by ion-exchange chromatography on CM-Sephadex followed by chromato-focussing. The purified protein migrated on SDS-polyacrylamide gels as one band with an estimated molecular mass of 14 kD. Antibodies obtained by injecting the purified protein, bound to nitrocellulose, into rabbits had high affinity for the protein on western blots and little or no interactions with other protein bands. In compatible C. fulvum-tomato interactions the protein could be detected in apoplastic fluid 8 days after inoculation. The protein was not detected in the mycelium or culture filtrates obtained from C. fulvum grown in culture, nor in apoplastic fluids from tomato leaves inoculated with the tomato strain of Phytophthora infestans. Furthermore, it was not detectable in old tomato leaves.

Phenolic compounds accumulate in numerous plant species following infection with plant pathogens (Friend, 1977; Bazzalo et al., 1985; Baker et al., 1989; Mahadevan, 1991). There is often a greater increase in phenolic biosynthesis in resistant host species than in susceptible host 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. Polyphenols are major constituents of tea leaves and their involvement in the resistance mechanism has been described by Chakraborty et al. (1995a).

In the present study, the levels of phenolics in leaves of resistant and susceptible tea varieties were estimated after 48h of inoculation with P. theae. Host responses could be differentiated by changes in content of phenolic compounds. Both the total phenol and orthodihydroxy phenol content increased in resistant varieties (CP-1 and TV-27) but decreased in susceptible varieties (TV-23, Teen Ali 17/1/54 and TV-25) in comparison to their healthy controls.

Present results substantiate the findings of previous workers who have also recorded similar results. Sridhar and Ou (1974) reported differences in total phenolics accumulation in the interaction of Pyricularia oryzae with rice. Purushothaman (1974) also reported that the resistant rice cultivar synsthesized more phenol, when inoculated with Xanthomonas campestris pr. oryzae, than the susceptible one. Hammerschmidt and Nicholson (1977) demonstrated a clear difference between resistant and susceptible interactions of maize to Colletotrichum graminicola based on accumulation of phenols. However, no differences were found in the phenolic content in the interaction of Helminthosporium maydis race T with N and T cytoplasms of a single maize genotype (Macri et al., 1974). On the other hand, resistant cotton cultivar contained fairly high amount of total as well as orthodihydroxy phenol than susceptible cultivar. After inoculation with Xanthomonas campestris pr. malvacearum, total and orthodihydroxy phenol increased in resistant interaction, whereas, it decreased in susceptible interaction (Borkar and Verma, 1991). Greater accumulation of orthodihydroxy phenol in resistant interaction of P. theae and tea varieties indicated that this may play a role in disease resistance mechanism. Eswaran (1971) has also considered that 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 are involved in phytoalexin

accumulation (Mansfield et al., 1974; Langcake and Pryce, 1976; Langcake and McCarthy, 1979; Holliday et al., 1981; Pierce and Ersenberg, 1987; Baker et al., 1989).

In the present investigation, diffusates were collected from the adaxial surface of the leaves of resistant (CP-1 and TV-27) and susceptible (TV-23, Teen Ali 17/1/54, TV-18, TV-26 and TV-25) varieties after 48h of incubation following drop diffusate method and their biological activities were evaluated on spore germination and germ tube length of P. theae. The diffusates collected from the leaves of resistant tea varieties were more fungitoxic than those from the susceptible varieties. Fungitoxicity of leaf diffusates has been implicated in natural defense mechanism of plants against attack by fungal pathogens in several instances (Purkayastha and Ray, 1975; Mukhopadhyay and Purkayastha, 1981; Sinha and Hait, 1982; Purkayastha and Ghosh, 1983; Purkayastha et al., 1983; Hait and Sinha, 1986; Chakraborty and Saha, 1989). Although the drop diffusate method has often been criticised as biologically unnatural, the advantage it has over other techniques is that a relatively pure phytoalexin preparation can be obtained without maceration of the plant tissues. However, unstable phytoalexins might decompose during isolation. On the other hand, diffusates do not give any indication of the phytoalexin concentrations within inoculated tissues. Moreover, phytoalexins which are not diffusible into the inoculum droplet also can not be detected by this method.

Hence, facilitated diffusion technique as suggested by Keen (1978) was followed to detect the antifungal substance (phytoalexin) from tea leaves inoculated with P. theae. On the 'Chromatogram-Inhibition-Assay', two compounds I and II at Rf 0.63 and 0.58 respectively were found to be fungitoxic against B. carbonum. However compound I could be detected mainly from the healthy tea leaf extracts of all the four varieties tested (TV-23, Teen Ali 17/1/54, TV-27 and

CP-1) which exhibited prominent inhibition zone on TLC plate and showed the highest fungitoxic activity in the spore germination assay. No such fungitoxic activity was evident on TLC plate in leaf extracts from susceptible variety (TV-23 and Teen Ali 17/1/54) inoculated with P. theae, but traces of the inhibition zone was evident in resistant varieties (CP-1 and TV-27) even after 48h of inoculation. Rf value and colour reaction of this antifungal compound corresponded with catechin. Catechins are flavon-3-ols with two hydroxyl groups in the side ring. These include gallic acid esters with the acid moiety attached to the hydroxyl groups. Kawamura and Takeo (1989) showed the antimicrobial activity of tea catechin towards Streptococcus mutans. Wang (1991) have reported the presence of four forms of catechins such as, (-) epicatechin (EC), (-) epicatechin gallate (ECG), (-) epigallocatechin (EGC) and (-) epigallocatechingallate (EGCG).

The compound II showed positive colour reaction of phenolics with the chromogenic sprays on TLC plates at Rf 0.58 and exhibited prominent inhibition zone in TLC plate bioassay as well as inhibited markedly the spore germination of P. theae. This compound was identical to an authentic pyrocatechol as determined by thin layer chromatography and UV-spectrophotometry. 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 dioxygenase) 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 48h of inoculation. Accumulation of catechol in rsistant varieties increased significantly (477-612 µg/g fresh wt.) after 48h of inoculation with P. theae. Concentration of this compound in healthy leaf tissue is very low (60-96 µg/g fresh wt.). Accumulation of pyrocatechol in susceptible variety was not greater than the resistant ones even though complete breakdown of

catechin was detected in the former case. Increased level of pyrocatechol may be associated with the differential host responses to disease production.

Two phenolic antifungal compounds in leaf diffusates and water extracts of maize leaves after inoculation with Helminthosporium turcicum have been demonstrated by Lim et al. (1970). However, Hammerschmidt and Nicholson (1977) reported accumulation of three phenolic compounds in maize leaves after inoculation with Colletotrichum graminicola which increased earlier in the resistant interaction than in the susceptible one. Two of these three compounds inhibited the germination of C. graminicola spores in vitro. Role of phenolic metabolism in the resistance of maize to Helminthosporium carbonum have been investigated by Werder and Kern (1985). Accumulation of antifungal compounds in tea leaf tissue infected with Bipolaris carbonum has also been discussed by Chakraborty and Saha (1994a). Resistant varieties accumulated more pyrocatechol than the susceptible varieties 2 days after inoculation with B. carbonum.

Although less is known with certainty about the specific recognitional events that predicate 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 plant that dictate the ultimate accumulation of antifungal compound at the infection site (Albersheim and Anderson-Prouty, 1975; Keen and Bruegger, 1977). Cell recognition has been defined as the initial event of cell-cell communication which elicits morphological, physiological and biochemical response (Clarke and Knox, 1978). Surface molecules of eukaryote cells have been involved in cell-cell recognition and/or adhesion and as receptors for various effects (Snary and Hudson, 1979). 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; Beissmann, 1992; Ransom

et al., 1992). In this study, P. theae was found to elicit greater amount of antifungal compound in the resistant varieties than the susceptible ones. Cell-wall was isolated from mycelia of P. theae and their role in host response and elicitation of antifungal compound were determined. Finally the chemical nature of mycelial wall extract was determined by SDS-PAGE and ConA-FITC binding which confirmed its nature as glycoprotein. Six glycoprotein bands of molecular weights 113, 105, 46, 39, 28 and 26 kD were detected in the extract. When these mycelial wall extracts were mounted on the adaxial surface of tea leaves of resistant variety (CP-1 and TV-27) they elicited a similar response to that shown by the spore suspension. In subsequent experiments mycelial wall extract also elicited production of antifungal compound in tea leaves. Keen and Legrand (1980) isolated two low molecular weights (14 and 34 kD) surface glycoprotein from the cell wall of Phytophthora megasperma f.sp. glycinea which were involved in phytoalexin elicitation. Ricci et al. (1992) demonstrated that P. parasitica could be differentiated on the basis of their ability to produce a proteinaceous elicitor active on tobacco and of their pathogenicity to tobacco and there is relationship between these two properties. Isolation of mycelial wall from Drechslera longistrata and its characterization as polysaccharide was also reported by Kumar et al. (1993) which elicited glyceollin production. Results of this investigation along with those of other workers clearly demonstrate that the cell walls of P. theae contain glycoprotein which have a role to play in the initial recognition leading to the activation of the defense mechanisms by accumulating the antifungal compounds.

The host-pathogen interaction is also influenced by the epiphytic microorganisms occurring on the leaf surfaces. In order to get a thorough knowledge of the microbial communities occurring on the tea leaf surfaces further studies were conducted on the phylloplane microorganisms of tea and their interaction with P. theae. At the onset, microorganisms were isolated from tea phyllosphere of eighteen tea estates of Dooars and Darjeeling hill. These microorganisms

included mainly fungi and bacteria. The isolated microorganisms which could be identified are Acremonium fusidioides, Alternaria alternata, Aspergillus candidus, A. carneus, A. flavus, A. fumigatus, A. melleus, A. nidulans, A. niger, A. sydowii, A. terreus, A. ustus, A. versicolor, A. wentii, Bipolaris carbonum, Cochliobolus sativus, Colletotrichum gloeosporioides, Curvularia lunata, Fusarium avenaceum, F. chlamydosporum, F. equiseti, F. udum, Glomerella cingulata, Penicillium oxalicum, Pestalotiopsis theae, Phoma exigua, Rhizopus stolonifer, Trichoderma viride, Bacillus cereus, B. pumilus, Bacillus sp., Micrococcus sp., M. luteus and Coryneform bacterium. Filamentous fungi and bacteria have been recorded to form resident populations on leaves (Blakeman and Fokkema, 1982). Bacterial population so far reported includes Bacillus, Corynebacterium, Erwinia, Flavobacterium, Pseudomonas and Xanthomonas. In addition to the above mentioned saprophytic bacteria, pathogenic bacteria such as, Erwinia amylovora, E. cartovora, Pseudomonas glycinea and P. syringae can also live on the foliar surface (Blakeman and Brodie, 1976). Early in the growing season bacteria have been found to be predominant on leaves. Throughout the growing season spores of filamentous fungi such as Alternaria, Cladosporium and Epicoccum can land on leaves but they germinate to form colonies only towards end of the season when leaf senescence starts (Blakeman, 1981). The original sequence in colonization on the phylloplane is a function of available inocula, the environment and host phenology, the pattern of which on individual leaves is localized and heterogeneous (Andrews, 1992).

In vitro interaction studies between isolated microorganisms and the pathogen (P. theae) resulted into four different types of reaction such as homogeneous, overgrowth, cessation at line of contact and aversion. Among the tested bacteria Bacillus pumilus (359389) and Micrococcus sp. (359384) which exhibited strong antagonistic reaction against P. theae were selected for further study. Such antagonistic behaviour of other bacteria have also been reported. Bacillus subtilis isolated from soybean showed antagonistic reaction in dual culture

against 26 fungi commonly associated with soybean seeds (Cubeta et al., 1985). Erwinia hrbicola and Bacillus cereus were found to be antagonistic to Ulocladium botrytis, the causal agent of tomato leaf spot (Zaher et al., 1985). There is evidence that seed bacterization with Bacillus pumilus protected sheath blight disease of rice in the glass house trials (Rosales et al., 1993). Five isolates of Bacillus sp. showed antagonistic reaction against Botrytis cinerea and Pythium mamillatum (Walker et al., 1994). On the basis of in vitro and in vivo tests, E. herbicola was selected as a potential antagonist against Leptosphaeria maculans causing black leg of Canola (Chakraborty et al., 1994a). Pseudomonas sp. when applied to tea plants (TV-18) prior to inoculation with Glomerella cingulata, it reduced disease (brown blight) development (Chakraborty et al., 1994c, 1995b).

Selected microorganisms were tested for their effect on the development of grey blight disease. For in vivo tests, aqueous cell suspension, bacterial cell-free culture filtrates and washed cells were used as foliar sprays. The aqueous cell suspensions were found to be most effective in reducing lesion production than the other treatments. Cell-free culture filtrate of B. pumilus was more effective than its washed cell. However, cell-free culture filtrate of Micrococcus sp. was not as effective as its washed cells. Records of growth inhibition of other pathogen in vitro and disease reduction by the culture filtrates of antagonistic microorganisms substantiate the present findings. Application of sterilized culture filtrate of B. cereus on alfalfa seedling were found to be effective in protecting from damping-off of seedlings (Handelsman et al., 1990). A cell-free culture filtrate of Bacillus subtilis also significantly reduced disease incidence and severity on alfalfa seedling, although treatment of seedlings with washed cell suspension had no influence on disease development (Douville and Boland, 1992). Suspensions from washed or non-washed cells of Saccharomyces cerevisiae or the filtrates when applied on the maize leaves prior to inoculation with Colletotrichum graminicola reduced the development of anthracnose (Da Silva and Pascholati, 1992). Culture filtrate of Streptomyces pulcher or S.

canescens significantly inhibited spore germination, mycelial growth and sporulation of Fusarium oxysporum f. sp. lycopersici, Verticillium albo-atrum and Alternaria solani. Culture filtrates were also effective in reducing disease severity in vivo (El-Abyad et al., 1993).

Different ratios of antagonists and P. theae on the tea leaf surface (TV-23) also influenced the activity of the antagonists. The inhibitory effect of the two tested microorganisms increased with increase in their concentration. At the highest ratio of antagonists and pathogen (50:1) disease development could not be noticed. Similar results have also been reported by Sekhawat and Chakraborty (1977). They reported 100% control of bacterial leaf spot of chilli by applying antagonistic bacteria mixed with the pathogen (Xanthomonas vesicatoria) in the ratio of 64:1. It has also been reported by Saikia and Chowdhury (1993) that Erwinia herbicola controlled Xanthomonas oryzae pr. oryzae on rice most effectively even at lowest ratio 1:1 and registered more than 90% reduction in disease development.

The antagonistic interaction between the phylloplane microflora and pathogen may be brought about by different mechanisms including parasitism, nutrient competition, antibiotic production or interaction with the host (Blakeman and Fokkema, 1982). Some of the saprophytes are hyperparasites and they may affect plant pathogens in two main ways - either by penetration of fungal tissues and production of metabolic substances which result in destruction by lysis of spores, or hyphae; or by displacement of tissues of the pathogen within pustules or by the formation of crusts of mycelium which overlay fruiting structures (Barnett, 1963). One of the hyperparasites, Ampelomyces telliopsis have been reported to infect and kill mildew pathogens (Sphaerotheca fulliginea and Erysiphe cichoracearum) that attack cucumbers (Sutton and Peng, 1993).

In nature, intraspecific or interspecific competition is known to be one of the most important factors determining the population density (Schroth and Hancock, 1981). Water films on leaf surfaces often lead to low levels of available nutrients. However, because of the more favourable surface to volume ratio bacteria are able to take up nutrients even from the dilute solution of an infection droplet more rapidly and in greater quantity than the germ tubes of fungal pathogens. The exogenous nutrients available to the pathogen are thus reduced and some of the endogenous nutrient reserves leaked from the germinating spores are also preferentially absorbed by the competing epiphytes surround in the spores (Brodie and Blakeman, 1975). Under these conditions, spores of fungi such as Botrytis cinerea or Phoma betae either do not germinate or germinate poorly, failing to give rise to infections.

The most important mechanism of antagonistic reaction is perhaps that of production of antibiotics by such microorganisms which inhibit the growth of pathogens (Douville and Boland, 1992; Koomen and Jeffries, 1993; Hodges et al., 1993; Chakraborty et al.,1994a,c). In vivo activity of Erwinia herbicola against Fusarium culmorum have been attributed to antibiosis (Kempf and Wolf, 1989). They suggested that competition between F. culmorum and E. herbicola may also play a role in the antagonistic interaction. The possible activation of resistance mechanisms in coffee against Hemileia vastatrix have been reflected by the reduction in disease expression due to application of filtrates of S. cerevisiae (Martins et al., 1986).

Results of replacement culture media test confirmed that observed reduction of growth of P. theae in presence of antagonistic bacteria (B. pumilus and Micrococcus sp.) was not due to competition for nutrients but due to the presence of antifungal compound(s) in the culture filtrates. In order to determine whether the antagonistic bacteria secrete any antifungal compounds into the culture medium, the cell-free culture filtrates were bioassayed against P. theae.

Inhibition in spore germination of P. theae by the culture filtrates indicated that these contained some toxic metabolites which are fungitoxic and partly thermolabile as they lost some of their activity by heat sterilization.

Having established that B. pumilus and Micrococcus sp. secrete antifungal compounds into culture, further attempts were made for partial purification of these compounds. Cold sterilized cell-free culture filtrates obtained from those antagonistic bacteria were extracted with three different solvents - chloroform, diethyl ether and ethyl acetate and each extract was bioassayed separately against P. theae. Results revealed that the solubilities of these compounds from B. pumilus and Micrococcus sp. varied, former being more soluble in diethyl ether while the latter was found more soluble in ethyl acetate.

Presence of an ethyl acetate soluble partially thermolabile antifungal substance in the culture filtrate of Bacillus megaterium was reported by Purkayastha and Bhattacharyya (1982) which was antagonistic to Colletotrichum corchori causing anthracnose disease on jute. However, the culture filtrate extracts of Bacillus subtilis contained antifungal compounds that were soluble in ethanol, methanol, isopropanol and water above pH 7.5, but not soluble in ethyl acetate, ether, acetone or methylene chloride (McKeen et al., 1986).

Among the two antagonistic bacteria, B. pumilus was selected for further studies on the production of antifungal compound and its probable use as a biocontrol agent against P. theae. For partial purification, the B. pumilus was grown in a specific medium for 7 days, adsorbed with active carbon at pH 8.0, eluted with methanol and finally thin layer chromatography (TLC) was performed. Bioassay of the eluates from different zones revealed the antifungal compound to be present predominantly in fraction number 4 (Rf 0.39). Maximum UV-

absorption of this compound was found at 220 and 272 nm. When the crude extract from B. subtilis was developed in TLC, it separated into four occasionally active bands which inhibited M. fructicola. The bands with Rf values of 0.48 and 0.55 were ninhydrin positive while the ones at 0.60 and 0.67 were not (Mc Keen et al., 1986).

In the present investigation it has been established that B. pumilus is antagonistic to P. theae and the mechanism of antagonism has been shown to be by production of antifungal metabolite. This microorganism being a naturally occurring resident on the tea leaf surface has become adapted to survive and grow in this habitat. It is well known that if such organisms have been found to possess an effective antagonistic action against a pathogen, then their use for biocontrol purpose should be preferred to organisms from other habitats which may be equally antagonistic to the pathogen (Blakeman and Fokkema, 1982) . In this respect, B. pumilus seems to be a good choice as biocontrol agent.

Besides, instances where antibiotic production has been identified as a main cause of antagonism by a saprophyte, a cell-free culture filtrate or semi-purified antibiotics production may be used for biocontrol purpose as opposed to using a living inoculum. This procedure may be more advantageous if the antagonists fail to effectively colonize host surfaces. Keeping this in mind, the semi-purified extract from B. pumilus was sprayed on tea leaves prior to inoculation with P. theae. Lesion production on detached leaves or cut shoots as well as disease development on whole plants were effectively controlled by the application of semi-purified extract of B. pumilus.

It can be generalized from the aforesaid considerations that the final appearance of disease on a host is dependent on a multitude of factors. Detailed studies on grey blight disease and its causal agent, P. theae have shown that the pathogen is influenced by a number

of biochemical responses of the host as well as on its interaction with the phylloplane microorganisms present on the leaf surface. As an outcome of this study, a phyllosphere antagonist has been selected which could be used as a potential biocontrol agent against tea pathogens.

Biocontrol in the phyllosphere has not yet met with outstanding success so far, but the use of introduced antagonistic microorganism, for control of plant pathogens has attracted worldwide attention over the past few decades (Cook, 1993). After about three decades of phyllosphere research, the challenge still remains - one of converting a wealth of circumstantial evidence that microbial biocontrol operates in nature into effective and dependable strategies. Biocontrol by microbial antagonism will expand provided that effective formulations can be developed for the phyllosphere and that innovative approaches emerge for deploying endophytes as well as epiphytes.