

Literature-Review

Any plantation programme needs fresh healthy seedlings or clonal cuttings. The seedlings in nurseries face several disease problems. To control diseases of plants effectively, it is necessary to know about host-parasite interactions. Pathogenesis and disease resistance are closely related to each other because they treat host-parasite interactions from different points of view. Pathogenesis is related to compatible interactions while resistance related to incompatible ones. At the onset of the present study it was considered to review the works of the previous workers in a selective manner. The observations of the different workers in concord with the present line of investigations are being presented briefly in the following paragraphs, however, for convenience the observations have been divided into some groups or aspects. The different aspects of this review are:

- Diseases.
- Growth and physiology of the pathogens.
- Common antigenic relationship.
- Plant disease alteration by chemical treatment.
- Disease control by fungicides.
- Disease control by botanicals.
- Disease control by antagonistic organisms.

Diseases

Like any other cultivated crop, tea plants [*Camellia sinensis* (L.) O. Kuntze.] in northeast India are prone to a number of serious diseases (Fig.1 & 2). The fact that it is a perennial crop grown remote from its natural habitat over vast areas as a homogenous mass under varying soil and climatic conditions makes it a happy hunting ground for many fungi and other disease causing organisms. All the common and economically important diseases of tea with the exception of 'red rust' are incited by fungal pathogens. As early as in the sixties, Agnihothrudu (1964) listed 385 species of fungi on tea plants. After a thorough revision, Chen and Chen (1989) reported that a total of 507 fungi had been recorded on tea. Barua (1989) reported that out of 385 species of fungi occurring on tea all over the world, 190 were recorded in tea in northeast India alone (Fig. 2).

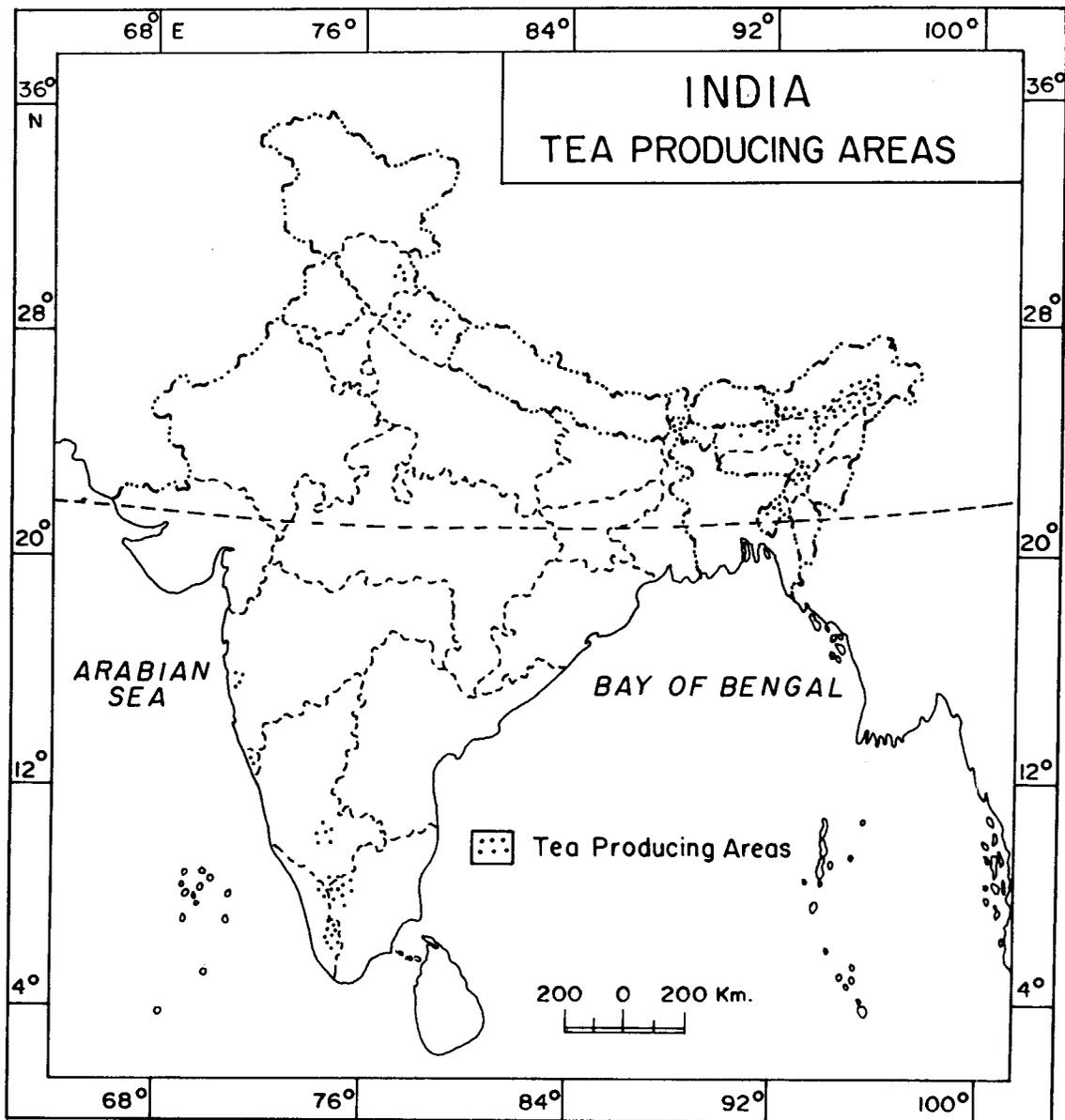


Fig.1

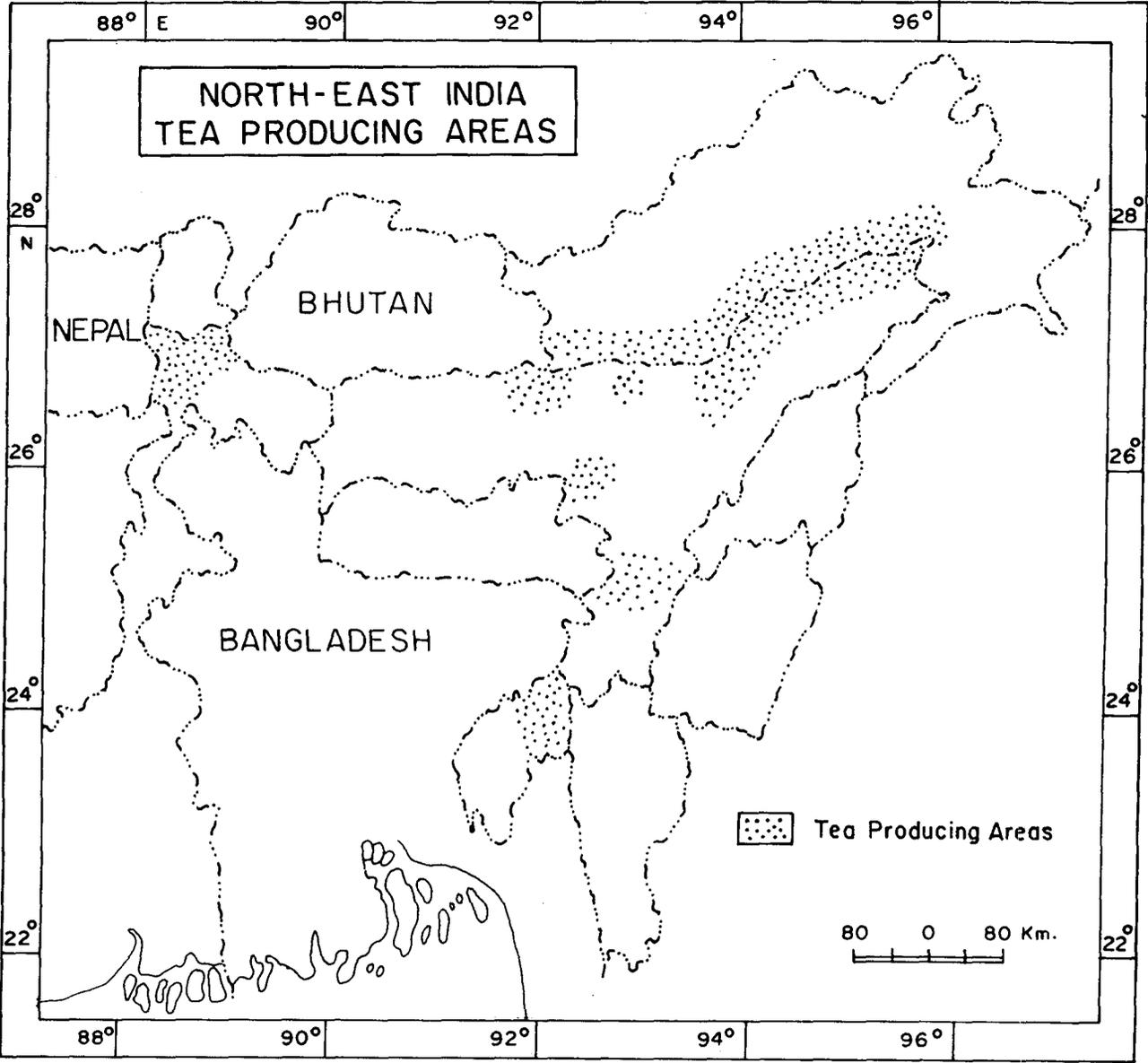


Fig. 2

Diplodia disease caused by *Botryodiplodia theobromae* Pat. is probably the commonest of all the fungi recorded on tea in northeast India. The pathogen can attack any part of the plant, young or old, only when the plant is debilitated by other causes. Although the disease may be produced in the leaves, roots and stems, the symptoms are of different types; in case of leaves and collar regions it is grayish-black to coal-black, hairy cushions, giving a shooty appearance. The infection is believed to take place through air borne spores. Practically all woody parts, dying roots and twigs show *Botryodiplodia* infection. Sarmah (1960) suggested that improving general health of the plants, by subsoil irrigation, manurial and cultural practices would greatly help in protecting the plants from diplodia root disease. Venkata Ram (1960) observed the predisposing factors like low starch reserves; high soil temperature and low soil moisture were essential as a requisite for fungal invasion to cause diplodia disease. Chandra Mouli (1988) reported that diplodia root disease (a secondary root disease of tea), caused by *B. theobromae* was very common and occurred both in north India as well as south India.

Beside *Botryodiplodia*, several other diseases are reported to affect the tea plants throughout the world. Arpon and Supachi (1980) reported that blister blight of tea caused by *Exobasidium vexans* Masee., was the most serious disease in many tea plantations on the mountains. Saha *et al.* (1980) observed that red rust of tea caused by *Cephaleuros parasiticus* Karst. is the most important and lone algal disease of tea. Smit and Devis (1989) reported about crown and root rot disease caused by *Macrophomina phaseolina* (MP) and *Neocosmospora vesinfecta* (NV) which occurred in rooibos tea throughout the main production areas. Tissue infected by MP was gray with numerous black sclerotia when broken open, and the branches were characteristically twisted. Tissue infected by NV was typically maroon to black with superficial orange to red perithecia. Disease developed on plants inoculated during summer when subjected to moisture stress, but not on plants inoculated during winter. Number of propagules recovered from the soil varied greatly from field to field with the height levels being 32 viable MP sclerotia and 315 visible ascospores per gram of soil.

Wang *et al.* (1990) described diseases of tea in 6 zones of Zhejiang province of Peoples Republic of China from 1985 to 1988. Symptoms and biology

of pathogens of 20 diseases were described. Among them, 5 of the 20 diseases caused by *Colletotrichum camelliae*, *Monochaetia camelliae*, *Pestalotia guepini*, *Phyllosticta theicola* and *Fusarium ventricosum* were distributed more widely and cause more severe damages and those caused by *Pestalotia algeriensis*.

Barthakur (1994) observed that among the common diseases of tea in the hills of Darjeeling, West Bengal, India blister blight caused by *Exobasidium vexans* was the most serious; root diseases caused by *Ustilina zonata*, *Fomes lamaoensis*, *Rosellina arcnata* and *Armillaria mellea* and stem diseases caused by *Tunstallia aculeate* and *Poria hypobrunnea* were also common in Darjeeling gardens but they were difficult to control.

Park (1995) reported that in May and June, 1992-1994 tea with white scab symptoms, i.e., numerous small, circular, reddish or yellowish brown spots on young tea leaves was observed in a plantation in Boseung, Chonnum Province, Korea Republic. At the late growth stages the center of the spot became light gray. The causal agent was identified as *Sphaceloma theae*. Similar symptoms occurred on leaves 5-6 days after inoculation with *S. theae*. Park *et al.* (1996) reported the occurrence of gray blight of tea in several tea plantations in Boseung, Chonnam Province, Korea Republic, during 1992-94 after harvesting and pruning of the second crop. Circular to irregularly shaped dark brown spots developed in concentric rings on leaves and black, dot-like acervuli formed in concentric rings on the lesions. The pruned twigs were blackened and killed and acervuli formed randomly on them. The causal fungus of gray blight was identified as *Pestalotiopsis longiseta*. Typical symptoms by *P. longiseta* appeared 11 days after inoculation.

Khodaparast and Hedjaroude (1996) reported that during 1991-1993, several tea plantations were surveyed in the north of Iran in order to determine the main fungal diseases of tea. The result of the survey and pathogenicity tests showed that *Botrytis* sp., *Glomerella cingulata*, *Fusarium solani*, *Botryodiplodia theobromae*, *Pestalotiopsis longiseta*, *P. natrassii*, *P. theae*, *Phyllosticta theacearum* and *Corticium rolfsii* were pathogens of tea.

Mouli (1997) gave a brief account on abiotic and biotic problems in the nurseries in India, and suggested several remedial measures. The diseases covered

included stalk rot caused by *Pestalotia theae* and *Colletotrichum camelliae*, root rot by *Pythium* spp., *Cylindrocladium* spp. or *Fusarium* spp., blister blight caused by *Exobasidium vexans* and leaf spot caused by *Cercospora theae*.

Onsando *et al.* (1997) reported that among the 12 tea growing districts of Kenya, *Armillaria* root rot disease was most severe in the districts east of rift valley. Investigation showed that infection of tea bushes required primarily the mycelial growth from residual tree roots and from infected tea roots rather than from rhizomorphs. Inoculum from residual tree in debris in the soil was the most important source of infection in plantations of seed origin.

Yamaguchi *et al.* (1992) reported the superiority of Fushan variety of green tea over Yabukita (green tea) based on productivity, disease and pest resistance. Hu-ShuXia (1996) found two highly resistant cultivars to *Pestalotiopsis theae* among the 18 cultivars tested in Anhui Province of Peoples Republic of China.

Verma and Balasundaran (1990) investigated shoot-die back in cashew. In addition to feeding injury caused by *Helopeltis antonii* Sign. A fungus viz. *Botryodiplodia theobromae* was also isolated consistently from the dead tissues. The primary cause for entry and establishment of the pathogen seemed to be infestation of the insects. Controlled experiment revealed that die back occurred only when the fungus was inoculated in the lesion caused by feeding of *H. antonii* Sign.

Roux *et al.* (2001) reported the survey of diseases of *Eucalyptus* plantations (mostly *E. grandis*) in southern Uganda during June, 1999. They collected root, stem and leaf samples from the trees ranging in age from a few months to approximately 10 years. The most commonly isolated pathogen was *Lasiodiplodia theobromae* (*Botryodiplodia theobromae*), which was frequently associated with stem cankers and die back. Bacterial wilt, caused by *Ralstonia solanacearum*, was the most common cause of death of trees less than two years old, in the warmer areas around Kampala. In the eastern part of Uganda, the wilt pathogen *Ceratocystis fimbriata* was isolated from dying *Eucalyptus grandis* and, together with *Lasiodiplodia theobromae*, was considered the greatest threat to *Eucalyptus* plantations in Uganda.

Growth and physiology of the pathogens

Pathogens are attaining increasing importance in various economic fields like agriculture and industries. A thorough understanding of the physiological processes of pathogens is also of immense use in understanding the host-parasite relationship and mechanism of pathogenicity. Physiological studies are also helpful in chemotherapy and other control measures.

Saha and Chakraborty (1990) reported the effect of some environmental factors on spore germination of *Bipolaris carbonum* Nelson, a pathogen of tea. Under identical humid condition, the optimal concentration of spores, temperature, and pH for spore germination were recorded to be 11.2×10^5 spores.ml⁻¹, 32 °C and pH 6.75 respectively. Temperature pretreatment at 50 °C for 20 minutes significantly reduced spore germination, whereas pretreatment at 0 °C for even 12 hours had no effect on spore germination and germ tube elongation. Light condition and age of the conidia did not affect the spore germination.

Chakraborty *et al.* (1995) shown that factors associated with conidial germination and appressoria formation of *Glomerella cingulata* causing the brown blight disease of tea were studied *in vitro*. Spore germination and appressoria formation were optimum at a temperature of 25 °C, pH 5.0, a 7 hours light/day regime and a 24 hours incubation period. At a concentration of conidia of 1200/10 days old culture, *G. cingulata* exhibited a maximum germination and appressoria formation. Maximum production of lesions was also evident on detached tea leaves at this spore concentration and in diffuse light. Diffusates of phenolic nature collected from tea varieties susceptible and resistant to *G. cingulata* inhibited spore germination and appressoria formation. Diffusates from resistant varieties were more fungitoxic than from susceptible varieties.

Achar (2000) reported that the mycelial growth of three isolates of *Stenocarpella maydis* from maize seeds increased progressively from 15 °C to a maximum of 30 °C. The maximum number of conidia were produced by all three isolates after 8 days of incubation at temperatures ranging from 22 °C to 30 °C.

The effects of temperature and pH on the growth and sporangial production of isolates from each of the four known races of *Phytophthora clandestina* Taylor,

Pascoe & Greenhalgh were investigated. Mycelial growth occurred at temperatures from 10 °C-30 °C and pH 3.5-9.0 with highest growth rates of all isolates being at 25 °C with a pH of 6.0-6.5. Sporangial production was greatest between 20 °C-25 °C and pH 5.0-7.0 with all races. However, sporulation occurred over a temperature range from 10 to 30°C and from pH 4.0-9.0 with all isolates. There were no consistent differences between the four pathogenic races of *P. clandestina* in their relative growth rate or extent of sporangial production over a range of temperatures and pH values (Harden *et al.*, 2002).

Conidial germination *in vitro* and foliar lesion expansion were studied for *Sphaerotheca macularis* f. sp. *fragariae*. Detached strawberry (*Fragaria × ananassa*) leaves were inoculated, then held in controlled environments of constant temperatures (4 °C-36 °C) and relative humidity (RH, 32-100%) representing the range of these variables observed under California commercial production conditions. Percent germination and lesion expansion rate were determined by destructive sub sampling over time. Conidia germinated at all temperatures by 6 hours and reached a maximum by 48 hours, with the optimum near 20 °C. Lesions were marked with the aid of a microscope and measured by computer-assisted image-analysis to determine expansion rate. Maximal rates occurred at 25 °C. Several growth models were fit to the expansion rate data with high significance. Predicted optima from these models ranged from 22 °C-27 °C and/or 17-27 mm Hg VP (water @ 100% RH). Neither RH, partial vapor pressure of water (VP (water)), nor vapor pressure deficit (VPD) correlated with lesion expansion rate, adding to studies minimizing the importance of RH and VPD as determinants of asexual phase powdery mildew growth other than specifically at spore germination. (Miller *et al.*, 2003).

Common antigenic relationship

It has been evidenced by several workers that the similarity and disparity of the antigenic determinants of a host and a parasite determines the resistance and susceptibility of the host plant (DeVay and Adler, 1976; Chakraborty, 1988; Purkayastha, 1989; Chakraborty and Saha, 1994). Cross-reactive antigens, have also been suggested, to be involved in determining host-parasite compatibility (Alba *et al.*, 1983; Alba and DeVay, 1985; Chakraborty and Saha, 1994).

Ala-EI-Dein and EI-Kady (1985) used crossed immunoelectrophoresis (CIE) techniques to show that the tested isolates of *Botrytis cinerea* were serologically different; some antigens were specific for each isolate, *Botrytis cinerea* isolate no.1 had four specific antigens; these antigens were absent in other isolates. At least sixteen antigens were common in the isolates tested. Some isolates were serologically similar when tested by double gel diffusion test while they were distinguishable when CIE techniques were used. Numbers of precipitin peaks obtained with CIE techniques were more than double the number of precipitin lines detected with double gel diffusion test. Results revealed that CIE techniques could be used as valuable analytical tools in resolving the spectrum of antigens present in *Botrytis cinerea* isolates. Antigenic structures of *B.cinerea*, *B.tulipae*, *B.paeoniae* and *B.allii* isolates were also compared by using CIE techniques. Antisera against antigens of these isolates gave 24, 15, 20 and 15 precipitin peaks respectively, when analysed in homologous reactions. CIE with an intermediate gel and CIE with antibody absorption *in situ* reacted that each isolate was serologically different from the other and had species-specific antigens. Eight antigens distinguished *B. cinerea* from the other species of *Botrytis*, these were present only in the former species. *B. allii* had less common antigens than the other species.

Antiserum obtained against the mycelial proteins of a strain of *Phytophthora fragariae* could detect 11 different strains of *P. fragariae* in pure culture and pathogen in naturally infected or inoculated roots. The antiserum failed to react with 18 fungal species isolated from underground parts of strawberry but reacted with some strains of *P. cactorum*, which parasitized only rhizomes but not roots. In inoculated strawberry roots, *P. fragariae* was detected reliably by ELISA several days before oospores were found and before symptoms developed (Amouzon-Alladaye *et al.*, 1988)

Evaluation of antisera raised against pooled mycelial suspensions from five isolates (Pf-1, Pf-2, Pf-3, Pf-10 and Pf-11) representing five physiologic races of *Phytophthora fragariae* for detecting the red core disease of strawberries by enzyme-linked immunosorbent assay (ELISA) was done by Mohan (1988). Cross-reactivity of antiserum raised against *P. fragariae* with other *Phytophthora* as a

genus detecting antiserum has also been discussed by Mohan (1989). Antiserum of *P. fragariae* isolates (Anti-PfM) reacted strongly with antigens from several *Phytophthora* species. Some cross-reaction with antigens from *Pythium* species was decreased by fractionating on an affinity column of sepharose 4 B bound to extracts of *Fragaria vesca* roots infected with *P. fragariae*. The affinity purified anti-PfM retained its high cross-reactivity with the various *Phytophthora* species. Anti-PfM could not be made specific for *P. fragariae* because it was raised against components shown to be antigenically similar in all *Phytophthora* species tested. However, immunoblotting with the affinity purified anti-PfM produced distinct patterns for *P. fragariae*, *P. erythrosetpica* and *P. cactorum*.

Kitagawa *et al.* (1989) has also developed competitive types of two novel enzyme-linked immunosorbent assays (ELISA) for specific detection of *Fusarium oxysporum* f.sp. *cucumerinum* as well as for general detection of ten strains of common *Fusarium* species that show specific pathogenicities to different plants. Antiserum against a strain of *Fusarium oxysporum* f. sp. *cucumerinum* (F 504) was elicited in rabbits and a highly specific, sensitive and accurate ELISA for the homologous strain was developed by using the antiserum with β -D-galactosidase-labelled anti-rabbit IgG as a secondary antibody and cell fragments of the strain attached to amino-Dylark balls as the solid-phase antigens. This assay was specific for strain F 504 and showed little cross-reactivity with nine other strains of *Fusarium* species including strain F 501 of *F. oxysporum* f.sp. *cucumerinum* (FO). F 501 possess pathogenicity against cucumber similar to that of strain F 504, although slight differences have been observed between these two strains regarding their spore formation and pigment production. Cell fragments of strain F 501 absorbed on amino-Dylark balls possessed sufficient immune activity against anti-FO antibody to use in a heterologous ELISA for general detection of ten *Fusarium* species with high sensitivity.

Common antigenic relationships between soybean and *Colletotrichum dematium* var. *truncata* was also studied by Purkayastha and Banerjee (1990) using immunodiffusion, immunoelectrophoresis and indirect ELISA technique. Cross-reactive antigens were detected between susceptible soybean cultivars and the virulent strain of *C. dematium* var. *truncata* but no cross-reactive antigen was

detected between soybean cultivars and avirulent pathogen (*C. dematium*) of non-pathogen *C. corchori*. Results of immunodiffusion and immunoelectrophoresis showed absence of common antigen between resistant cultivars (UPS M-19) and the pathogen, while the results of indirect ELISA indicated the presence of common antigen between the two at a very low level. They compared antigenic patterns of untreated and cloxacillin treated soybean leaves which induced resistance of soybean against anthracnose disease. The disappearance of one antigen from cloxacillin treated leaves of susceptible soybean cv. "Soymax" was correlated with alternation of disease reaction.

Polyclonal antiserum raised against mycelial extracts of the rot fungus *Phialophora mutabilis* reacted strongly with its homologous antigen and cross-reacted strongly to moderately with six other *Phialophora* soft rot spp. in ELISA (Daniel and Nilsson, 1991). With the help of an indirect ELISA technique, Ricker *et al.* (1991) showed that increase in cross-reactivity in late bled antiserum (anti-Bc IgG), raised against water soluble antigens from *Botrytis cinerea* corresponded with an increase in the overall serum titres for anti-Bc IgG to antigens of *B. cinerea*. Sundaram *et al.* (1991) reported that polyclonal antiserum of mycelial proteins of *Verticillium dahliae* reacted positively with 11 of 12 isolates of *V. dahliae* from potato, cotton and soil but negatively with one isolate from tomato in indirect ELISA. He also found positive results in detecting *V. dahliae* and *V. albo-atrum* from infected roots and stems of potato in an double antibody sandwich enzyme linked immunosorbent assay (DAS-ELISA).

Results of conventional isolation techniques for *Pythium violae* were compared with the assay of cavity spot lesions using polyclonal antibodies raised to *P. violae* or *P. sulcatum* in competition ELISA (Lyons and White, 1992). A double antibody sandwich ELISA test was developed for the detection of *Pseudocercospora herpotrichoides* using a highly specific monoclonal antibody pH 10 as the capture antibody and genus specific polyclonal rabbit antisera as test antibody. The assay recognized extracts from plants both artificially and naturally infected with *P. herpotrichoides*, at least three-fold higher absorbance values with extracts of *P. herpotrichoides* infected tissue than with extracts from healthy tissues. The high molecular weight fraction of immunogen (mycelial extracts) was shown

to contain cross-reactive antigens: it induced antiserum in mice that cross-reacted with the other stem base fungi even at high dilution (Priestley and Deway, 1993).

Polyclonal antibodies (PABs) produced against culture filtrates and mycelial extracts immunogen preparations from the soybean (*Glycine max*) and fungal pathogen *Phomopsis longicolla* were purified to the immunoglobulin fraction and tested in indirect ELISA and in direct DAS-ELISA (Brill *et al.*, 1994). The PABs raised to culture filtrate were more specific but less active in binding to members of Diaparth-Phomopsis complex than were those to mycelial extract immunogen preparation. DAS-ELISA was more specific and 100-fold more sensitive in detecting members of the complex than was indirect ELISA. Variability in specificity between different PABs was lower in DAS-ELISA compared to indirect ELISA. Janaux and Spire (1994) used double DAS-ELISA to screen the cross-reactivity of soluble mycelial extracts of *Sclerotinia sclerotiorum*, an important pathogen of rapeseed with other cross-reacting fungal species such as *Botrytis cinerea*, a pathogen commonly present in rapeseed petals using a polyclonal anti-*B. cinerea* serum. An extensive cross reaction was found when two monoclonal and three polyclonal antisera, raised against the cell wall/membrane fractions of *Pythium violae* and *P. sulcatum* screened with a collection of 40 isolates of the genus *Pythium* including 20 species and the H-S group. However, when the binding of the antibodies was assessed in an enzyme-linked immunosorbent assay (ELISA) using cytoplasmic fraction antigens, the combined recognition patterns produced profiles unique to each species (White *et al.*, 1994).

Wakeham and White (1996) raised polyclonal antisera against whole (coded: 16/2) and sonicated (coded: 15/2) resting spores of *Plasmodiophora brassicae* as well as soluble components prepared by filtration and ultracentrifugation (coded: SF/2), cross-reactivity of all three antisera with a range of soil fungi, including *Spongospora subterranean* was low. Test formats including western blotting, dipstick, dot blot, indirect ELISA and indirect immunofluorescence were assessed for their potential to detect resting spores of *P. brassicae* in soil. Dot blot was least sensitive, with a limit of detection level of 1×10^7 resting spores/g in soil. With western blotting the lower limit of detection with antiserum 15/2 was 1×10^5 . This antiserum showed the greatest sensitivity in a dipstick assay, indirect



ELISA and indirect immunofluorescence, for all of which there was a limit of detection of 1×10^2 . Of the assays tested, indirect immunofluorescence appears to be the most rapid and amenable assay for the detection in soil of low levels of resting spores of *P. brassicae*.

Das and Mitra (1999) produced monoclonal antibodies (MAbs) produced against clover phyllody phytoplasma which were used in immunofluorescence test for the detection of brinjal little leaf phytoplasma in periwinkle. The monoclonal antibodies reacted with the antigen from little leaf infected periwinkle plants, but not with that from healthy plants, indicating a close serological relationship between these two phytoplasmas.

Besides fungus, virus (Petrunak *et al.*, 1991; Abou-Jawdah *et al.* 2001; Hema *et al.*, 2001) and bacterial (Mazarei and Kerr, 1990) pathogens of plants could be successfully detected by various ELISA formats. Double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) and direct antigen coating (DAC)-ELISA tests were evaluated for detection of sugarcane streak mosaic virus (SCSMV-AP), a new member of Tritimovirus genus in the family Potyviridae, in leaf extracts, sugarcane juice and purified virus. The virus was detected up to 1/3125 and 1/625 dilutions in infected sugarcane leaf, 5 μ l and 10 μ l/well in sugarcane juice, 1/3125 and 1/3125 dilutions in infected sorghum leaf and 10 ng and 50 ng/ml for purified virus in DAS-ELISA and DAC-ELISA tests, respectively (Hema *et al.*, 2001). Abou-Jawdah *et al.* (2001) in a survey detected potato virus Y (PVY), potato virus A (PVA), potato virus X (PVX), potato virus M (PVM), potato virus S (PVS) and potato leaf roll virus (PLRV) by ELISA from potato fields in the two main production areas of Lebanon, the Bekaa and Akkar plains.

Plant disease alteration by chemical treatment

Several agents both physical and chemical have been used for alteration of disease reaction. The agents mostly include X-rays, UV-rays and biological agents. Chemicals of diverse nature without any direct toxic action were used in the past. Systemic acquired resistance (SAR) of plants against pathogens is a widespread phenomenon with respect to the underlined signalling pathways as well as to its potential use in plant protection. Plants respond with a salicylic-

dependent signalling cascade that leads to the systemic expression of a broad spectrum and long-lasting disease resistance (Heil and Bostock, 2002).

Yalpini *et al.* (1991) suggested salicylic acid as an endogenous signaling molecule that mediates systemic acquired resistance (SAR) in several host-pathogen systems. Pautot *et al.* (1991) studied the differential expression of tomato proteinase inhibitor I (Pin I) and proteinase inhibitor II (Pin II) genes during infection by *Pseudomonas syringae* PV tomato, the causal agent of bacterial speck disease in tomato. Inoculation of *P. syringae* PV tomato to resistant and susceptible tomato leaves resulted in the accumulation of Pin I mRNA and Pin II mRNA more rapidly in disease resistant variety than in disease-susceptible plants.

Artemisia tridentate (sagebrush), a plant shown to possess methyl jasmonate in leaf surface structures when incubated at chambers with tomato plants, proteinase inhibitor accumulation is induced in tomato leaves, demonstrating that interplant communication, through airborne methyl jasmonate can occur from leaves of one species of plants to leaves of another species and simultaneously the defensive genes are also expressed (Farmer and Ryan, 1992). Schneider-Muller *et al.* (1994) reported Ca^{2+} ions playing an important role in the induced production of salicylic acid and chitinase, one of the pathogenesis-related proteins.

Leroux (1996) reported that SAR can be accomplished by the exogenous application of aspirin or its derivatives. This biorational approach has been the basis of the development of a new fungicide, BTH (CGA 245704) by Ciba Geigy. BTH has been shown to offer prophylactic protection when applied @ 20-30 g.ha⁻¹ at early tillering stage against powdery mildew in wheat, rice blust in rice and blue mould in tobacco.

A single spray of solutions of 0.005 M H_3BO_3 , 0.0025 M CuSO_4 and 0.0025M MnCl_2 , on the upper surface of the first true leaf of cucumber plants 2 hours before inoculation with a conidial suspension of *Sphaerotheca fuliginea*, induced systemic protection against powdery mildew in leaves 2 and 3 without causing any damage on the induced leaf (first leaf). A similar level of systemic protection was observed when plants were induced by a variety of micronutrients or microelements with various concentrations, 2, 24 and 72 hours before challenge with *S. fuliginea* (Renveni *et al.*, 1997).

Induced systemic resistance is mediated by a jasmonate/ethylene sensitive pathway and does not involve expression of PR proteins (Pieterse *et al.*, 1998 and VanLoon *et al.*, 1998). Ding *et al.* (1999) reported that pretreatment of tomato fruit with low concentration (0.01 mM) of methyl jasmonate (MeJA) or methyl salicylate (MeSA) induces the synthesis of some stress proteins such as PR proteins which leads to increased tolerance to chilling temperature and resistance to pathogens, thereby decreasing the incidence of decay. Dombrowski *et al.* (1999) indicated that prosystemin and/or large fragment of prosystemin can be active inducers of defense responses in both tomato leaves and suspension cultured cells.

Besides chemicals several virulent and avirulent pathogens, nonpathogens, herbivore attack and wounding might induce resistance. Hammerschmidt (1999) reported the local or systemic induction of disease resistance in the treated plant to subsequent pathogen attack.

Signal transduction pathways that operate both at the site of wounding and undamaged distal leaves regulate plant defense responses to wounding and herbivore attack. Genetic analysis in tomato indicates that systemin and its precursor protein prosystemin are upstream components of a wound-induced inter-cellular signaling pathway that involves both the biosynthesis and action of jasmonic acid. Activation of jasmonate biosynthetic in response to wounding or prosystemin is required for the production of a long distance signal, whose recognition in distal leaves depends on jasmonate signaling, which may act as a transmissible wound signal (Lil *et al.*, 1999). Systemin, an 18-amino acid polypeptide wound signal activate defense gene in wounded leaves of young tomato plants and induces alkalization of media containing suspension-cultured *Lycopersicon peruvianum*. (Scherre and Ryan, 1999).

Jasmonic acid, oligopeptide systemin, oligosaccharides and other phytohormones such as ABA and ethylene as well as physical factors such as hydraulic pressure or electrical pulses have been proposed to play a role in wound signalling. Components of different jasmonic acid dependent and independent wound signalling transduction pathways are mostly similar to those implicated in other signalling cascades in eukaryotes and include reversible protein

phosphorylation steps, calcium/calmodulin-regulated events and production of active oxygen species (Leon *et al.*, 2001).

A central signalling molecule in induced responses against herbivores is jasmonic acid (JA) (Creelman and Mullet, 1997; Wasternack and Parthier, 1997). In response to wounding and/or insect feeding, linolenic acid is released from membrane lipids and then converted enzymatically into JA. JA, in turn, causes the transcriptional activation of genes encoding proteinase inhibitors (PIs) and of enzymes involved in the production of volatile compounds, or of secondary compounds such as nicotine and numerous phenolics, and other defence-related compounds (Creelman and Mullet, 1997; Karban and Baldwin, 1997; Wasternack and Parthier, 1997; Boland *et al.*, 1999). Oligosaccharides (Bishop *et al.*, 1981) and oligogalacturonides (Doares *et al.*, 1995; Norman *et al.*, 1999) released from damaged cell walls might play a role in the elicitation of the general wound response, but specific elicitors such as systemin have also been reported (Pearce *et al.*, 1991). Systemin is an 18-amino acid polypeptide that is released upon wounding from a 200-amino acid precursor ('prosystemin') and that leads to the release of linolenic acid. This activates the octadecanoid signalling cascade (Ryan, 2000). Both JA (Zhang and Baldwin, 1997) and systemin (Ryan, 2000) can be transported in the phloem and thus might act as systemic signals. To date, systemin has been described for tomato only, and not even for other solanaceous plants such as tobacco (Ryan, 2000; León *et al.*, 2001). The importance of cell wall fragments in elicitation was supported by the finding that cellulysin, a mixture of several cell wall-degrading enzymes from the plant parasitic fungus *Trichoderma viride*, can induce several JA-responsive volatiles in lima bean (*Phaseolus lunatus*) (Piel *et al.*, 1997). The action of cellulysin is followed by a rapid increase in endogenous JA (Koch *et al.*, 1999).

Disease control by fungicides

Chemical control continues to play an important role in the integrated control of tea diseases (Muraleedharan and Chen, 1997). Several workers have suggested chemical control of tea diseases. Some of the previous works are being included in this review.

Sulphur compounds were not seriously considered as they imparted undesirable taint to tea (De Jong, 1954). Mulder (1961a,b) reported that nickel chloride when used either alone or in combination with Zineb controlled blister blight satisfactorily. Of the various nickel salts tested nickel chloride was found to be the best (Venkata Ram, 1962). De Silva (1965) stated that nickel chloride though gave satisfactory control was found to be less effective than perenox. In tea recovering from pruning nickel chloride can not be used due to phytotoxicity (Chandra Mouli and Venkata Ram, 1979). Pasaribu and Sinaga (1981) reported the usefulness of nickel chloride in controlling disease in Indonesia.

A combination of nickel chloride with copper oxychloride offered better disease control to either of them sprayed alone (Venkata Ram, 1966). Systemic fungicides like derivatives of 1,4-oxathins, carboxin and oxycarboxin controlled the disease when tested in potted plants (Venkata Ram, 1969). However, these systemic fungicides had poor retention under severe wet weather conditions and they could not be used during periods of heavy rainfall (Venkata Ram, 1977).

Copper fungicide formulations containing 50% metallic copper either as copper oxychloride or cuprous oxide have been the best choice for protective control of blister blight disease. Of the two copper formulations, the latter had an edge over the former (Venkata Ram and Chandra Mouli, 1981). Nickel sulphate was useful in controlling blister blight (Tzong- Maochen and Shin-funchen, 1982). Three different concentrations of copper chloride and two different concentrations of nickel chloride and three different concentrations of the combination of the two chemicals were used to check *Exobasidium vexans* (Venkata Ram and Chandra Mouli, 1983).

Out of many protectant-therapeutant organic fungicides used as alternatives to copper oxychloride and nickel chloride, only chlorothalonil and dithianon provided satisfactory control (Chandra Mouli and PremKumar, 1986). In case of red rust (caused by *Cephaleuros parasiticus*) of tea, copper fungicides having 50% metallic copper are most effective with 1:400 dilution in four rounds of hand spray (Barua, 1988).

Application of copper-antibiotic treatment was inferior to copper plus nickel combination in tea under regular plucking (Venkata Ram and Chandra Mouli, 1979;

Chandra Mouli and Premkumar, 1989). Onsando and Langat (1989) reported the wood rot of tea caused by *Hypoxylon serpens*. The study showed that the normal surgical prunes caused comparatively lower disease incidences while medium prune aggravated the incidence and severity of wood rot and reduced yields. Among the fungicides tested, only benlate significantly reduced disease incidence and severity.

Hypoxylon wood rot, caused by *Hypoxylon serpens* is one of the major disease of tea in which the pathogen has been found to exist in both sexual and asexual states. Superficial fructifications of stromata of the fungus that appear as irregular dark-grey or black slightly raised patches of various sizes, and gradual decline that culminates in the death of the plant are symptoms of infection of tea bush by *Hypoxylon serpens*. Surgical removal of all infected bushes and treating the resultant wounds with copper oxychloride in raw linseed oil and use of systemic fungicides were suggested in control of the disease (Otieno, 1993). Otieno *et al.* (1994) screened five fungicides and three pruning modes for management of *Hypoxylon* wood rot of tea. The screening results revealed that normal and surgical prune were superior to medium prune in suppressing spread of the disease. The use of benomyl, a systemic fungicide also lowered disease incidence to levels sufficiently different from that of the other fungicides.

Several chemicals have also been used against pathogens of different plants. Matheron and Porchas (1999) compared the *in vitro* activity of azoxystrobin, dimethomorph, and fluazinam on growth, sporulation, and zoospore cyst germination of *Phytophthora capsici*, *P. citrophthora*, and *P. parasitica* to that of fosetyl-AI and metalaxyl. The 50% effective concentration (EC(50)) values for inhibition of mycelial growth of the three pathogens usually were lowest for dimethomorph and metalaxyl, ranging from < 0.1 to 0.38 µg/ml. However, the 90% effective concentration (EC(90)) levels for dimethomorph always were lower than the other four tested compounds, with values ranging from 0.32 to 1.6 µg/ml. Mycelial growth of *P. capsici*, *P. citrophthora*, and *P. parasitica* was least affected by azoxystrobin and fluazinam, with estimated EC(90) values > 3,000 µg/ml. Reduction of sporangium formation by *P. capsici*, *P. citrophthora*, and *P. parasitica* in the presence of dimethomorph at 1 µg/ml was significantly greater than that recorded for the same concentration of azoxystrobin, fluazinam, and fosetyl-AI. For the three

species of *Phytophthora*, zoospore motility was most sensitive to fluazinam (EC(50) and EC(90)) values of $< 0.001 \mu\text{g/ml}$ and least sensitive to fosetyl-AI, with EC(50) and EC(90) values ranging from 299 to 334 and 518 to 680 $\mu\text{g/ml}$, respectively. Germination of encysted zoospores of *P. capsici*, *P. citrophthora*, and *P. parasitica* was most sensitive to dimethomorph (EC(50) and EC(90)) values ranging from 3.3 to 7.2 and 5.6 to 21 $\mu\text{g/ml}$, respectively), intermediate in sensitivity to fluazinam (EC(50) and EC(90) from 18 to 108 and 67 to $> 1,000 \mu\text{g/ml}$, respectively) and metalaxyl (EC(50) and EC(90)) from 32 to 280 and 49 to 529 $\mu\text{g/ml}$, respectively), and lowest in sensitivity to azoxystrobin and fosetyl-AI (EC(50) and EC(90) from 256 to $> 1,000 \mu\text{g/ml}$). The activity of azoxystrobin, dimethomorph, and fluazinam on one or more stages of the life cycle of *P. capsici*, *P. citrophthora*, and *P. parasitica* suggested that these compounds potentially could provide *Phytophthora* spp. disease control comparable to that of the established fungicides fosetyl-AI and metalaxyl.

The control of *Fusarium* head blight (FHB) of wheat using fungicides was investigated in two field trials by Cromey *et al* (2001). The first trial examined the effects of tebuconazole applied at a range of crop growth stages around flowering, whereas the second trial compared nil fungicide, tebuconazole, carbendazim, and azoxystrobin, applied at full ear emergence or mid anthesis. Moderate FHB levels were recorded in untreated plots in both trials. In the first trial, FHB incidence was reduced by up to 90% and yield increased by 14% following two applications of tebuconazole. Levels of *Fusarium* in harvested grain were not affected but mycotoxin levels were reduced by some treatments. In the second trial FHB incidence was decreased and grain weight increased with all fungicides at one or both application stages. High levels of *Fusarium* were recorded in harvested grain in the nil fungicide treatment. Levels of both *Fusarium* and resulting mycotoxins were substantially reduced following treatment with tebuconazole or carbendazim but were not affected by treatment with azoxystrobin.

In a series of experiments, fungicides with different modes of action to the commonly used phenylamide-based products were examined against Downy mildew of rose (*Rosa spp.*) and blackberry (*Rubus fruticosus*), caused by *Peronospora sparsa*. Cymoxanil + mancozeb + oxadixyl and fluazinam gave good

downy mildew control on both rose and blackberry. On outdoor, container-grown rose, high volume sprays of fosetyl-aluminium were also effective, but on young micropropagated blackberry plants, application as a drench treatment was better than as a spray. Good control was also achieved on blackberry with chlorothalonil and with metalaxyl in formulation with either thiram or mancozeb. (O' Neill *et al.*, 2002).

Disease control by botanicals

There are certain advantages in the deployment of botanical pesticides. These are biodegradable, safe to non-target organisms, renewable and suit to sustainability of local ecology and environment. Several authors have demonstrated the use of botanicals to control the plant diseases.

Synergistic enhancement by 2- to 73- folds of antifungal activity of wheat thionins when combined with 2 S albumins of radish or rape was noticed being effective against filamentous fungi and some gram-positive bacteria. Permeabilization of the hyphal plasmalemma of thionins has been shown to be the mode of action (Terras *et al.* 1993). Soil amendments with crop residues lead to build up of allelochemicals and plant nutrients. In a comparative study, Prew *et al.*, (1995) shown that incorporation of straw was found more effective than burning of straw in containing the symptoms of eye spot disease (*Pseudocercospora herpotrichiodes*) and sharp eye spot disease (*Rhizoctonia cerealis*) of wheat.

Evaluation of rape and Indian mustard as companion crop showed that the latter was more effective in minimizing the incidence not only of take-all disease of wheat but also *Rhizoctonia solani*, *Pythium* and *Cochliobolus sorokiniana*. The tissue extract of Indian mustard was equally effective and hence the role of volatile isothiocyanates is implied (Kirkwaad *et al.*, 1996). Certain phytochemicals like gallic acid and abscisic acid have been shown to be antifungal. For instance, abscisic acid was shown to inhibit mycelial growth and sporidial formation and also germination of teliospores (Singh *et al.*, 1997).

The hexane and methanol extracts of sixteen plants of the family Caesalpiniaceae, collected around Karachi, Pakistan, were phytochemically screened and tested for their antibacterial and antimicrobial activity. As compared

to hexane extracts, the methanol extracts of all the examined plants showed stronger growth inhibitions against both bacteria and fungi, *Cassia* species being the biologically more active plants (Ali *et al.*, 1999). Carpinella *et al.* (1999) reported that the ethanol extract of *Melia azedarach* ripe fruits showed fungistatic (MIC 50-300mg/ml) and fungicidal (MFC60-500mg/ml) activity against *Aspergillus flavus*, *Fusarium moniliforme*, *Microsporium canis* and *Candida albicans*.

The antimicrobial activities of *Valex* (the extract of valonia), the extracts of mimosa bark, gullnut powders, *Salvia ancheri* Benthum. var. *ancheri* and *Phlomis bourgei* Boiss. were studied. The results of the study indicated that mimosa bark extracts had the greatest antibacterial activity, followed by the *Valex*, gullnut powders, *Salvia ancheri* var. *ancheri* and *Phlomis bourgeie* extracts, respectively. Furthermore, it was found that gullnut powders and the extracts of mimosa bark contained high amounts of tannins and showed antifungal activity (Digrak *et al.*, 1999).

Two hundred and four species of traditional Chinese herbal medicines belonging to 80 families were collected from Yunnan Province in People's Republic of China and tested for antifungal activities using a *Pyricularia oryzae* bioassay. Twenty-six herbal medicines from 23 families were active against *P. oryzae* and the ethanol extract of *Dioscorea camposita* (dioscoreaceae) exhibited the most bioactivity among all the tested samples (Ke Hu *et al.*, 1999).

The antibacterial activity of ethanol extracts of 15 plant species used in the traditional medicine in Jordan and other middle east countries were tested. Extracts of certain parts of these plants were tested *in vitro* against 14 pathogenic bacterial species and strains using the agar diffusion methods. Three plants exhibited broad spectrum antibacterial activity: *Punica Granatum* L., *Quercus infectoria* Olive., and *Rhus coriaria* L. The most susceptible bacteria were *Pseudomonas aeruginosa*, *Bacillus cereus* and *Streptococcus pyogenes* (ATCC 12351), and the most resistant species were *Escherichia coli* (ATCC 25922 and clinical isolates), *Klebsiella pneumoniae*, *Shigella dysenteriae* (ATCC 49345), and *Yersinia enterocolitica* (ATCC 9610) (Nimri *et al.*, 1999).

Three thiosulfinates with antimicrobial activity were isolated from oil-macerated garlic extract and their structures were identified by Yoshida *et al.* (1999a)

as 2-propene-1-sulfinothioic acid S-(Z,E)-1-propenyl ester [AII S(O)SPn-(Z,E)], 2-propenesulfinothioic acid S-methyl ester [AII S(O)SMe], and methane sulfinothioic acid S-(Z,E)-1-propenyl ester [MeS(O)SPn-(Z,E)]. Antimicrobial activities of AII S(O)SPn-(Z,E) and AII S(O)SMe against gram-positive and negative bacteria and yeasts were compared with 2-propene-1-sulfinothioic acids-2-propenylester [AII S(O)SAII, allicin]. Antimicrobial activity of AII S(O)S Me and AII S(O)S Pn-(Z,E) were comparable and inferior to that of allicin, respectively. In another study, an organosulfur compound was isolated and identified from oil-macerated garlic extract by silica gel column chromatography and preparative TLC. The antimicrobial activity of isoE-10-DA was inferior to those of similar oil-macerated garlic extract compounds such as E-ajoene, Z-ajoene and Z-10-DA (Yoshida *et al.*, 1999b).

The leaves of five *Betula* species, *B. pendula*, *B. browicziana*, *B. medwediewii*, *B. litwinowii* and *B. recurvata* collected from different parts of Turkey were hydrodistilled to yield the consequent essential oils. The essential oils showed antifungal activity against various phytopathogenic fungi like *Cephalosporium aphidicola*, *Drechslera sorokiniana*, *Fusarium solani* and *Rhizoctonia cereals* (Demirci *et al.*, 2000).

Rao *et al.* (2000) reported about Limonene, the major constituents of essential oil of exocarpic part of *Citrus sinensis* which possessed strong and broad-spectrum antifungal activity against important fungal pathogens of sugarcane. The mycelial growth of *Ceratocystis paradoxa* at 2000ppm and that of *Fusarium moliniforme* and *Curvularia lunata* at 3000ppm concentration of limonene were completely inhibited. It proved fungistatic at minimum inhibitory concentration and exhibited non-phytotoxicity or germination and growth of sugarcane.

Analysis of methanol extracts from leaves, stem bark, root bark, fruits and seed kernels of *Butyrospermum. pradoxum* (*Vitellaria paradoxa*) by Ogunwande *et al.* (2001) revealed the presence of alkaloids (in leaves and stem barks), flavones (in stem and root bark), saponins (in root bark), steroids (in stem bark, fruits and seed kernels) and tannins (in leaves and root bark) which have antimicrobial activity against different bacteria (*Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella*

typhi, *Staphylococcus aureus*, *Ralstonia solanacearum* and *Bacillus cereus*) and fungi (*Fusarium oxysporum* and *Candida albicans*).

Control of *Botrytis cinerea* Pers. leaf colonisation and bunch rot in grapes with oils was studied in laboratory and field tests. In detached lateral experiments, the essential oils from thyme (*Thymus vulgaris* L.) and clove (*Syzygium aromaticum* L.), as well as massoialactone (derived from the bark of the tree *Cryptocarya massoia* R.Br.) were not phytotoxic on leaves at concentrations of 0.33% or less. *B. cinerea* sporulation on artificially induced necrotic leaf lesions was significantly reduced by thyme (Thyme R) and massoialactone oils at 0.33%. A single application at veraison (1997/98) of either compound at concentrations of 0.33% controlled bunch rot and necrotic leaf lesion colonisation by *B. cinerea* compared with *Botrytis* control treatments. Spray applications of Thyme R oil (0.33%) at 8–10 day intervals (1998/99) from flowering to harvest controlled *B. cinerea* bunch rot but also caused floral tissues to senesce (Jaspers *et al.*, 2002).

Crude extract of the bulb of *Eucomis autumnalis* showed *in vitro* mycelial growth inhibition of *Mycosphaerella pinodes*, the cause of black spot or *Ascochyta* blight, in peas. The control of *Ascochyta* blight by different concentrations of the crude *E. autumnalis* extract was followed *in vivo* by leaf symptoms over a 6 day period at 20°C in a growth cabinet. 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 (Pretorius *et al.*, 2002).

Disease control by antagonistic organisms

To develop biological control strategies of any disease, a thorough knowledge of life cycle of the pathogen(s), their mode of survival, the plant pathogen interaction processes starting from, the physical relationship of the pathogen to its host during pathogenesis, and the time and factors leading to infection and disease development are needed.

Leach (1939) showed that tea bush prunings left on the soil surface for a while to permit their colonization by airborne saprophytes were then protected

from colonization by *Armillaria mellea* when subsequently buried. This resulted in lesser *Armillaria* inoculum and hence less root rot of tea. Kerr (1980) showed that the K84 strain of *Agrobacterium radiobacter* produces a bacteriocin, agrocin, achieves a population higher than that of the pathogen and prevents transfer of the tumour inducing (Ti) plasmid (TDNA) from the pathogen to the host. Ayers and Adams (1981) reported Deuteromycetes fungus, *Coniothyrium minitans*, a biocontrol agent produces several enzymes which cause lysis and kill upto 99% of the sclerotia of *Sclerotinia sclerotiorum*. Upadhyay and Rai (1985) reported that *Trichoderma viride* and *Trichoderma harzianum* significantly suppressed *Fusarium udum* in soil and roots of pigeon pea.

Panday *et al.* (1997) reported that a large number of bacteria and fungi were isolated from the rhizosphere of established tea bushes over a period of one year. Fiftyone of these bacterial isolates were tested for their antifungal activity against 12 test fungi, which include 9 minor and 3 major pathogens of tea. The bacterial isolates exhibiting the highest antifungal activity were also the most dominant bacterial species in the rhizosphere.

Similarly, several pathogens of other crops were also controlled by several workers such as mycostop was a biofungicide that had been effectively used to control a number of soil and seed-borne pathogens like *Botrytis cinerea*, *Rhizoctonia solani* etc. and seed borne foot rot disease of wheat and barley (Tahvonen and Lahdenperä, 1988; Tahvonen and Avikainen, 1990). The active component of mycostop was the spores and mycelium of *Streptomyces griseoviridis*. The product has been used successfully in seed treatment, soil drench, drip irrigation and as a transplant dip to control various disease causing fungi (Lahdenperä, 1987; Lahdenperä *et al.*, 1990 and Mohammadi, 1992). Suleman *et al.* (2002) used Mycostop at the rate of 0.35g/l or greater which reduced spore germination, plasmolysed germlings and reduced sporulation of *C. radicola*. In essence, it reduced the inoculum potential of *C. radicola*.

The antagonistic effect of *Aspergillus niger*, *A. fumigatus*, *A. flavus* and *Trichoderma viride* was well established as reported by several workers. Wu *et al.*, (1986) and Vinod (1988) reported the antagonistic effect of *A. niger*. The antagonistic property of *A. flavus* against many pathogenic microorganisms has been reported

by Massoor and Chandra (1987) and Deb (1990) though not specific against *Pythium* spp. The hyphal coiling and production of inhibitory substances by different species of *Trichoderma*, resulting in dieback and disintegration of *Pythium* spp were reported by Raju (1991) and Vinod *et al.* (1991). Mukherjee and Sen (1992) observed that the culture filtrate of *A. fumigatus* inhibited the growth of *Macrophomina phaseolina* and sclerotial germination.

Mukhopadhyay *et al.* (1992) found good control of chickpea wilt complex when seeds were treated with *Gliocladium virens* (10^7 conidia/ml) and carboxin 0.1%. Ghizalberti and Rowland (1993) shown that alkylpyrones, cycloneroditol etc., the metabolites isolated from *Trichoderma harzianum*, were active against take-all disease of wheat. Similarly, 2,14-diacetyl phloroglucinol, isolated from fluorescent *Pseudomonads*, was responsible for controlling the take-all disease of wheat.

Several authors have shown considerable potential of *Trichoderma* and *Gliocladium* in controlling disease caused by *Sclerotium rolfsii* in snapbean, sugarbeet, tomato, chickpea and cotton in greenhouse and field studies (Elade *et al.*, 1983; Upadhyay and Mukhopadhyay, 1983; Henis, 1984; Punja, 1985; Papavizus and Lewis, 1989; Wokocho, 1990; Ciccarese *et al.*, 1992 and Latunda Dada 1993).

The fungus *Ulocladium atrum* Preuss, indigenous to Netherlands, was found to exclude *Botrytis cinerea* from necrotic plant tissue more effectively under field conditions than other antagonists such as *Gliocladium catenulatum* Gilman and E. Abbott, *Aureobasidium pullulans* (deBary) Arnaud or *Chaetomium globosum* Kunze: Fr. (Köhl *et al.*, 1995). Sutton (1995) and Lima *et al.* (1997) reported that biological control of various filamentous fungi and yeasts is one of the methods that has shown good perspectives for non-chemical management of gray mould in strawberry.

Different isolates of *Trichoderma harzianum* showed differential antagonistic potential as biocontrol agent against *Sclerotium rolfsii* as reported by Maity and Sen (1985) and Biswas (1999).

Filonow (1998) observed that three antagonistic yeasts competed successfully for sugars since their uptake was faster and higher than that of *Botrytis*

cinerea. He concluded from this that high competitiveness plays a central role in antagonism. The antagonistic yeast *Cryptococcus albidus* were highly effective when applied to the strawberry fruits after harvest by colonizing the wounds and competing for nutrients or space and thereby reducing the infection (Droby *et al.*, 1989; Benbow and Sugar, 1999 and Helbig, 2002).

Pandey and Upadhyay (1999) reported the comparative performance of chemical, biological and integrated control of wilt of pigeonpea caused by *Fusarium udum*. In chemical control, bavistin was found highly effective, while *Trichoderma viride* and *T. harzianum*-C isolates were found best among biocontrol agents. Integration of biocontrol agents with bavistin was not beneficial. However, integration of the bioagents with thiram reduced wilt incidence significantly. Thus seed coating with bioagents proved better and safe for the management of wilt of pigeonpea.

Prasad *et al.* (1999) tested 14 isolates of *Trichoderma* and *Gliocladium* species were tested in vitro against *Sclerotium rolfsii*, the causal organism of root/collar rot of sunflower. Two isolates of *T. viride*, four isolates of *T. harzianum*, one each of *T. hamatum*, *T. koningii*, *T. polysporum*, *G. virens*, *G. deliquescens* and *G. roseum* inhibited mycelial growth of the pathogen significantly. Among *Trichoderma* species, *T. harzianum*, isolates PDBCTH2 gave 61.4% inhibition of mycelial growth followed by PDBCTH 8 (55.2%) and PDBCTH 7 (54.9%). Among *Gliocladium* isolates, *G. virens* gave maximum inhibition (39.9%) of mycelial growth. Suppression of sclerotial production by antagonists ranged from 31.8 to 97.8%. Complete inhibition of sclerotial germination was obtained with the culture filtrates of *T. harzianum* (PDBCTH 2, 7 and 8), *T. pseudokoningii* and *G. deliquescens*. The three *T. harzianum* isolates and the *T. viride* isolate (PDBCTV 4) were superior under greenhouse conditions with PDBCTH 8 showing maximum disease control (66.8%) followed by PDBCTH 7 (66.0%), PDBCTV 4 (65.4%), PDBCTH 2 (61.6%) and were even superior to fungicide captan. *G. deliquescens* gave maximum (55.7%) disease control among *Gliocladium* spp.

A modified granular formulation containing powdered wheat bran, kaolin, acacia powder and biomass of isolates of *Trichoderma harzianum* (PDBCTH 10 and PDBCTH 8), *T. virens* (PDBCTV_s 3 and ITCC 4177) and *Gliocladium deliquescens* (ITCC 3450) were evaluated (Prasad and Rangeshwaran, 1999) for

their effect on the reduction of chickpea damping off caused by *Rhizoctonia solani*, reduction of pathogen inoculum and proliferation of the bioagents in the soil. Granules with all isolates of bioagents significantly reduced damping off. At 4 weeks, PDBCTH 10 and PDBCTH 8 isolates treatments have recorded better plant stands (63 and 53%) than fungicide (captan) treatment (43%). But none of the isolates of bioagents have recorded plant stands comparable to non-infested control (83%). The above two *T. harzianum* isolates were more effective in reducing saprophytic growth of the pathogen compared to other bioagents.

Native microorganisms were isolated from the rhizosphere of healthy ginger plants, in the rhizome rot affected fields and screened *in vitro* for their antagonistic effects against the pathogen *Pythium aphanidermatum* by dual culture and cell free culture filtrate studies. *Aspergillus niger*, *A. fumigatus*, *A. flavus* and *Trichoderma viride* were found to be potential antagonists (Shanmugam and Sukunara Verma, 1999).

The antagonistic potential of eight isolates of *Trichoderma harzianum* against four fungal pathogens of betelvine (*Phytophthora parasitica*, *Colletotrichum capsici*, *Sclerotium rolfsii* and *Rhizoctonia solani*) were shown. Isolates T₁, T₂ and T₃ had highest promise under *in vitro* conditions (D'Souza *et al.*, 2001).

Saprophytic fungus *Ulocladium atrum* Preuss was a promising biological control agent for control of *Botrytis cinerea* in strawberry and other crops (Boff *et al.* 2002).

The microbial basis of specific suppression to four diseases, Fusarium wilts, potato scab, apple replant disease, and take-all, was reported by Weller *et al.* (2002). One of the best-described examples occurs in take-all decline soils. In Washington State, take-all decline results from the buildup of fluorescent *Pseudomonas* spp. that produce the antifungal metabolite 2,4-diacetylphloroglucinol. Producers of this metabolite may have a broader role in disease-suppressive soils worldwide.

During the past ten years, over 80 biocontrol products have been marketed worldwide. A large percentage of these have been developed for greenhouse crops. Products to control soilborne pathogens such as *Sclerotinia*, *Pythium*, *Rhizoctonia*

and *Fusarium* include *Coniothyrium minitans*, species of *Gliocladium*, *Trichoderma*, *Streptomyces*, and *Bacillus*, and nonpathogenic *Fusarium*. Products containing *Trichoderma*, *Ampelomyces quisqualis*, *Bacillus*, and *Ulocladium* are being developed to control the primary foliar diseases, *Botrytis* and powdery mildew. The development of *Pseudomonas* for the control of *Pythium* diseases in hydroponics and *Pseudozyma flocculosa* for the control of powdery mildew was also reported. (Paulitz and Bélanger, 2002).

Candida guilliermondii (strains 101 and US 7) and *C. oleophila* (strain I-182) were screened for biocontrol activity (BA) against *Botrytis cinerea*. *In vivo* application of both *C. oleophila* (strain I-182) and *C. guilliermondii* (strains 101 and US 7) gave significant control of *B. cinerea* (Saligkarias *et al.*, 2002).