Literature Review

Microorganisms incited plant diseases are numerous, diverse and omnipresent. More than 1,00,000 plant diseases are known to be caused by plant pathogens (fungi, bacteria, viruses, mycoplasmas, viroids etc). They are equally diverse in their ecological niches. Fungi are most damaging and sometimes highly destructive too (Gupta and Mukherjee, 2006). Diseases caused by *Sclerotium rolfsii* are especially rampant in the tropics and subtropics where temperatures are sufficiently high to permit the growth and survival of the fungus. Despite continuous research over the years since the report by Rolfs on *S. rolfsii* on tomato, this pathogen continues to plague growers and cause considerable economic loss. Eruptive germination on soil is greatest at 21-30^{0}C and is less common below 15^{0}C and above 36^{0}C. Although percentage of germination is lower at soil depth below 2.5 cm, than it is at the soil surface and is nil below 8 cm, results from controlled gaseous studies suggest that the inhibition is not due to oxygen depletion or ethylene and carbon dioxide buildup (Punja, 1985). Sclerotia formed in non-sterile soil germinate poorly and less consistently than those produced in laboratory cultures. Mycelium survives better in sandy soils than in fine-texture soils. Sclerotia survive better in soil near the surface rather than buried beneath it. Factors that increase nutrient leakage and the activities of soil microorganisms near sclerotia, or that predispose sclerotia to antagonism, may accelerate the death of sclerotia. The effective plant disease management lies in rapid diagnosis of the disease and detection of its causative agent. The prevalence, the extent of spread and damage is assessed through surveys on the basis of symptoms followed by detection of pathogen in laboratories, which helps to assess, alter and modify the effectiveness of the plant protection measures that are being followed. Apart from the role of detection and diagnosis in plant disease management, detection of pathogen(s) can act as an important tool to localize and prevent the spread of the disease(s). Recognition of the diagnostic potential of such determinants for both experimental and applied investigations in plant pathology has resulted in a bewildering array of techniques being developed referred to as immunoassays. The presence of cross-reactive antigens (CRA) between plant hosts and parasites have been reviewed (De Vay and Adler, 1976; Chakraborty, 1988; Purkayastha, 1994). Immunological techniques used for detection of fungal pathogens in soil, water and plant tissues

have also been reviewed by various workers (Hansen and Wick, 1993; Werres and Steffens, 1994, Chakraborty and Chakraborty, 2003; Gawande *et al.*, 2006). On the other hand detection and quantification of pathogenic inoculum has considerable applicability in diagnosis and management of existing and emerging plant diseases within agricultural crop production systems (Kennedy *et al.*, 1999; 2000). Detecting target particles or spores of plant pathogens in air samples can be used predictively to forecast the likelihood of important disease transmission events. A range of methods have been developed by Kennedy and Wakeham (2006), which can be used to detect inoculum within air samples. In the era of globalization and WTO regime, detection of plant pathogens has an immense role to play because the free movement of materials create chances for introduction of new pathogens across the countries.

A short, selective review is presented on the observation of previous workers in concordance with the present line of investigation on three major aspects (A) Serological relatedness between host and pathogens; (B) Biochemical changes in plants following infection and (C) The effective integrated disease management practices.

Serological relatedness between host and pathogens

Taking advantage of the serological relationship between host and pathogen, the antiserum raised against the pathogen is being used for the detection of the pathogen in the host tissues beginning from the early stages of host pathogen interaction. Commercial diagnostic kits have been offered in recent years for the rapid diagnosis of several fungi in plant tissues, soil and water.

Using the serological techniques of agglutination, gel diffusion and immunofluorescence, Amos and Burrell (1966) identified eight species of the genus *Ceratocystis*. The immunofluorescence technique proved to be the most useful in differentiating among these species. Root antigens from four cotton varieties and isolates of *Fusarium* and *Verticillium* species exhibited common antigen relationship in immunodiffusion tests (Charudattan and De Vay, 1972). Five to eight precipitin bands were observed in the homologous reactions; of these only one or two bands

were present in heterologous reactions. The common antigenic determinant shared by cotton fungal isolates did not appear to be related to the severity of wilt symptoms, but it had affected host-pathogen compatibility during the process of root infection. Using fluorescein isothiocynate (FITC), De Vay *et al.* (1981) demonstrated indirect immunofluorescence in cross-sections of cotton roots. Cross reactive antigenic (CRA) substance was concentrated mainly on xylem elements, the endodermis, epidermal cells and around the cortical tissue. Protoplasts prepared from cross-sections of young cotton roots also contained the CRA concentrated in the region of the plasmalemma. Treatment of conidia and mycelia of *Fusarium oxysporum* with antiserum to cotton and followed by labelling with FITC indicated that CRA was mainly present in hyphal tips and in patch like areas on conidia. Ishizaki *et al.* (1981) investigated the serological cross reactivity of *Sporothrix schenkii* serum with various unrelated fungi by use of immunodiffusion tests. A rabbit anti *S. schenkii* serum was obtained, which related with *Cladosporium werneckii, C. carrionii, C. bantianum, Coccidioides immitis, Phialophora jeanselmi, P. gougerotii, P. dermattidis, Fonsecaea pedrosoi, Asperigillus fumigatus, Histoplasma capsulatum* and *Trichophyton mentagrophytes,* but not with *Saccharomyces cerevisiae* antigens. The serological determinates responsible for the cross-reactions were suggested to be D-galactosyl residue.

Rabbit antisera were raised by Chakraborty and Purkayastha (1983) against the antigens of *Macrophomina phaseolina* (isolate MPI) and roots of soybean cultivars *viz.*, Soymax and UPSM-19, susceptible and resistant to charcoal rot disease respectively. These antisera were used in immunodiffusion and immuno-electrophoretic tests for the presence of common antigens between isolates of *M. phaseolina* and soybean cultivars. Four antigenic substances were found common between the susceptible soybean cultivars and isolates of *M. phaseolina* but no common antigens were detected between resistant cultivars and the fungus. Cross-reactive antigens were also detected between *Phytophthora infestans* and potato cultivars (King Edward and Pentland Dell) using ELISA (Alba and De Vay, 1985). Immunodiffusion, immuno-electrophoretic and cross immuno-electrophoretic analysis of rice antigens using polyclonal antisera raised against *Acrocylindrium oryzae* was done by Purkayastha and Ghoshal (1985). When the antigen preparation

of *A. oryzae* was cross-reacted with its own antiserum or against the antisera of four susceptible rice cultivars, one precipitin band was detected. Precipitin band was detected when antiserum of the resistant cultivar was cross reacted with antigen preparations of three isolates of *A. oryzae*.

Purkayastha and Ghoshal (1987) also compared the antigenic preparations from two isolates of *Macrophomina phaseolina* (causal agent of root rot of groundnut), four non-pathogens of groundnut (*viz., Corticium sasaki, Colletotrichum lindemuthianum, C. corchori* and *Botrytis alii*), and five cultivars of groundnut using immunodiffusion, immunoelectrophoresis and crossed immunoelectrophoresis in order to detect CRA. Common antigens were found among the susceptible cultivars of groundnut and two isolates of *M. phaseolina* but not between non pathogens and groundnut cultivars. No antigenic similarity was found between non pathogens and *M. phaseolina* isolates. Competitive types of two novel enzyme linked immunosorbent assays (ELISA) for *Fusarium* species were developed by Kitagawa *et al.* (1989). Antiserum against a strain (F504) of *F. oxysporum* was elicited in rabbits, and a highly specific, sensitive, and accurate ELISA for the homologous strains was developed by using the antiserum with β-D-galactosidase-labeled anti-rabbit IgG as the secondary antibody and cell fragments of the strain attached to amino-dylark balls as the solid-phase antigen. All other micro-organisms tested, including nine other strains of *Fusarium*, showed little cross-reactivity. When cell fragments of *F. oxysporum* (F504) attached to the balls were used as a solid phase antigen in a heterologous competitive ELISA, the modified system was a general assay for 10 strains of four *Fusarium* species with a high sensitivity. The specificity of the heterologous competitive ELISA was shown to be limited to *Fusarium* species.

Fuhrmann *et al.* (1989) raised antisera from a rabbit immunized with *Penicillium verrucosum* var. *verrucosum*. These antisera were characterized by immunofluorescence and by indirect enzyme-linked immunosorbent assay for their reactivity with 44 strains of moulds. Antigenically, *P. verrucosum* var. *verrucosum* (subgenus *Penicillium*) appeared to be similar to strains belonging to subgenus *Furcatum*, but strongly different from *Penicillium frequentans* (subgenus

Aspergillides) specific absorption of antibodies to antigens confirmed the existence of similar biochemical structures on *Penicillium frequentans, Aspergillus versicolor* and *Aspergillus fumigatus.* Immunological procedures may thus significantly contribute to refine the taxonomic classification of moulds. Purkayastha and Banerjee (1990) studied common antigenic relationships between seven soybean cultivars, their pathogens and non pathogens using immunodiffusion, immunoelectrophoresis and indirect ELISA technique. CRA between susceptible soybean cultivars and the virulent strain of *Colletotrichum dematium* var. *truncate* were detected but no CRA could be detected between avirulent pathogen or non-pathogen and soybean cultivars.

Antibodies to three isolates each of *Armillaria mellea, A. ostoyae, A. tabescens* and *Lentinula edodes* were isolated from eggs of immunized laying hens. The reactivity of each antibody preparation with the isolates was examined using an enzyme-linked immunosorbent assay (ELISA). The cross-reactivity of the antibody preparations to a given *Armillaria* species varied considerably when tested against isolates of other *Armillaria* species. Several antibody preparations were capable of distinguishing isolates of homologous species from isolates of heterologous species. The specificity of the antibodies present in eggs was dependent on time elapses since immunization (Burdsall *et al.,* 1990). Rabbit antisera were raised against one resistant cultivar (UPSM-19), two susceptible cultivars (DS-74-24-2 and PK-327) of soybean and three isolates of *Myrothecium roridum* (M-1, ITCC-1143 and ITCC-1409) for analysis of CRA shared between host and pathogen. Results of immunodiffusion revealed that common antigens were present only between the virulent strain and susceptible host cultivars which was confirmed by presence of one common antigen in immunoelectrophoretic analysis. In case of resistant cultivars (UPSM-19 and DS-73-16) no CRA was detected (Ghose and Purkayastha, 1990).

Sundaram *et al.* (1991) prepared antisera against purified mycelial proteins from *Verticillium dahliae,* the predominant fungus species in the potato early dying complex. The tested antisera against crude mycelial preparations of *Verticillium* spp. using indirect enzyme-linked immunosorbent assay (ELISA) reacted positively with 11 of 12 *V. dahliae* isolates from potato, cotton and soil but negatively with one isolate from tomato. The antisera did not react with mycelial proteins from *Fusarium*

spp. from potato and cotton, with a *Colletotrichum* sp. from potato, or with one isolate of *Rhizoctonia solani* from sugarbeet. Double antibody sandwich (DAS) - ELISA, using polyclonal antisera, detected *V. dahliae* and *V. albo-atrum* in infected roots and stems of potato. Ricker *et al.* (1991) detected water soluble antigens produced by *Botrytis cinerea* in spiked and naturally infected grape juice by using an enzyme immunoassay with an indirect format of antibody horseradish-peroxidase conjugate bound to polyclonal rabbit antibodies directed against *B. cinerea*. Daniel and Nilsson (1991) raised polyclonal antiserum against mycelial extracts from *Phialophora mutabilis a* wood degrading soft rot fungus. In ELISA, the antiserum reacted strongly to moderately with six soft rot *Phialophora* species. With exception of *Cerotocystis albida,* the serum reacted weakly or not at all with 11 other mold, and rot fungi occurring frequently in or on wood. The antiserum was cross-reacted strongly with antigens in extracellular filtrates from *P. mutabilis* cultures that contained about 40mg / ml of protein. Ultrastructural and immunocytochemical studies on wood degraded by *P. mutabilis* showed specific localization of the fungal cell wall and certain intracellular structures. Extracellular labeling within soft rot cavities and sites of erosion decay of wood also were noted. The antiserum was assessed by ELISA for detecting the presence of fungus and soft rot in untreated and preservative treated wood blocks of pine and birch degraded for periods of 1-12 months. *P. mutabilis* was detected in samples from all wood blocks degraded to low or high weight loss. Highest ELISA readings were recorded for wood blocks with highest substrate losses and vice versa.

A polyclonal, enzyme-linked immunosorbent assay of *Phytophthora infestans* has been developed by Beckman *et al.* (1994) for use in the determination of fungal biomass during the early stages of infection of tuber disc of *Solanum tuberosum.* By optimizing the dilution of sample extracts and the dilution of primary anti *P. infestans* antiserum, quantification of the biomass of *P. infestans* in zoospore inoculated tuber disc could be achieved by 8-18h after inoculation. Differences in growth between avirulent and virulent isolates of *P. infestans* on the resistant potato cv. Kennebec were quantified by 32-48h after inoculation. Together with a comparison of growth of the same isolates on the susceptible cv. King Edward, these results comprised on ELISA of the Quadratic Check. On the resistant host, the

growth of the avirulent isolate was essentially arrested by 16h after inoculation, whereas that of the virulent isolate continued throughout the time course. On the susceptible host, however, the avirulent isolate appeared more aggressive than the virulent isolate. These results demonstrated that ELISA, which is often simpler to perform than other procedures for estimating fungal growth, may be used to complement biochemical studies of rapidly induced plant defence response.

Purkayastha and Pradhan (1994) studied serological differences between three strains of *Sclerotium rolfsii* and groundnut cultivars. Among three strains of *S. rolfsii*, 266 was most virulent and exhibited antigenic relationship with susceptible groundnut cultivars (AK-12-24, Gangapuri and J-11). However, resistant cultivars (ICGS-26 and JL-24) showed no antigenic relationship with fungal strains in either immunodiffusion or immunoelectrophoretic test.

Brill *et al.* (1994) produced polyclonal antibodies against immunogen preparations from culture filtrate and mycelial extract of *Phomopsis longicalla* and soybean. The PAbs were purified to the immunoglobulin fraction and tested in indirect ELISA and in DAS-ELISA. The PAbs raised to culture filtrate were more specific but less active in binding to members of the *Diaporthe- Phomopsis* complex than were those raised to the 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 with indirect ELISA. Immunization of one rabbit with culture filtrate over an extended time resulted in maximum anti- *P. longicolla* activity after three immunizations and the activity became constant against most members of the complex at the same time.

CRA were found among the isolates of *Bipolaris carbonum* and susceptible tea varieties (Chakraborty and Saha, 1994a). Such antigens were not detected between isolates of *B. carbonum* and resistant varieties, non pathogens and tea varieties as well as non pathogens and isolates of *B. carbonum*. Indirect staining of antibodies using FITC indicated that in cross sections of tea leaves the CRA were concentrated mainly around epidermal cells. Treatment of mycelia and conidia of

B. carbonum with antisera to tea leaves (TV-18) and indirect staining with FITC indicated the presence of CRA in the young growing hyphal tips of conidia.

Polyclonal antisera were raised against mycelial suspensions of *P. theae* (isolate Pt-2) causal agent of gray blight disease and leaf antigens of Teen Ali 17/1/54 and CP-1 and immunological tests were performed in order to detect CRA shared by the host and parasite (Chakraborty *et al.*,1995a). CRA were found among the susceptible varieties and isolates of *P. theae*. Such antigens were not detected between isolates of *P. theae* and resistant varieties, *B. tetramera* and tea varieties or isolates of *P. theae*. Indirect staining of antibodies using FITC also indicated the presence of CRA in the epidermal cells and mesophyll tissue of tea leaves. CRA was evident in the young hyphal tips of the mycelia and on the setulae and appendages of the conidia of *P. theae*.

Chakraborty *et al.* (1996b) raised polyclonal antisera against leaf antigens to tea varieties (TV-18, Teen Ali 17/1/54 and CP-1) and mycelial antigens of *G. cingulata* (isolate GC-1) separately in white rabbits. CRA were found among the susceptible varieties and *G. cingulata* isolates. Such antigens were not detected between *G. cingulata* and resistant varieties of tea, non pathogen and tea varities as well as *G. cingulata* and non pathogens. In cross sections of tea leaves (TV-18), the CRA was found to be concentrated in epidermal cells, mesophyll tissue and vascular elements. Chakraborty *et al.* (1997b) also determined the presence of CRA between *Fusarium oxysporum* and soybean cultivars. Antigens were prepared from the roots of ten varieties of soybean and mycelium of *F. oxysporum*. Polyclonal antisera were raised against the mycelial suspension of *F. oxysporum* and root antigen of susceptible soybean cultivar (UPSM-19). The immunoglobulin (IgG) fraction of those antisera were purified by ammonium sulfate precipitation & DEAE-Sephadex column chromatography. In enzyme-linked immunosorbent assay, antigens of susceptible cultivars showed higher absorbance values when tested against the purified anti-*F. oxysporum* antiserum. Antiserum produced against UPSM-19 showed cross-reactivity with the antigens of other cultivars. Indirect staining of antibodies using FITC indicated that CRA were concentrated around xylem elements, endodermis and epidermal cells in cross-section of roots (UPSM-19), while in the resistant variety, fluorescence was concentrated mainly around

epidermal cells and distributed in the cortical tissues. CRAs were also present in microconidia, macroconidia and chlamydospores of the fungus. Polyspecific antisera were raised against a plant cell supernatant fraction from homogenized naturally blister infected tea leaf tissue (AV-2) and immunoprecipitated with healthy leaf antigens of AV-2 in order to separate antibodies unique to *E. vexans*. Immunoglobulin (IgG) was purified following ammonium sulphate fractionation and chromatography on DEAE-Sephadex. CRA were detected between the pathogen and the susceptible Darjeeling tea varieties in immunodiffusion tests and enzyme-linked immunosorbent assay (Chakraborty *et al.* 1997a).

Gupta *et al.* (2000) had done immunodetection of teliospores of *Telletia indica* which is a causal agent of Karnal bunt (KB) of wheat, by using fluorescent staining test. Polyclonal antibodies were raised against teliospores and indirect immunofluorescence (IIF) test was developed using anti-teliospores serum and binding was monitored by goat-rabbit antibody conjugated to FITC. The standardization of IIF test was carried out by optimization of dilution of anti-teliospores antibodies, fluorescent probe and exposure time. The teliospores of *T. indica* showed bright green, patchy and ring shaped fluorescence around the teliospore. The spore exhibited uniform distribution in discrete regions of spore probably in spore episporium. Similar fluorescence pattern in the teliospores of KB isolated from infected wheat seeds of cultivars HD 23328, UP 2338, PBW 393, WH 542, as well as RR 21 (susceptible cultivars) respectively, is an indication of the presence of similar antigenic configuration of teliospores. Again, they did not exhibit variation in the expression of teliospore associated molecular pattern during previous and subsequent years of infection. Polyclonal antiserum raised against *T. indica* also reacted strongly in agglutination reaction with intact teliospores of Pantnagar isolate. The wheat grains with different grades of infection could be readily detected by Seed Immunoblot Binding Assay (SIBA). The teliospores of karnal bunt infected wheat seeds when kept for vigour reacting on nitrocellulose paper, formed a coloured imprint after the paper was assayed. The SIBA developed was not only a better indication of teliospores load on seed but also quality of seed in terms of vigour. The developed immunodetection method apparently proved to be useful in routine

monitoring of wheat lots for the presence of karnal bunt pathogen (Kumar *et al.*, 2000)

Viswanathan *et al.* (2000) performed ELISA using PAb raised against *Colletotrichum falcatum* to detect pathogen before the symptom development. When 20 different sugarcane varieties were subjected to ELISA test after pathogen inoculation, it showed a clear variation in disease resistance among them as in field testing. Chakraborty *et al.* (2000) had demonstrated immunological detection of *Sphaerostilbe repens, Trichoderma viride* and *T. harzianum* using DAC-ELISA formats to develop strategies for management of violet root rot of tea. Polyclonal antibody based immunoassay for detecting *Fomes lamaoensis,* causing brown root rot disease of tea has also been developed (Chakraborty *et al.*, 2001c). Eight blood samples were collected and IgG were purified using DEAE cellulose. Immunodiffusion tests were performed in order to check the effectiveness of mycelial antigen preparations of *F. lamaoensis* for raising PAbs. Optimization of PAbs was done using indirect ELISA. Increased activity of PAbs against *F. lamaoensis* could be noticed from second bleedings, which continued up to fourth bleeding. Root antigens prepared from healthy and artificially inoculated (with *F. lamaoensis*) tea plants (Teen Ali 17/1/54, TV-18, TV-22, TV-26, TV-27, TV-28, TV-30, S-449, BSS-2) were analysed following DAC-ELISA format. Such format was also used to detect the pathogen in infested soil. Young mycelia of *F. lamaoensis* gave bright fluorescence in indirect immunofluorescence tests using PAbs and FITC-conjugates of goat specific for rabbit globulin. Such immunological assays developed for detection of *F. lamaoensis* in rhizosphere of tea plantations can enable disease prevention at an early stage.

Immunodiagnostic kits were developed for detection of *Ustulina zonata,* causing charcoal stump rot disease, in the soil and tea root tissues. PAbs were raised separately against mycelial and cell wall antigens prepared form 10-day-old cultures of *U. zonata.* Optimization of PAbs was done using indirect ELISA. Two different ELISA formats such as direct antigen coated (DAC) and double antibody sandwich (DAS) were tested to detect the pathogen in soil and artificially inoculated root tissues. Indirect immunofluorescence using PAbs and FITC conjugates of goat

specific for rabbit globulin were assessed for their potential to detect mycelia and spores in soil (Chakraborty *et al.*, 2002b).

Chakraborty *et al.* (2002d) studied serological cross reactivity between *Glomerella cingulata* and *Camellia sinensis.* PAbs were raised against antigen preparations from mycelia and cell wall of *G. cingulata* (isolate Gc-1), causal agent of brown blight of tea, mycelia of *Fusarium oxysporum* (non pathogen of tea) and leaf antigens of TV-18 and CP-1. CRA were found among the susceptible varieties of tea and isolates of *G. cingulata* (Gc-1, 2 and 3). Such antigens were not detected between resistant varieties of tea and isolates of *G. cingulata* (Gc-1, 2 and 3); non pathogen (*F. oxysporum*) and tea varieties; isolates of *G. cingulata* and *F. oxysporum* and between non host (*Glycine max*, *Cicer arietinum* and *Camellia japonicum*) and *G. cingulata.* Antisera raised against cell wall preparations gave better recognition than that against mycelial preparations as observed in ELISA test with antigens of tea leaves of different ages.

Biochemical changes in plants following infection

Disease develops in individual plants by a series of sequential steps beginning with the arrival of inoculum at the plant surface and ending with the terminal stages of pathogens. There are many defense barriers in plants such as the cuticle, cell wall or constitutive antimicrobial compounds as well as defenses triggered by the invader. The success or failure of infection is determined by dynamic competition and the final outcome is determined by the sum of favourable and unfavourable conditions for both the pathogen and host cells. One of the most important and well documented host responses is the biochemical changes following infection. Phenols in plants which occur constitutively are thought to function as preformed inhibitors associated with non host resistance. Since the phenolic intermediates have a role in the active expression of resistance, an underlying problem in ascertaining that such secondary metabolites are of primary importance has been the localization and timing of the host response (Nicholson and Hammerschmidt, 1992).

In potato tubers chlorogenic acid was reported to accumulate slower following inoculation with *P. infestans* than in non-inoculated controls, regardless of cultivar resistance (Gans, 1978). In contrast, in some susceptible cultivars chlorogenic acid accumulates at an accelerated rate after inoculation (Henderson and Friend, 1979). The differentiation of the responses of plants to pathogens based on host and non host interactions has been argued by Heath (1980).

Chlorogenic acid acts as a reservoir for the caffeoyl moiety that, as an activated phenylpropanoid, could be shunted to the synthesis of other phenolics possibly involved in containment of the pathogen (Friend, 1981). The accumulation of chlorogenic acid may represent a general rise in phenolic biosynthesis which can ultimately result in the accumulation of compounds with sufficient toxicity to be involved in resistance. When carrot root slice is infected with *Botrytis cinerea*, the infection leads to the production of inhibitors such as 6-methoxymellein, p-hydroxybenzoic acid and falcarinol (Harding and Heale, 1981). Oat produces nitrogen containing phenolic phytoalexins, the avenalumins, and these compounds accumulate only in incompatible host pathogen interactions (Mayama *et al.*, 1981).

Mayama and Tani (1982) took advantage of the UV-absorbance and auto fluorescence spectra of the avenalumins and used microspectrophotometry to reveal the presence of intense fluorescence only in cells immediately associated with the infection site. Rapid accumulation of phenols may result in the effective isolation of the pathogen (or non pathogen) at the original site of ingress (Legrand, 1983; Ride, 1983). In potato, phenols accumulate as an initial response to infection (Hammerschmidt, 1984; Hachler and Hohl, 1984). The accumulation of polymerized phenols also occurs as a rapid response to infection. Hydroxycinnamic acids and their derivatives are thought to contribute to the discoloration and autofluorescence of host tissues at the site of infection (Farmer, 1985; Bolwell *et al.*, 1985).

Werder and Kern (1985) demonstrated resistance of maize to *Helminthosporium carbonum* and subsequent changes in host phenolics and their antifungal activity. Maize inbreds Prl (resistance) and Pr (susceptible) to *B. zeicola* race 1 were inoculated and phenolic material was extracted from maize leaf tissue. The components were then analyzed and resistance was studied with respect to

phenol metabolism and accumulation of fungitoxic compounds. Host responses could be differentiated by changes in content of phenolic compounds. The pattern of changes of total phenolic content (hydrolyzed and unhydrolyzed ethylacetate soluble phenols) of resistant and susceptible inbreeds did not differ much between 0 h and 96 h. after inoculation. However, phenolics content in the resistant inbreed increased between 96 and 120 h after inoculation to a level two to three times higher than that of susceptible and non-infected control in breeds. They isolated four antifungal compounds, A, B, C and D from hydrolyzed maize leaf extracts. All four compounds were fungitoxic to *B. zeicola* in spore germination and chromatographic bioassays. Compounds A and B were inhibitory to *B. zeicola* only in high concentrations. The investigators suggested a role of the phenol metabolism in the resistance of maize to *B. zeicola* based on different content of total phenolics in resistance and susceptible inbreeds. The compounds C and D were supposed to play a role in the resistance mechanism as fungitoxic components.

Saxena *et al.* (1986) evaluated the changes in phenolics of two each of resistant and susceptible varieties of wheat leaves in response to *Puccinia recondita* causing brown rust. They found that resistant varieties exhibited higher concentration of phenolics than the susceptible ones. Esterification of phenols to cell-wall materials has been considered as a primary theme in the expression of resistance (Fry, 1986; 1987). Biochemical analysis of pea varieties resistant and susceptible to *Erysiphe polygoni* causing powdery mildew disease revealed that the quantity of total phenol and ortho-dihydroxyphenol was higher in stem and leaves of resistant varieties as compared to susceptible ones which decreased as the age of plant increased in all the varieties (Parashar and Sindhan, 1987).

The temporal and spatial differences in the accumulation of phenylalanine ammonia lyase (PAL) mRNA occurred as a response on incompatible race of fungus, whereas a significantly different profile of mRNA accumulation occurred in interactions involving a compatible race (Cuypers *et al.*, 1988). The kinds of phenolic compounds that accumulate prior to the active defence response as well as their origin has been addressed by Matern and Kneusel (1988) using parsley leaves with *P. megasperma* f. sp. *glycinea* (Pmg) or treatment of parsley cell suspensions with a Pmg elicitor results in the accumulation of substantial concentrations of

coumarin phytoalexins as well as esterification of phenylpropanoids, in particular ferulic acid, to cell walls. Treatment of parsley cells with the Pmg elicitor causes the synthesis of the coumarin phytoalexins isopimpinellin, psoralen, bergapten, xanthotoxin and graveolone. The healthy leaves of *Morinda tomentosa* contained the two methoxyflavonols 4'–OMe Kaempferol and 3', 4' – dia OMe quercetin, and the four phenolic acids – vanillic, syringic, gentisic and ferulic. The *Colletotrichum gloeosporoides* infected leaves contained the hydroxyflavonols kaempferol and quercetin along with four phenolic acids found in healthy leaves. The diffusates of both the pathogen and non-pathogen (*F. solani*) treated leaves contained quercetin and kaempferol (Abraham and Daniel, 1988).

Matern and Kneusel (1988) have proposed that the defensive strategy of plants exists in two stages. The first is assumed to involve the rapid accumulation of phenols at the infection site, which function to slow (or even has) the growth of the pathogen and to allow for the activation of "secondary" strategies that would more thoroughly restrict the pathogen. Secondary responses would involve the activation of specific defenses as the *de novo* synthesis of phytoalexins or other stress-related substances. They argue that the initial defense response must occur so rapidly that it is unlikely to involve *de novo* transcription and translation of genes, which would be characteristic of the second level of defence. The sequence of events in a defence response can be thought to include – host cell death and necrosis, accumulation of toxic phenols, modification of cell walls by phenolic substituents or physical barriers such as appositions or papillae, and finally, synthesis of specific antibiotics such as phytoalexins.

Prasada *et al.* (1988) also reported that after infection total phenol increased in green and ripe tomato fruits in case of rotting due to *Sclerotium rolfsii*. There is often a greater increase in phenolic biosynthesis in resistant host species than in susceptible hosts and it is sometimes postulated that the increase in phenolic compounds is part of the resistance mechanism. Some of these compounds are toxic to pathogenic and non-pathogenic fungi and have been considered to play an important role in disease resistance (Vidyasekharan, 1988). Changes in phenol contents were determined by Oke (1988) in healthy and *Colletotrichum nicotianae* infected leaves of tobacco. After infection the quantity of total phenols and ortho

dihydroxy phenol increased in both stem and leaves of susceptible and resistant varieties.

The changes in phenolic and nitrogen metabolism were investigated by Tore and Tossi (1989) in healthy and infected (with *Thielaviopsis basicola*) tobacco roots and leaves. The cholorgenic acid content increased in infected root and leaves compared with the control beginning on the 8^{th} day after inoculation. Polyphenol content in sweet cherry bark was drastically changed after infection by *Cercospora personii* (Bayer, 1989). Infected tissue and closely neighboring areas were characterized by the appearance of phenolic aglycones which inhibited growth of both the pathogen. Mechanically wounded bark tissues showed different phenolic patterns than infected ones.

Etenbarian (1989) detected quantitative changes in phenolic compounds at different time intervals on barley varieties inoculated with *Puccinia hordei*. Luthra (1989) detemined the levels of total phenol in sorghum leaves, resistant and susceptible to *Ramulispora sorghicola* at 15-day-intervals after 25 day of sowing. Resistant varieties exhibited high phenol content in comparison to susceptible ones at all stages of growth.

Phenolic compounds inhibitory to the germination of spores of *Colletotrichum graminicola* were shown to leach from necrotic lesions on corn leaves caused by the fungus. Primary components of the phenolic mixture were identified as esters and glycosides of p-coumaric and ferulic acids as well as the free compound themselves. Spores of *C. graminicola* produced in acervuli of infected leaves were shown to be surrounded by a mucilaginous matrix as in the case when the fungus is cultured *in vitro*. It is suggested that the mucilage protects spores from the inhibitory effects of the phenols by the presence of proline rich proteins that have been shown to have a high binding affinity for a variety of phenols (Nicholson *et al.*, 1989). The relatively non-specific disruptive effects on cells that result from wounding lead almost immediately to a variety of physiological changes, including oxidation of secondary metabolites. The accumulation of these esters preceded the onset of visible necrosis of infection sites, the concentration of the compound fell substantially after the onset of necrosis both of which strengthen the argument for

their involvement in the browning response (Bostock and Stermer, 1989). Toxic phenylpropanoids, such as ferulic acid, can form rapidly without the involvement of the traditionally accepted route of phenyl-propanoid synthesis and conversion to CoA esters (Hahlbrock and Scheel, 1989). It has long been recognized that responses are characterized by the early accumulation of phenolic compounds at the infection site and that limited development of the pathogen occurs as a result of rapid (hypersensitive) cell death (Fernandez and Heath, 1989).

Baker *et al.* (1989) examined specific race interaction with clones of resistant and susceptible genotypes and they found greater accumulation of phenolic compounds in resistant reaction than in susceptible reaction. They suggested that accumulation of phenolics may play a role in natural and induced interaction involving *Colletotrichum trifolii* and *Medicago sativa.*

Kumar *et al.* (1990) analysed certain biochemical changes in the pearl millet shoots infected with downy mildew pathogen (*Sclerospora graminicola*). The estimation revealed that the total phenol and free amino acids content were found to be low both in diseased shoot and roots of pearl millet (*Pennisetum glaucum*). A marked accumulation of two caffeic acid esters after inoculation of maize with *Glomerella graminicola* or *Cochliobolus heterostrophus* was reported in both compatible and incompatible combinations (Lyons *et al.*, 1990). One compound was identified as caffeoyl glucose, whereas the other was a caffeoyl ester of an unknown organic acid moiety. Although neither compound was fungitoxic, a pattern of rapid accumulation followed by a sharp decrease in the amount of both compounds in the tissue suggested that they may serve as a pool of phenols required for diversion to other products. Seasonal changes in the phenolic constituents of jack pine seedlings (*Pinus banksiana*) in relation to the purpling phenomenon were studied by Nozzolillo *et al.* (1990).

Mansfield (1990) has proposed that cell death results from irreversible membrane damage that may occur in response to pathogen recognition or as a result of activated host response. Niemann *et al.*, (1991) demonstrated that low molecular weight phenols, such as benzoic acids and the phenylpropanoids, are formed in the initial response to infection. Early after infection, low molecular weight phenols

accumulate in both incompatible (resistant) and compatible (susceptible) interactions. Whether these compounds, are significant in the ultimate host response presents a perplexing problem. Bruzzese and Hasan (1991) demonstrated that accumulation of phenols at the infection site occurred as early as 3h after inoculation, indicating an association of phenols with the initial stages of the response. The contents of phenols, o-dihydroxy phenols and peroxidase activity in healthy and *Curvularia andrepogonis* infected leaves of *Java citronella* (*Cymbopogan winterianus*) were determined by Alam *et al.* (1991). As a result of infection the content of phenols and peroxidase increased two and four fold respectively, in necrotic lesions compared to healthy leaves. It has been suggested by Permulla and Heath (1991) that the accumulation of phenolics as an initial response to infection may reflect a general increase in host metabolism as well as an accumulation of relatively non-toxic secondary metabolites, which could ultimately serve as precursors for compounds essential for expression of resistance. In the interaction of potato tubers with *Verticillium dahliae* hypersensitive browning and suberization are characteristic of the initial events in resistance rather than production and accumulation of phytoalexins (Vaughn and Lulai, 1991).

The *Fusarium* sp. infected leaves of *Trianthema portulacastrum* contained 6, 7, dimethoxy-3, 5, 4' – trihydroxy flavone, vanillic acid, p-hydroxybenzoic acid, quercetin and ferulic acid. By using drop diffusate technique it was found that the pathogen induces the formation of quercetin and ferulic acid (Darshika and Daniel, 1992). Changes in carbohydrates, amino acid and phenolic contents in jute plant on inoculation with *Macrophomina phaseolina, Colletotrichum corchori* and *Lasiodiplodia theobromae* were studied by Sahabuddin and Anwar (1992). Total sugars, non-reducing sugars, starch and total free amino acids were found to decrease on inoculation with all the three test pathoges of jute, while reducing sugars, total phenols and orthodihydric phenols increased.

Fifteen isolates of *Phytophthora parasitica,* nine from tobacco (causing black shank disease) and six from other host plants were compared by root inoculation with regard to their pathogenicity to young tobacco plants. A progressive invasion of the aerial parts over 1 week was observed only with the black shank isolates, while the non-tobacco isolates induced leaf necrosis within 2 days. Similar necrosis

occurred when the roots of tobacco plants were dipped in diluted culture filtrates from non-tobacco isolates, but not in those from tobacco isolates. The necrosis inducing filtrates contain 10 kDa protein band which was not present in the other filtrates. This protein (named parasiticein) was purified by ion exchange chromatography to homogenicity in SDS-PAGE and reverse phase HPLC. Parasiticein was serologically related to cryptogein, a member of the elicitin family of proteinaceous elicitors. Like the other elicitins, parasiticein induced necrosis in tobacco plants and protected them against black shanks. It most closely resembled little leaf necrosis. Ricci *et al.* (1992) suggested that the absence of parasiticein production by the black shank isolates might be a factor involved in their specific pathogenicity to tobacco.

A glycoprotein elicitor of phytoalexin accumulation in leaves of *Phaseolus vulgaris* produced well before lysis in the medium of cultures of *Colletotrichum lindemuthianum* was purified to homogeneity by Coleman *et al.* (1992). The glycoprotein was a monomer of M.W. 28 kDa. The glycosyl side chains which accounted for 43% of the weight of the holoprotein, were composed principally of galactose, mannose and rhamnose exhibited a minimum degree of polymerization of eight and were apparently O-linked to abundant serine and / or threonine residues of the peptide backbone. In a *P. vulgaris* leaf infection bioassay the purified glycoprotein had activity easily detectable at nanomolar concentrations and induced browning of the treated tissue and also the accumulation of both phenylalanine ammonia-lyase and the isoflavanoid phytoalexins phaseollin isoflavone. For these three linked defence responses, sub optimal concentrations of the glycoprotein induced respectively 4.2, 7.6 and 9.7 fold more activity in the cultivar resistant to race delta (cv. Kievit) than in a cultivar susceptible to that race (cv. Pinto). Protein integrity was not required for elicitor activity and glycosyl side-chains isolated from the protein were shown to be active elicitors. The effects of an elicitor from *Colletotrichum graminicola* was studied by Ransom *et al.* (1992). Roots of sorghum (*Sorghum biocolor*) accumulated 3-deoxyanthocyanidin phytoalexins in response to CG elicitor. Elicitation of the phytoalexins prior to treatment with the elicitor did not prevent infection and development of disease symptoms in susceptible seedlings inoculated with conidia of *Periconia circinata*. However, treatment of roots with the

CG elicitor enhanced the synthesis of 16 kDa proteins in both resistant and susceptible genotypes without expression of disease symptoms.

Effect of the elicitor and the suppressor from a pea pathogen, *Mycosphaerella pinodes*, on polyphosphoinositide metabolism in pea plasma membrane were examined *in vitro* by Toyoda *et al.* (1992). Lipid phosphorylation in the isolated pea plasma membrane was drastically stimulated by the elicitor, but markedly inhibited by the suppressor. A similar inhibitory effect was observed by the treatment with orthovande or K-252a that blocked pisatin production induced by the elicitor. Neomycin, an aminoglycoside antibiotic that interacts with the polyphosphoinositide metabolism, also affected the lipid phosphorylation *in vitro* and blocked the elicitor induced accumulation of pisatin *in vivo*. Rapid changes of polyphosphoinositide metabolism in pea plasma membranes, in one of the indispensable processes during the elicitation of defence responses. Cell walls of germ tubes from wheat stem rust (*Puccinia graminis* f. sp. *tritici*) contain a glycoprotein with a residues in the carbohydrate moiety because periodate, but not trypsin or pronase destroyed activity. These results suggest that the Pgt elicitor is released from hyphal cell walls into the wheat protoplast during stem rust infection.

The elicitor induced incorporation of phenylpropanoid derivatives into the cell wall and the secretion of soluble coumarin derivatives (phytoalexins) by parsley (*Petroselinum crispum*) suspension cultures can be potentiated by pretreatment of the cultures with 2, 6-dichloroisonicotinic acid or derivatives of salicylic acid. The cell walls and an extra cellular soluble polymer were isolated by Kauss *et al.* (1993) from control cells or cells treated with an elicitor from *Phytophthora megasperma* f. sp. *glycinea*. After alkaline hydrolysis, both fractions from elicited cells showed a greatly increased content of 4-coumaric, ferulic, and 4-hydoxybenzoic acid, as well as 4-hydroxybenzaldehyde and vanillin. Two minor peaks were identified as tyrosol and methoxy tyrosol. The pretreatment effect is most pronounced at a low elicitor concentration. Its specificity was elaborate for coumarin secretion. When the parsley suspension cultures were pre-incubated for 1 day, with 2, 6-dichloroisonicotinic, 4- or 5-chlorosalicylic, or 3, 5- dichlorosalicylic acid, the cells exhibited greatly increased elicitor response. Pretreatment with isonicotinic, salicylic, acetylsalicylic, or 2, 6-dihydroxybenzoic acid was less efficient in enhancing the response, and some

other isomers were inactive. This increase in elicitor response was also observed for the above mentioned monomeric phenolics, which were liberated from cell walls upon alkaline hydrolysis and for "lignin like" cell walls polymers determined by the thioglycolic acid method. It was shown for 5-chlorosalicylic acid that conditioning most likely improves the signal transduction leading to the activation of genes encoding phenylalanine ammonia lyase and 4-coumarate: coenzyme A ligase. The conditioning thus sensitizes the parsley suspension cells to respond lower elicitor concentration. If a similar mechanism were to apply to whole plants treated with 2, 6-dichloroisonicotic acid, a known inducer of systemic acquired resistance, one can hypothesize that fungal pathogens might be recognized more readily and effectively.

The elicitor molecules that function *in vivo* for phytoalexin elicitation in soybean (*Glycine max*) infected with *Phytophthora megasperma* f. sp. *glycinea* have been identified as β-1, 6-and β-1, 3-linked glucans that are released from fungal cell walls by β-1,3-endoglucanase contained in host tissue. Yoshikawa and Sugimoto (1993) identified the putative receptor like target sites for glucanase-released elicitor in soybean membranes. The binding was dependent on the pH of the incubation chamber, as well as on the duration and temperature of the incubation. The binding of the glucanase released elicitor to membranes was abolished by both heat and proteolytic enzymes. Therefore, the binding site was probably composed of proteinaceous molecules.

Resistance or virulence are modelled by multiple biochemical components of two living organisms. *Costus speciosus* a major sapogenin bearing medicinal plant was severely affected by *Drechslera rostrata* causing leaf blight disease. An interesting interaction phenomenon was noticed by Kumar *et al.* (1995). The HPLC analysis indicated the accumulation of glyceollin II and III as potent phytoalexins by *C. speciosus* in response of non pathogenic *D. longirostrata.* Further the presence of a polysaccharide elicitor or mycelial wall component seems to be detrimental cause of phytoalexin accumulation. The same elicitor was also present in mycelial wall of pathogenic *D. rostrata* but in much lower concentration. Additionally it was associated with another polysaccharide component with different identity. The bioassay method of elicitor preparation was expressed in terms of antimicrobial activity mediated through glyceollins. It was determined to be 88.6% in incompatible

which was considerably low (13.7%) in pathogenic reaction. During the pathogenesis of *D. rostrata* the susceptibility was not only exercised with low concentration of elicitor but also being mediated with the association of additional carbohydrate component of mycelial wall hence expressing the involvement of multiple biochemical components to regular susceptibility. The non specific elicitors (which include proteins, glycoproteins, various types of oligosaccharides and unsaturated fatty acids) are more difficult to assign a role in the induction of phytoalexin production by pathogens (Hahn, 1996). A race specific elicitor has been isolated from *Uromyces vigna*. This elicitor can induce phytoalexin production in cowpea resistant to this race of the pathogen based on hypersensitive response (HR) – like symptoms induced by treatment of resistant cowpea leaves with the elicitor (D'Silva and Heath, 1997). The presence of phenolic acids in cell wall - esterified p-coumaric acid and ferulic acids bound to cell wall polysaccharides are widespread in Gramineae. Cell wall bound phenolics in resistance to rice blast disease was demonstrated by Kumar *et al.* (1997). The relative roles of glyceollin, lignin and the hypersensitive response (HR) in pathogen containment and restriction were investigated in soybean cultivars that were inoculated with *Phytophthora sojae*. Incompatible interactions in leaves and hypocotyls were characterized by HR, phenolic and lignin deposition and glyceollin accumulation. The uncoupling of glyceollin synthesis is a major factor in restriction of the pathogen during these interactions (Mohr and Cahill, 2001).

The response of bavistin on disease incidence, phenolic compounds and their oxidative enzymes, non-structural carbohydrates, different forms of nitrogen and mineral content in cowpea roots susceptible to *Rhizoctonia solani* and *R. bataticola* was reported by Kalim *et al.* (2000). Bavistin (0.2%) as seed treatment significantly reduced the incidence of root rot of cowpea to the extent of 57.5 and 58.9 percent in case of *Rhizoctonia solani* and *R. bataticola,* respectively. Reduction in disease incidence has been attributed to the increased activities of polyphenol oxidase (PPO) and peroxidase (PO) along with higher amounts of total phenols. PO activity was several times more as compared to PPO specific activity. Contrary to PPO and PO the specific activity of catalase declined sharply. Bavistin seed treatment also caused

an increase in reducing sugars, Cu, Zn and Mn but a decrease in o-dihydric phenols, flavanols, total soluble sugars, non-reducing sugar and Fe contents.

The effect of phenolics and related compounds on pectinolytic enzymes of *Sclerotinia sclerotiorum,* a phytopathogenic fungus causing white rot in pea (*Pisum sativum*) had been studied by Sharma *et al.* (2001). Activities of both pectinases (polygalacturonase and pectin methyl esterase) from *S. sclerotiorum* increased with the growth period of fungus upto 7 days of growth and declined as the growth period was further progressed. Polygalacturonase (PG) and pectin methyl esterase (PME) had pH optima of 5.2 and 5.0 and maximum activity at 35^0C and 45^0C temperatures respectively. Activities of these enzymes were in general inhibited by divalent metal ions. However, Mg^{++} stimulated activities of both the enzymes. Both PG and PME were inhibited by phenolic compounds viz. m-coumaric, homo-vanillic and protocatechuic acid. The activities of these enzymes also decreased when phenolic extracts of resistance variety of pea seeds and neem leaves were incorporated in the culture medium. These results suggest the role of phenolics in disease resistance.

Biochemical study on peroxidase (PO) and polyphenol oxidase (PPO) activity; reducing, non reducing and total sugar; total phenol and potash content before and after powdery mildew infection in seven mungbean genotype was carried out by Gawande *et al.* (2002) to know their role in host parasite interaction. Resistant genotype had higher activities of PO and PPO, total phenol and potash content before and after infection and lower level of sugars than observed in susceptible genotype. Activity of enzymes total phenols and potash content were positively associated with resistance, whereas sugars had negative association with disease resistance.

Ten cultivars of soybean were tested for their disease reactions against *Fusarium oxysporum* Schlecht the causal agent of root rot disease. The different cultivars exhibited varying degrees of susceptibility with Soymax being the most and JS-2 and UPSM-19 being the least resistant. Seed bacterization with *Bradyrhizobium japonicum* reduced root rot intensity significantly. Application of *Trichoderma harzianum* to soil also reduced root rot intensity. Combined application of *B. japonicum* and *T. harzianum* gave the most significant disease reduction.

B. japonicum did not exhibit any antagonistic reaction against *F. oxysporum in vitro*, whereas *T. harzianum* inhibited growth of *F. oxysporum*. Phenylalanine ammonia layse and peroxidase activities were assayed in both resistant and susceptible cultivars following the different treatments. Activities were significantly higher in the infected roots in comparison to healthy ones. PAL activity was higher in the resistant cultivar but bacterization with *B. japonicum* prior to inoculation with *F. oxysporum* enhanced PAL activity in both the cultivars. Persoxidase activity did not show any increase following pre-inoculation with *B. japonicum*. Glyceollin accumulation which was significantly higher in the resistant cultivar also registered a marked increase due to pre-inoculation with *B. japonicum*. *T. harzianum* did not affect enzyme activities or glyceollin accumulation (Chakraborty *et al.*, 2003).

Accumulation of total and o-dihydroxy phenols in three maize varieties (Malan, Ganga-5 and VL-42) infected with *Helminthosporium maydis* and *H. turcicum* was recorded as compared to their healthy counterparts. Reaction of these varieties to both the pathogens varied significantly in terms of accumulation of phenolics. Ganga-5 showed three-fold increase in phenolic contents due to infection by *H. maydis* while double amount of total phenols was recorded in VL-42. *H. turcicum* induced maximum accumulation of phenolics in variety VL-42 followed by Ganga-5 and Malan. An increase in the activity of peroxidase, polyphenol oxidase and IAA-oxidase was noticed in all the three varieties of maize under infection of *H. maydis* and *H. turcicum*. The results have suggested that the accumulation of phenolics was higher in resistant varieties like 'Ganga-5' and "VL-42' as compared to susceptible Malan. Corresponding increase in the activities of oxidative enzymes suggested active metabolic reaction of the host to the pathogenesis and their possible role in an increased level of phenolics (Sukhwal *et al.*, 2003).

Six apple rootstocks, namely M7, M9, M25, MM103, MM104 and MM115 showed different reactions to *Pythium ultimum* causing collar rot of apple. The maximum amount of total and ortho-dihydroxy (OD) phenols and high activity of phenylalanine ammonia lyase (PAL), tyrosine ammonia lyase (TAL) and polyphenol oxidase (PPO) were detected in highly resistant healthy (uninoculated) apple rootstock (MM115) and minimum in highly susceptible ones (MM103 and MM104).

The peroxidase activity was, however, maximum in M9 (susceptible) and minimum in M25 (resistant). On infection, the levels of total phenols and activities of their synthesizing (PAL and TAL) and oxidizing enzymes (PPO and PO) increased rapidly in resistant root stocks (MM115 and M25) at the initial stages of pathogenesis and subsequently declined rapidly. The activity of these enzymes also continued to increase gradually with pathogenesis (up to 20^{th} or 25^{th} day of inoculation) in highly susceptible root stocks (MM103 and MM104). In resistant root stocks, the level of phenols and activities of enzymes (except peroxidase) remained higher during pathogenesis in comparison to that of susceptible ones (Sharma, 2003).

During cavity spot disease of carrot (*Daucus carota*), the surface of the root is penetrated by the fungus *Pythium violae* causing surface lesions and cell breakdown. Commercial varieties range from the very susceptible Bertan, to the less susceptible Bolero with Narbonne intermediate while the gene bank cultivar Purple Turkey was much less susceptible. Examination of the colonization process *in vitro* by scanning electron microscopy of Narbonne showed that fungal proliferation occurred in the first 2 days of colonization but this species had disappeared from lesions by day 7. No lesions were evident on Purple Turkey although the fungus had penetrated the root which itself was composed of small regularly arranged cells. Examination of the activity of defence related enzymes during *in vitro* colonization showed that phenylalanine ammonia lyase and chitinase activities remained low throughout the first 7 days of infection of commercial cultivars, Bolero and Bertan. Peroxidase and β-glucosidase activity in Bolero increased briefly on day 3 but otherwise were uniformly low. Enzyme activities were generally higher in Purple Turkey. The small cell size within the root and higher constitutive levels of the enzymes may constitute the basis for resistance in Purple Turkey. Potentially this cultivar may provide a source of germplasm for improving the resistance of commercial carrots to cavity spots (Cooper *et al.*, 2004).

Alternaria blight disease of cluster bean is caused by *Alternaria cucumerina* var. *cyamopsides*. The disease appears year after year in mild to severe form to cause yield losses, as the pathogen is seed borne in nature. An investigation was attempted to quantify biochemical changes in cluster bean using highly susceptible (IC 116835)

and moderately resistant (IC 116903) genotypes. The catalase activity (65 DAS) decreased with the increase in disease intensity in both genotypes. Activity of peroxidase (PO), polyphenol oxidase (PPO), phenylalanine ammonia lyase (PAL), tyrosine ammonia lyase (TAL) as well as quantity of phenols and lignin increased with the increase in disease intensity, indicating thereby that these enzymes play important roles in the defense mechanism against *Alternaria* blight in clusterbean (Joshi *et al.*, 2004).

A study was carried out by Sharma and Chakraborty (2004) on the association of defense enzymes with resistance in tea plants triggered by *Exobasidium vexans*. Results revealed significant changes in the level of enzymes mainly β-1, 3 glucanase and chitinase exhibiting antimicrobial activity. A wide variety in the activities of the enzymes involved in phenol metabolism including phenylalanine ammonia lyase, peroxidase and polyphenol oxidase were seen in compatible and incompatible interactions. Multicomponent coordinated responses of tea plants under biotic stress with special reference to *E. vexans* causing blister blight disease of tea have also been demonstrated by Chakraborty *et al.* (2004). The possibility of inducing resistance in susceptible tea varieties was achieved following foliar spray with salicylic acid and results established its potential in immunizing tea plants which was confirmed by immunoassays and immunolocalization of chitinase in tea leaf tissues by employing polyclonal antibodies raised against chitinase and labeled with FITC after induction of resistance. The accumulation of defense enzymes in tea plants in response to salicylic acid treatment suggests its role in the cellular protection mechanism which was also confirmed (Chakraborty *et al.*, 2005b).

The effective integrated disease management practices

As the management of some diseases is not possible through only one approach, efforts are being made to reduce environmental effects and rationalize the use of pesticides and manage diseases more effectively. This led to the emergence of the new discipline called Integrated Disease Management (IDM), which is an ecologically based, environmentally conscious method that combines or integrates

biological and non-biological control techniques to suppress weeds, insects and diseases (Anahosur, 2001). The five components of an IDM program are prevention, monitoring, correct disease and pest diagnosis, development and use of acceptable thresholds, and optimum selection of management tools. For sustainable crop production the components involved should be eco-friendly, so that beneficial organisms would be safe and IDM practices would go a long way helping stabilized crop production. Integrated disease management is the selection and hormonal combination of appropriate techniques to suppress the disease to a tolerable level (Gupta and Mukherjee, 2006).

Integration of *Trichoderma harzianum*, *Rhizobium* and carbendazim remarkably reduced the root rot of groundnut, caused by *Sclerotium rolfsii*. The antagonistic population increased with increasing time, and maximum population was recorded 75 days after sowing. At this time, while the native soil recorded only $2.8x10^3$ cfu/ g of *Rhizobium*, the seed treatments with *T. harzianum* and *Rhizobium* plus soil inoculation with *T. harzianum* recorded $44x10^3$ cfu/ g after 6 days, indicating a 16 fold increase. Maximum number of plants survived when the antagonist was applied as seed treatment or applied to soil at sowing. The nodule number and maximum population of $37x10^3$ cfu/g were recorded when *T. harzianum* inoculum was added to soil at sowing. Addition of *T. harzianum* inoculum at 2 and 5% were at par with 88 and 92% surviving plants, respectively. Addition of inoculum at 5% recorded a slight increase in nodule number. When the dose of inoculum was increased from 2 to 10%, there was no corresponding increase in population of antagonist in all intervals tested. In all experiments, plants died within 45 days after sowing in pathogen alone inoculated soils (Muthamilan et *al.*, 1996).

Crop losses caused by *Sclerotinia sclerotiorum*, *S. minor* and *S. rolfsii* were evaluated in 41 commercial peanut (*Arachis hypogaea*) fields located in the southern region. The incidence of either disease was generally higher in fields in which the sequencing of crops was the same during the last 15 years. Furthermore, the incidence of 'blight' and 'wilting' was often higher in peanut crops where the preceding crop had been peanut, soybean (*Glycine max*) or sunflower (*Helianthus annus*) than in peanut crops preceded by sorghum (*Sorghum bicolor*), maize (*Zea mays*), alfalfa (*Medicago sativa*), lovegrass (*Eragrostis curvula*) or grassland

(Marinelli *et al.*,1998). The soil borne diseases of crops incited by species of *Sclerotium, Rhizoctonia, Fusarium* and *Pythium* are difficult to be managed through one method of approach *viz.*, cultural practices or fungitoxicants or host plant resistance or bio-agents. Among them the disease caused by *Sclerotium rolfsii* are predominant under rainfed and assured moisture conditions and cause considerable loss to field crops, vegetables, fruit crops and plantation crops.

Fourteen 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 PDBCTH-2 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 (39.9%) inhibition of mycelial growth. Suppression of sclerotial production by the 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 (PDBCTV4) were superior under greenhouse conditions with PDBCTH 8 showing maximum (66.8%) disease control followed by PDBCTH 7 (66.0%), PDBCTH 4 (65.4%), PDBCTH 2 (61.6%) and were even superior to the fungicide - captan. *G. deliquescens* gave maximum (55.7%) disease control among *Gliocladium* spp. (Prasad *et al.*, 1999).

The antagonistic microbes *viz. Trichoderma harzianum, T. viride, Gliocladium virens, Penicillium* spp. *Bacillus subtilis, Pseudomonas fluorescens*, mycorrhizae and few others have been extensively evaluated as seed dress, soil application or plant spray or spot application against soil borne diseases *viz.*, root rots, foot rots, wilt; damping off of seedlings caused by *Sclerotium rolfsii, Pythium debarianum, Macrophomina phaseolina, Rhizoctonia solani and Fusarium* spp. As bio-control agents alone may not completely and effectively manage disease, it can be used as one of the components in the integrated management of diseases. (Anahosur,1999).

Broad spectrum biological control of diseases caused by soil borne plant pathogens such as *Pythium, Phytophthora,* and *Rhizoctonia solani* requires the introduction into or presence of edaphic sources of organic nutrients in soil for sustenance of biological agents. The decomposition level of organic matter critically affects the composition of bacterial taxa as well as the populations and activities of biocontrol agents. Composition, antibiosis, parasitism, and systemic induced resistance are all affected. Highly stabilized sources of *Sphagnum* peat consistently fail to support sustained biological control, even when inoculated with biocontrol agents. Composts, on the other hand, can serve as an ideal food base for biocontrol agents and offer an opportunity to introduce and establish specific biocontrol agents into soils, which in turn leads to sustained biological control based on the activities of microbial communities (Hoitink *et al.,* 1999). Species of *Trichoderma, Gliocladium* and *Pseudomonas* have been employed and some success has been achieved in selected crops against a few pathogens in restricted soil temperature and pH (Sen, 2000).

Three biocontrol agents (BCAs) were evaluated individually and in combinations, and in integration with bavistin seed treatment in pathogen infested soil in pots, for suppression of dry root rot pathogen *Rhizoctonia solani* in bell Pepper (*Capsicum frutescens* cv. California Wonder). Seed treatment with the bio-control agents was as effective as bavistin seed treatment. Integration of seed and soil application of individual BCA resulted in higher germination and reduced mortality due to disease. Combination of two biocontrol agents, particularly of *Trichoderma harzianum* and *T. aureoviride* was better than the individual ones. Population of BCAs in chilli rhizosphere and soil was directly related to suppression of *R. solani.* Application of mixture of *T. harzianum* and *T. aureoviride* as seed and soil treatment was the most promising in increasing the germination and suppression of chilli root rot pathogen and the disease (Bunker and Mathur, 2001).

Six varieties of ginger along with five fungicidal treatments were examined to find out the best ginger variety giving highest yield and it has been observed that 'Moran' variety yielded 114.08 q ha^{-1} which was followed by 'Rio-de-genario' (106,47 q ha^{-1}). Per cent disease incidence recorded was less in these two varieties. Effect of different fungicides on disease incidence showed that Ridomil MZ-72 @

0.2% effectively reduced the disease with only 18.63 per cent disease incidence. Ridomil MZ-72 treated rhizome also showed maximum germination as well as the highest yield. It was followed by copper oxychloride @ 0.3%, Bordeaux mixture @ 1%, mancozeb (indofil M-45) @ 0.25% and captan @ 0.25% (Das *et al.*, 2001).

Agricultural soils suppressive to soil borne plant pathogens occur world wide and for several of these soils the biological basis of suppressiveness has been described. Two classical types of suppressiveness are known. General suppression owes its activity to the total microbial biomass in soil and is not transferable between soils. Specific suppression owes its activity to the effects of individual or select groups of microorganisms and is transferable. The microbial basis of specific suppression to four diseases, *Fusarium* wilts, potato scab, apple diseases, and take-all, is discussed. One of the best described examples occurs in take-all decline soils. In Washington State, take-all decline results from the build up of fluorescence *Pseudomonas* spp. that produce the antifungal metabolite 2, 4-diacetyl phloro-glucinol. Producers of this metabolite may have a broader role in disease suppressive soils worldwide. By coupling molecular technologies with traditional approaches used in plant pathology and microbiology, it is possible to dissect the microbial composition and complex interactions in suppressive soils (Weller *et al.*, 2002).

Upmanyu *et al.* (2002) studied root rot and web blight (*Rhizoctonia solani*). Soil amendment with cotton, mustard and neem cakes was found effective in reducing the root rot (pre- and post-emergence) incidence both under glass house and field conditions and increased the yield. Foliar sprays of carbendazim (0.1%) and tebuconazole (0.05%) were most effective in reducing web blight severity while seed treatment with carbendazim (0.2%) in combination with foliar sprays were found effective the root rot incidence and web blight severity as well as increased the yields. *Trichoderma harzianum* and *T. virens* as seed treatment and foliar sprays were found effective in reducing the disease and resulted in increased pod yield. Among the bioagents, *T. viride* showed the maximum tolerance to carboxin, tebuconazole and carbendazim followed by *T. virens*, *T. harzianum* and *A. niger* and were used in integrated disease management along with fungicides and oil cakes both under glass house and field conditions. Soil amendment (cotton cake) + *T. virens* and carboxin (ST), mustard cake + *T. virens* and carbendazim (ST), seed

treatment with combination of *T. viride* and carboxin, *T. harzianum* + tebuconazole and soil amendment (mustard cake) + carbendazim (ST) were found effective in containing the root rot under glasshouse conditions while soil amendment (mustard cake) + carbendazim (ST) + carbendazim (FS) were found highly effective in reducing pre-and post – emergence root rot while web blight severity was best contained by soil amendment (mustard cake) + carbendazim (ST+FS) followed by tebuconazole + *T. virens* (ST) + carbendazim (FS). Sprays of carbendazim (0.1%) on mulch and foliage were found highly effective but pine needle mulch impregnated with floor application of carbendazim was also quite effective in avoiding the contamination with fungicide residues.

Biologically and chemically treated chickpea seeds observed after one month showed that vitavax (carboxin @ 1g kg^{-1} seed) did not significantly affect the spore viability of *Gliocladium virens* (Gv). Application of carboxymethyl cellulase (CMC) with *G. virens* powder (10^9 spores per g) in combination with vitavax provided maximum protection (81.9%) to the crop against chickpea root rot and collar rot pathogens in glasshouse. Chickpea seeds treated with Gv powder + CMC + vitavax significantly increased seedling emergence (47.9%); final plant stand (85.8%) and grain yield (79.7%) which was statistically at par with the treatment Gv powder + vitavax and Gv suspension + vitavax in a sick plot (Tiwari and Mukhopadhyay, 2003).

Ashwagandha (*Withania somnifera*) is an important medicinal plant and a major source of alkaloid and steroidal lactones (withanolide), which are regularly used in pharmaceutical industries. Plant growth retardation and gall formation in the root system indicated the presence of root – knot nematode, which was confirmed as *Meloidogyne incognita* race-2. Green house trials were conducted to determine the influence of different inoculum levels of *M. incognita* Chitwood on growth and yield of *W. somnifera*. Various organic materials *viz.* neem compound *Mentha* distillate, *Murraya koengii* distillate, *Artemisia annua* and vermicompost, bio-agents *viz.*, *Glomus aggregatum* and *Trichoderma harzianum* were tested individually as well as in different combinations for the management of root-knot nematode, *M. incognita* on *W. somnifera*. The experimental results indicated that most of the bio-agents and organic materials alone as well as in combination were root-knot nematode

suppressive and enhanced the growth and yield of *W. somnifera*. Maximum root-knot suppression was noticed in vermicompost and *T. harzianum* combination followed by *Mentha* distillate and *G. aggregatum*. Maximum increase in plant yield was noticed when the soil was amended with *Mentha* and *Murraya koengii* distilled *waste* along with bio-agents (Pandey *et al.*, 2003).

Sharma and Gupta (2003) reported soil solarization of infested soil with single and double polyethylene mulch alone and combination with soil amendment (mustard cake) increased soil temperature as compared to unmulched plots. This rise in temperature in mulching for 30 and 50 days eliminated the pathogen from 5 and 10 cm in soil depth, respectively. They also found antagonistic activity of *Bacillus subtilis, Trichoderma longibrachiatum* and *T. harzianum* against *Rhizoctonia solani*. Soil application of *B. subtilis* reduced pre and post-emergence root rot. Integrated management practices was done by using a combination of soil solarization + soil amendment (mustard cake) + combination of *A. sativum* and *B. subtilis* was found effective in reducing the incidence of root rot while web blight severity was best contained by combination of soil solarizatioin + soil amendment (mustard cake) + *A. sativum* (ST + FS) followed by soil solarization (SS) + soil amendment (mustard cake) + combination of *A. sativum* and *B. subtilis* + bavistin (0.1%) foliar sprays and increased green pod yields.

Rhizoctonia solani [*Thanatephorus cucumeris*] causes sheath blight, one of the most widely distributed and destructive diseases of rice. Three fungal bioagents *Trichoderma viride*, *T. harzianum* and *T. virens* and nine antagonistic rhizospheric bacteria were evaluated *in vitro* by dual culture method for their antagonistic activities against *Rhizoctonia solani*. A maximum inhibition in growth of *Rhizoctonia solani* was obtained by WRPf (62.96%) followed by WRb 8 (56.67%) and *T. virens*. Out of 4 plant extracts, extracts of *Allium sativum* inhibited maximum (100%) mycelial growth and sclerotial production at 10% concentration *in vitro* condition. Increase in epicotyl and hypocotyl length associated with plant growth promotion was observed in seed treatment with $FeCl_3$ (1mM) and IAA (0.001%) as compared to water treated control. Among abiotic elicitors, maximum reduction in sheath blight incidence was observed by seed treatment and seedling dipping with K_2HPO_4 (20mM) followed by $FeCl_3$ (10mM). On the other hand, among bioagents

maximum reduction in sheath blight incidence was observed in soil treated with WRPf alone or in combination with other bioagents and soil amendments. But, maximum reduction in sheath blight incidence as well as severity was observed in soil treated with *T. virens* + WRPf + *Gliricidia* compost (93% less disease) which was closely followed by combined application of soil amendments with *T. virens* and neem cake with seed and root dipping with K_2HPO_4 (20mM) in pot experiment under glass house condition (Chowdhury *et al.*, 2003).

Aqueous extracts of mustard cakes (5%), neem cake (1%), pine needles (5%), deodar needles (3%) and neem oil (3%) respectively, led to reduced *in vitro* germination of sclerotia of the pathogen *Sclerotium rolfsii* causing seedling blight disease in apple nurseries, as compared to control. Combinations of mustard cake (5%) with neem oil (3%), neem cake (1%) with deodar needles (3%) and neem oil (3%); and mustard cake (5%) with neem cake (1%), pine needles (5%) and neem oil (3%) resulted in total inhibition of sclerotial germination (Sonali and Gupta, 2004).

A survey of 277 farmers in three major potato growing areas in Kenya was conducted by Nyankanga *et al.* (2004) with the aim of assessing farmers' current perception and knowledge of late blight and practices for its management and identifying points of potential intervention in the development of integrated disease management (IDM) programmes. The problem of late blight was one of several constraints that growers faced such as lack of quality seed, markets, storage and prevalence of bacterial wilt. Most farmers (5.1%) regarded late blight as a serious biotic constraint upon production. Many farmers (79%) were able to recognize foliar symptoms of late blight but there was an evident lack of knowledge of tuber and stem infection, causes of leaf, stem and tuber infection, different inoculum sources, and accurate diagnosis of the disease. Most (81%) farmers associated the disease with cold weather. Farmers overwhelmingly (98%) relied on application of fungicides, mostly mancozeb (Dithane M45) and metalaxyl (Ridomil) as the main control methods, with most farmers knowing of no other method. High cost of fungicide, poor application techniques, and preference of susceptible cultivars were among the reasons contributing to inadequate control of late blight. Very few farmers showed the elements of IDM strategies, probably due to their limited knowledge of the biology of late blight. These results suggest that improvement of

late blight control could be achieved by enhancing farmers knowledge and developing and deploying IDM practices involving a multidisciplinary approach, which encompasses addressing other production constraints.

Crown rot (*Sclerotium rolfsii*) of French bean was found to be more severe in Solan district than Kullu of Himachal Pradesh. The disease incidence ranged from 11 to 56 per cent. Characteristic symptoms appeared as dark brown, water soaked lesions on the stem and collar region just below the soil surface followed by yellowing of leaves and production of a white mouldy growth interspersed with sclerotia at the base of the stem, under the surface of leaves and pods. *In vivo* evaluation of fungicides against the pathogen showed that penconazole, hexaconazole, propineb and mancozeb inhibited mycelial growth. Propineb was found to be the most effective in reducing disease incidence on crown and pods. Among the biocontrol agents *Gliocladium virens* and *Trichoderma viride* were found to be the most effective against the pathogen (Gupta and Sharma, 2004).

According to Bhatnagar *et al.*, (2004) cumin wilt, a serious disease induced by *F. oxysporum* f. sp. *cumini* causes heavy losses to the crop. A few compounds of plant origin have been provided to be possible alternatives to pesticides use. Out of 17 species tested plant extract from Datura (1.3 cm) and Isabgol (1.5 cm) were effective in reducing the radial growth of *F. oxysporum* f. sp. *cumini*. In a similar study, Ghasolia and Jain (2004) evaluated four commonly used fungicides, two bio-agents, two phyto-extracts and two physical seed treated agents, in both *in vitro* and *in vivo* conditions for fungitoxicity against *F. oxysporum* f. sp. *cumini*. Carbendazim (0.2%), thiram (0.25%), captan (0.25%), tebuconazole (0.2%), *Trichoderma viride*, *Euphorbia antiquorum* and hot water gave higher seed germination and vigour index and minimum pre – and post – emergence seedling mortality over check. Before maturity, all treatments showed reduced number of seedlings showing wilt symptoms in the field.

A number of plant species (*Azadirachta indica, Lantana camera, Dryopteris filix-mas*, *Eichhornia* sp.) have been reported to possess some natural substances in their leaves which were toxic to foliar fungal pathogens (*Pestalotiopsis theae, Glomerella cingulata*) of tea causing brown blight and grey blight disease

respectively. Attempts have also been made to use aqueous extracts of selected plants (*A. indica* and *Catharanthus roseus*) on tea plants for induction of resistance against *Alternaria alternata*, a newly recorded foliar fungal pathogen, causing leaf blight disease of tea as well as *E. vexans* causing blister blight of tea with special reference to the involvement of defense enzymes such as β-1,3- glucanase, chitinase and phenylalanine ammonia lyase and antifungal phenols. These extracts enhanced the level of defense enzymes, developed acquired resistance in tea plants and reduced blister blight disease incidence (Chakraborty *et al.*, 2004). Tea varieties treated with aqueous leaf extracts of *A. indica* exhibited high level of all three defense enzymes along with rapid and distinct accumulation of antifungal phenolics in comparison with *C. roseus*. Reduction in disease incidence by application of these extracts was also evident. Plant extracts from *A. indica* seem to act at various points in the defense activating networks and mimic all or part of the biological activities of resistance. The results support the hypothesis that neem extract may act indirectly by inducing plant defense reactions and it may be useful in integrated management of foliar disease of tea (Chakraborty *et al.*, 2005d).

Volatile and non-volatile metabolites of *Trichoderma* spp. significantly reduced the mycelial growth and germination of *Sclerotinia sclerotiorum*. Among the delivery systems evaluated by Kapil and Kapoor (2005) for controlling the white rot, sodium alginate pellet formulation (800 No./m^2) followed by soil application of wheat bran based *Trichoderma viride* formulation @ 11.2g /m^2 were found to be most effective delivery systems. Six neem based biopesticides were evaluated at two concentrations (0.5 and 0.1%) against *S. sclerotiorum*. Results revealed, Wanis as the most effective at both the concentrations followed by Neemgold. Out of eight organic substrates evaluated for mass multiplication of bioagents, maximum multiplication of bioagents was found in FYM followed by in *Lantana camara* and wheat bran. Population dynamics studies of *T. viride* revealed significant increase in population even after 60 days of application of bioagent.

Stalk rot of cauliflower caused by *S. sclerotiorum* could be reduced to 9.7% with 1.5 g/l bavistin 50% followed by 15.3% with 5.25g/l sailaxyl-MZ 72%, 16.7% with 8.34 g/l mancozeb 75%, 20.8% with 1.08 g/l topsin-M 70% and 34.7% with 6

ml/l neem extract 25%. Bavistin 50% also gave highest seed yield. Among bioagents, culture filtrate of *Trichoderma harzianum* was most effective in reducing disease incidence to 23.6% as compared to *Aspergillus niger*, kalisena or booty. The seed production was increased to 200.8 kg/ha after treatment with *T. harzianum*. The application of *A. niger* (194.1, 314.8 kg/ha), kalisena (192.2, 308.5 kg/ha), *T. harzianum* + *A. niger* (190.8, 302 kg/ha) booty + kalisena (186.3, 297 kg/ha) and booty (165.4, 258.5 kg/ha) also increased seed production. Intercropping with one and two rows of garlic reduced disease index significantly over control in first cropping season. However, intercropping with one and two rows of onion reduced disease index significantly in second season (Zewain *et al.*, 2005).

A severe crown and root rot of Chinese gooseberry (*Actimidia deliciosa*) caused by *Sclerotium rolfsii* was observed in the nursery at Kullu, Himachal Pradesh. The incidence was more pronounced in the areas previously occupied by strawberry or apple nursery. The infected plants showed water soaked areas at the base of the stem at soil level and drooping of the lower most leaf of the plant showed drooping as initial symptoms. White fan shaped mycelium developed which later disappeared and mustard-shaped sclerotia were formed. The fungus was isolated, and its pathogenecity was proved. In two years field tests *Trichoderma viride* (talc formulation 1.0%) proved very effective in providing 74 per cent disease control. Treatment with thiram (0.4%) and *Trichoderma viride* (0.5%) in combination was the best in controlling the disease (Khosla *et al.*, 2005).

Induction of chitinase in suspension-cultured tea cells following inoculation with *E. vexans* or treatment with hexaconazole, calixin and aqueous leaf extracts of *Catharanthus roseus* were characterized biochemically and immunologically in order to understand the mechanism of plant-pathogen recognition and the complex signaling networks mediating the activation of defense responses. The knowledge gained by such studies provides a base for the development of novel agrochemicals for disease control and also for the development of disease resistant crops by regulating the system in plants by integrated management that leads to development of systemic acquired resistance (Sharma and Chakraborty, 2005).