LITERATURE REVIEW

Different kind of pathogens are capable of infecting various types of crop plants and induce diseases that cause considerable economic loss. 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. This also helps us to assess, alter and modify the effectiveness of 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 important tool to localize and prevent the spread of the disease(s). In the era of globalization and WTO regime, detection of plant pathogens has immense role to play because of free movement of materials across the countries creates chances for introduction of new pathogens in to the important countries. Therefore, we need to have techniques that are rapid, accurate, sensitive, robust and easy to adopt. The emerging technologies, both serological and molecular, satisfy many of these requirements.

Serological detection of plant pathogen in diseased tissue and soil is based on study of antibody-antigen interaction. The different forms of serological assay include gel diffusion, enzyme linked immunosorbent assay (ELISA), immunofluorescence (IF), immuno-fluorescent colony staining, dot immunobinding assay (DIBA) and western blot analysis. Serological methods are being used regularly in agricultural research practices such as localization of pathogens within tissues; identification of specific antigens in electrophoretically separated components (Clark and Adams, 1977; Hansen and Wick, 1993; Werres and Steffens, 1994; Chakraborty and Chakraborty, 2003; Gadewar and Singh, 2006) as well as for quantification of defense related proteins such as PR- proteins (Chakraborty, 2005).

A short comprehensive review on the various serological techniques used in detection of plant pathogenic fungi and biochemical changes in plants following fungal infection has been presented below.

Immunodetection of plant pathogens

Charudattan and DeVay(1972) compared common antigenic relationship between four cotton varieties and isolates of *Fusarium* and *Verticillium* species. One antigenic substance was found to be common among the varieties of cotton and isolates of

V.nigrescens. Cotton varieties which were resistant or susceptible to *Fusarium* wilt as well as pathogenic and non-pathogenic isolates of *F.oxysporum f.sp. vasinfectum* shared the common antigen. The common antigen was not shared between *F.moniliforme* (non pathogenic) and cotton. In gel-diffusion tests, five to eight precipitin bands were observed in the homologous reactions; of these, only one or two bands were common in heterologous reactions between the fungal and cotton preparations. The common antigenic determinants shared by cotton and the fungal isolates does not appear related to the severity of wilt symptoms, but it may affect host-pathogen compatibility during the process of root infection.

MacDonald and Duniway (1979) used the fluorescent antibody to study the survival of *Phytophthora megasperma and P. cinnamomi* zoospore in soil. Zoospore cysts of *P. megasperma and P. cinnamomi* were adjusted to non sterile soil and its potential was adjusted to predetermined values. The infested soils were held under laboratory and field conditions and periodically sampled to determine cyst viability and soil water potential. During sampling, soil containing cysts was suspended in water and aliquots were placed on a selective agar medium to allow germination of viable cysts. After germination, the cysts were stained by an immunofluorescence detection technique and counted.

Duncan (1980) detected *Phytophthora fragariae* by a "root-tip bait" test in certified commercial stock strawberry plants, supposedly free of disease. Charudattan and DeVay (1981) further purified and partially characterized antigenic preparation from conidia of *F. oxysporum f.sp. vasinfectum* that cross-reacted with antiserum of cotton root tissue. When antiserum to cotton antigens was reacted with fungal antigens or when antiserum to fungal antigens was reacted with cotton antigens in immunodiffusion tests, one precipitin band was formed. Cross reactive antigen (CRA) migrated as a single band in polyacrylamide or agar-gel elcetrophoresis, and sedimented as a single band during analytical ultracentrifugation. DeVay *et.al.*(1981) also indicated the presence of CRA not only in *F.oxysporum f.sp. vasinfectum* and in cotton roots and seed but also in *Thielaviopsis basicola.* Indirect staining of antibodies using flourescein isothiocyanate (FITC) indicated that in cross-sections of roots, cut near or just below the root hair zone the CRA was concentrated mainly around xylem elements, the endodermis and epidermal

cells and was present throughout the cortex tissue. Protoplasts prepared from cross-sections of young cotton roots also contained the CRA which was concentrated in the region of the plasma lemma. Treatment of conidia and mycelia of *F. oxysporum f.sp vasinfectam* with antiserum to cotton and using indirect staining with FITC indicated that the CRA was mainly present in hyphal tips and in patch-like areas on conidia.

The occurrence of common antigens in leaf and root extracts of coffee plants and urediniospores of *Hemileia vestatrix* and their possible involvement in such interaction has been discussed by Alba *et.al.* (1983). Using antisera raised against antigens of *Macrophomina phaseolina* and roots of soybean cultivars, Chakraborty and Purkayastha (1983) demonstrated serological relationship between *M. phaseolina* and soybean cultivars following immunodiffusion and immunoelectrophoretic tests. Four antigenic substances were found to be common between the susceptible soybean cultivars and isolates of *M. phaseolina* but no such common antigens were detected between resistant cultivars and the fungus (Purkayastha and Chakraborty ,1983).

Aguelon amd Dunez (1984) developed an immuno enzymatic technique for the detection of *Phoma exigua* in infected potato tissues. Specific antibodies were raised against *P. exigua* solated and DAS-ELISA was used to test for the fungus in inoculated tubers and spores and in stems grown from these tubers. The fungus was detected in these different tissues demonstrating the applicability and sensitivity of the techniques. The presence of *Ganoderma lucidum* in roots of betelnut was detectable by the induction of fluorescent antibodies as developed by Koti Reddy and Ananthanarayanan (1984). *Phaeolus schweinitzii*, which causes a root-and butt-rot of conifers, secretes a number of species-specific and strain-specific polypeptides which are detectable by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and isoelectric focusing (IEF). Indirect immunoflourescence microscopy was used by Dewey *et.al* (1984) to detect the binding of species-specific antisera to these antigens to extracellular macromolecules secreted by the fungus, to the cell surface of basidiospores and chlamydospores, and to the cell surface and cross walls of mycelia. Common antigenic determinants were also present in extracellular culture filtlrate material and walls of mycelia, chlamydospores and basidiospores. Indirect immunofluorescence, performed by using antisera to culture filtrate molecules has been used to demonstrate the presence of mycelium, and on

occasions chlamydospores, in naturally and artificially infested soil samples. Authors suggested that this method could be used for identification of the kind of propagule most likely to be the source of field isolates of the organisms; this information, which cannot be obtained by using selective media, strongly suggests that the pathogen can survive saprophytically in the soil. In contrast, isolated mycelial cell wall preparation did not prove to be a suitable source of immunogenic material for these studies.

Cross-reactive antigens were detected by Alba and DeVay (1985) in crude preparations and in purified preparations from mycelia of *Phytohthora infestans* Race 4. and Race 1.2.3.4.7. with antisera for potatoes cv. King Edward and cv. Pentland Dell by using an indirect ELISA technique. They suggested that the fungal mycelia do not easily release cross- reactive antigens (CRA) into synthetic media where they grow. Most of *P. infestans* CRA are thermo labile and can be concentrated by precipitation in the presence of 40% saturated ammonium sulphate (SAS). An antigenic disparity was also evident when 40% SAS from *P. infestans* Race 4 mycelial preparation was assayed with antisera for King Edward and Pentland Dell. The adaptation of ELISA assay for the detection of *Sclerotiania sclerotiorum* from both artificially and naturally infected host plants was described by Walez *et.al.* (1985).

Major CRA shared by soybean roots and *M. phaseolina* were evident in comaptable interaction. However, an antigenic disparity was noticed in the susceptible cultivar (cv.soymax) after induction of resistance by sodium azide (Chakraborty and Purkayastha,1987). Using immunodiffusion, immunoelectrophoresis and crossed imminoelectrophoretic techniques, antigens prepared from roots of five cutivars of groundnut and two isolates of the antigens from two pathogen isolates (*M. phaseolina*), four non-pathogens of groundnut (*Corticium sasakii, Colletotrichum lindemuthianum, C.corchori* and *Botrytis alii* were analysed for the presence of cross-reactive antigens Purkayastha and Ghosh (1987). 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.

Colonization of cotton roots by *Verticillium dahliae* was studied by Gerik and Huisman(1988) using immunoenzymatic staining technique. Colony density of *V. dahliae*

increased with distance from the root tip, with the maximal density occurring more than 1cm from the root apex. Colony densities at distances more than 1cm from the tip were relatively constant. *Hyphae of V.dahliae* were present through the entire depth of the cortex, and were greatest in the interior of the root cortex at the surface of the vascular cylinder. The colony appearance was consistent with growth of hyphae from the root surface toward the stele. Colonies of *Fusarium oxysporum*, similarly stained, were found to be mostly confined to the root surface and the outer cortex. Yuen *et.al.*(1988) developed a double antibody sandwich indirect enzyme-linked immunsorbent assay (ELISA) for the detection and quantification of *Pythium ultimum.* A polyclonal antibody produced to cell walls of *P. ultimum* was used as the capture antibody, while a *P. ultimum* specific monoclonal antibody (MAbE5) was used for recognition of the fungus. In the ELISA, culture extracts of 7 isolates of *P. ultimum* exhibited strong positive reactions, where as none of the 37 isolates of other *Pythium spp.* and fungal genera had positive reactions. *P. ultimum* was detected by ELISA in roots of bean, cabbage, and sugar beet seedlings grown in pathogen infested soil. Mohan (1988), raised polyclonal antibody against mycelial antigens of five isolates of *Phytophthora fragriae* (Pf1, Pf2, Pf3, Pf10 and Pf11) to detect the pathogen in extracts of strawberry plants infected with *P. fragariae* following enzyme linked immunosorbent assay (ELISA). Root extracts prepared from the alpine strawberry (*Fragaria vesca* and *F. ananssa*) infected with any of the five isolates produced strong reactions in ELISA. In *F. vesca*, ELISA-positive material could be detected before macroscopic symptoms appeared. The cultivar Red Gauntlet, resistant to Pf1, 2 and 3 but susceptible to Pf10 and 11, reflected this differential response in ELISA. Cultivars (viz. Hapil, Ostara and Providence) were to all the isolates were also positive in ELISA. The antiserum also detected *P. cactorum* infections.

Polyclonal antibody was raised against semipurifed spore suspension of *Plasmodiophora brassiceae* causing club root of cabbage by Lange *et.al.* (1989). Crude root suspension samples were tested following dot immunobinding assay. The individual steps of the serological procedure were examined with a scanning electron microscope. The surface of the resting spores of *P. brassiceae*, race 7, appeared smooth, while the dot immunobinding processed spores had a heavy, irregular coating, which was demonstrated to originate from incubation in the primary antiserum. Normal serum did not give rise to

aphanidermatum. Samples consisted of (a) whole plants picked by hand and assayed as whole plants ; (b) whole plant sectioned into lower,middle, upper strata components; and (c) leaf clipping collected with a reel mower set at a 1.2cm cutting height. ELISA readings for mowed samples generally matched those for whole-plucked samples. Fluctuation in detectable *Pythium* antigens were most pronounced on the uppermost stratum compared with moderate to very little change in ELISA reading for the two lower strata. Two commercial serological assay Kits (multiwell E Kit and the rapid assay Kit) were compared to a cultlure plate method for detection of *Phytophthora cinnamomi* in root samples from inoculated azalea (Benson, 1991). Both the kits detected *P. cinnamomi* on azalea roots beginning 1 wk after inoculation. There was a positive correlation between root rot severity in greenhouse trails and root sample absorbance (multiwell) or meter reading (rapid assay) but not between symptom severity and immunoassay results. Although color reactions in the rapid assay detectors became increasingly darker after completion of the test, results after 5 min were as reliable as those after 60 min since readings for uninoculated controls used to determine test thresholds also increased with time. The multiwell Kit detected *P. cinnamomi* in root samples containing as little as 1% infected root tissue. In a commercial nursery survey, 5 and 15% of the azalea root samples at two nurseries had positive ELISA values that were unconfirmed by culture plate. The rapid assay kit detected *P. cinnamomi*, was easy to use and gave results in a short time.

Distribution of the endophytic fungus, *Acremonium coenophialum*, with in the tissues of infected tall fescue (*Festuca arundinacea*) can be determined with tissue print-immunoblot (TPIB), however, the accuracy of this method in the determination of endophytic infestation levels of pastures and seed lots has not been evaluated (Gwinn *et.al.* 1991). The purpose of this study was to compare TPIB to other methods used for endophytic detection. Seed lots of known endophyte infestation levels were tested with TPIB. Percentage endophyte infestation did not differ significantly from the expected values. Also, no significant differences were found between results of protein A-sandwich ELISA and TPIB tests when both were used to estimate endophyte infestation levels in pastures. Therefore, accuracy of TPIB in comparable to other endophyte detection techniques and can be used for routine detection of endophyte in tall fescue tissues.

Ribosomal protein differences among snow mold fungi and wheat were utilized to detect the causal fungi in infected wheat leaves by indirect enzyme linked immunosorbent assay (ELISA) and western blot analysis (Takenaka, 1992). Polyclonal antisera wera raised against the ribosomes of *Pythium paddicum, P. iwayamai, Typhula incarnate and Microduchium nivale,* and tested for sensitivity and specificity with ribosomes of these four pathogens, *Typhula ishikariensis*, and wheat. Using polyclonal antiserums, ribosomes of *P. paddicum and P.iwayamai* were serologically identical ; the ribosomes of *T. incarnate and T. ishikariensis* had partial common antigenic determinants, but there were apparent serological differences among *Pythium spp. Typhula spp., M. nivale* and wheat. With indirect ELISA, ribosomes of *Pythium* spp. were detectable at a concentration of 69 ng/ml and ribosomes of *T. incarnate* and *M. nivale* at a concentration of 210 ng/ml. The ribosomes for each target pathogen were detected from completely rotted wheat leaf homogentates diluted up 1:1,000 or 1:10,000. The indirect ELISA could not differentiate, *P. paddicum* from *P. iwayamai*-infected plants or *T. incarnate* from *T. ishikariensis*-infected plants but could detect and differentiate snow mold fungi at the genus level in wheat plants. Western blot analysis with these antisera also could not differentiate *P. paddicum* from *P. iwayamai* but could differentiate wheat leaves infected with *T. incrnata* form those infected with *T. ishikariensis.*

Linfield (1993) developed a specific and sensitive enzyme-liked immunosorbent assay using polyclonal antiserum raised against *Fusarium oxysporum f.sp. narcissi.* Antiserum raised against cell wall fractions gave better recognition than that to cytoplasmic fractions. Recognition was equally good in artificially and naturally infected bulbs. Nine isolates of *F.oxysporium f.sp.narcissi* from a wide geographic area gave similar results in an indirect ELISA test, although some cross-reactivity was observed with two other *Fusarium spp.* Ten days after inoculation the pathogen was readily detected in the base plate area of three *Narcissus* cultivars and points remote from the inoculation site in the most susceptible cultivar. A direct correlation was observed between positive results in ELISA and recovery of the pathogen on selective medium. Purkayastha and Ghosal(1993) analysed the cross-reactive antigens of *Acrocylindrium oryzae* and rice in relation to sheath rot disease. Immunodiffusion, immunoelectrophoresis and crossed immunoelectrophoretic analysis of rice antigens in

relation to sheath rot disease revealed a serological relationship between susceptible rice cultivars and isolates of *A. oryzae*. One precipitin band was observed when the antigen preparation of *A.oryzae* was cross-reacted with its own antiserum or against the antisera of four susceptible rice cultivars. No precipitin band was detected between the antiserum of the resistant cv. Mashuri and antigen preparations from three isolates of *A.oryzae* or in reciprocal cross reaction between the antigens of the resistant cv. Mashuri and the antiserum of the pathogen.

Monoclonal antibody (MAb) was raised against haustorial complexes isolated from pea leaves infected with the powdery mildew fungus *Erysiphe pisi* by Mackie *et.al.* (1993). The antibody designated UB10 recognises a 45 kDa N-linked glycoprotein which is specially located in the haustorial plasma membrane. This glycoprotein is clearly distinct from a previously characterized 62 kDa glycoprotein (indentified with MAb UBB) which is also specially located in the houstorial plasma membrane. These antibodies were used, along with MAb UB7 which binds to a major 62kDa glycoprotein in the cell wall plasma membrane of both haustoria and surface hyphae, to label haustoria with in epidermal strips from infected pea leaves using indirect immunofluorescence. Different stages of haustorial development can be observed using this procedure and the results showed that all three glycoproteins recognized by the MAbs are expressed early in haustorial development.

Roberts *et.al.*(1993) further described two new monoclonal antibodies (MAbs) which recognize glycoprotein components of the extrahaustorial membrane (ehm) that surrounds the haustorium formed during the infection of pea leaves by the powdery mildew fungus *Erysiphe pisi.* One of the MAbs, UB9, raised against enriched pea leaf plasma membranes, recognizes a large (200kDa) glycoprotein in this membrane within infected and uninfected epidermal cells. It does not label haustoria at early stages of development showing that at least one plasma membrane glycoprotein is excluded from the elm. However, UB9 binds to the elm of a subpopulation (approx 20%) of HCs late in the infection process and it is possible that the insertion of this glycoprotein into the ehm may represent a breakdown of biotrophy during senescence of haustoria or could be a late defence response by plant. A second MAb, UB11, was raised against isolates haustorial complexes derived from the pea powdery mildew system. This antibody recognises a

glycoprotein (250kDa) which is present in the elm of the haustorial complex and is expressed very early in haustorial development. UB11 does not bind to plant membranes of infected or uninfected plants and does not cross-react with other fungal components. This antibody therefore identifies a glycoprotein specifically located to the elm of haustorial complexes and shows that there is molecular differentiation of this key interface between the biotrophic partners.

Polyclonal antibodies (PAbs) were produced in New Zealand white rabbits with culture filtrate and mycelial extract immunogen preparations from the soybean (*Glycine max*) fungal pathogen *Phomopsis longicolla* (Brill *et.al.* 1994). The PAbs were purified to the immunoglobulin fraction and tested in indirect ELISA and in double antibody sandwich (DAS) -ELISA . The PAbs raised to culture filtrate were more specific but less active in binding than those raised to mycelial extract immunogen preparation. DAS-ELISA was more specific and 100-fold more sensitive in pathogen detection than 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 member of the complex at the some time. PAb raised against *Bipolaris carbonum* was cross reacted with antigens obtained from tea varieties, isolates of *B.carbonum* and nonpathogens of tea (*Biolaris tetramera and Bipolaris setariae*), following immunodiffusion, immunoelectrophoresis and ELISA in order to detect CRA shared by the host and the parasite. CRA were found among the susceptible varieties (TV-9,17 and 18) and isolates of *B. carbonum*(BC-1,2,3 and 4). Such antigens were not detected between *B. carbomum* and resistant varieties (TV-16,25 and 26), nonpathogens and tea varieties, as well as nonpathogens and isolates of *B. carbonum.* Indirect staining of antibodies using fluoresein isothiociyanate (FITC) indicated that in cross sections of leaves (TV-18), the CRA were concentrated mainly around epidermal cells. Treatment of mycelia and condia of *B.carbonum* with antisera to leaves (TV-18) and indirect staining with FITC indicated the presence of CRA in the young growing hyphal tips and conidia (Chakraborty and Saha,1994).

Purkayastha and Pradhan (1994) studied serological differences among three strains (266, 23, M) of *Sclerotium rolfsii* and their antigenic relationship with five

groundnut cultivars. Changes in antigenic patterns of one of its cvs. AK-12-24 and disease intensity were noted after treatment with a systematic fungicide Kitazin. Results reveal that significant variation exists among the strains of the pathogen. The strain 266 was most virulent having antigenic relationship with susceptible cvs. (Gangpuri, J-11 and AK-12-24) of groundnut. The strain 23 also exhibited common antigenic relationship with cv. AK-12-24. Resistant cvs. JL-24 and ICGS-26, however, showed no antigenic relationship with fungal strains in either immunodiffusion or immunoelectrophoretic test. Alteration of antigenic pattern and reduction in disease incidence in susceptibl cultivar was evident following application of kitazin, a systemic fungicide. Polyclonal antibodies were prepared against crude cell wall fractions of *Pythium* isolate with filamentous non-inflated sporangia, associated with root rot of tomato in soilless cultures. In ELISA tests, the antiserum showed sensitivity and specificity against original antigens and those from closely related isolates with filamentous sporangia (such as *Pythium aquatile and Pythium coloratum*). The antiserum reacted weakly with mycelial preparations of several fungi commonly found on tomato roots such as *Fusarium spp.* or *Penicillium spp.* An immunocytochemical staining test was developed in order to study the colonization by filamentous noninflated sporangial isolates of *Pythium* in the presence of other fungi in soil less cultures. (Rafin *et.al.,* 1994). Soluble protein extracts of chlamydospores and mycelium of *Thielaviopsis basicola*, the causal agent of black root rot of cotton were used to raise polyclonal antibodies. The immunoglobulin G antibody fraction was purified and biotin-labelled to divise a fungal capture sandwich enzyme-linked immunosorbent assay (ELISA). ELISA detected both brown and grey cultural types of *T. basicola* and had negliglible cross-reactivity with other soil borne fungi commonly found in cotton field soils. The minimum detection limit of ELISA was between 1 and 20mg of *T.basicola* protein depending on the assay. *T. basicola* could be detected in cotton roots 2 days after inoculation when initial symptoms were apparent. The antibody also was used to observe *T. basicola* on cotton roots with immunofluorescence microscopy (Holtz *et.al.*, 1994).

Leaf antigens prepared from ten tea varieties, three isolates of *Pestalotiopsis theae* and a non-pathogen of tea (*Bipolaris tetramera*) were cross reacted with polyclonal antibody(PAb) raised against mycelial suspension of *P.theae* and reciprocal cross reaction was made with PAb raised against leaf antigens of Teen Ali-17/1/54 and CP-1

following immunodiffusion test and enzyme linked immunosorbent assay. CRA were found among the susceptible varieties and isolates of *P. theae* (Pt-1,2 and 3). 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 fluorescein isothiocyanate (FITC) indicated that in cross sections of tea leaves, CRA was concentrated in the epidermal cells and mesophyll tissues. CRA was present in the young hyphal tips of the mycelia and on the setulae and appendages of the conidia of *P. theae* (Chakraborty *et.al.*,1995)

Optimization of purified immunoglobulin fractions of PAb raised against *Pestalotiopsis theae* was done using ELISA formats. Antiserum dilution up to 1:16000 detected homologous antigen at a 5 mg/L concentration, and at 1:125 antiserum dilution fungal antigens could be detected at concentration as low as 25 µg/L. Absorbance values of infected leaf extracts of fifteen tea varieties were significantly higher than those healthy extracts at a concentration of 40 mg/L. in indirect ELISA. ELISA positive material was detected in tea leaves as early as 12h after inoculation with *P. theae*. At antiserum dilution 1:125, the pathogen could be detected on inoculated leaf extracts upto 2mg/L. The antiserum reacted with two other isolates of *P. theae* tested but not with the antigens from mycelial extracts of *Glomerella cingulata* and *Corticium invisum* or with extracts of tea leaves inoculated with pathogen. The results demonstrated that ELISA can be used for early detection of *P. theae* in leaf tissues even at a very low level of infection (Chakraborty *et.al.* 1996a).

Wakeham and White (1996) raised polyclonal antisera were raised to whole (Coded:16/2),and sonicated (Coded: 15/2) resting spores of *Plasmodiophora brassicae*, and soluble components prepared by filtration and ultracentrifugation (Coded : SF/2) (). Cross- reactivity of all three antisera with range of soil fungi, including western blotting, dipstick, dot-blot, indirect enzyme-linked immunosorbent assay (ELISA) and indirect immunofluorescene 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 $1x\ 10^7$ resting spores g^{-1} in soil. With western blotting the lower limit of detection with antiserum 15/2 was $1\ x10^5$. This antiserum showed the greatest sensitivity in a dip-stick assay, indirect ELISA and indirect immunoflurescence, for all of which there was a limit

of detection of $1x10^2$. The indirect ELISA was successful only after the substitution of alkaline phosphatase by protein A conjugated horse radish peroxidase. 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.*

It was reported by Walsh *et.al.*(1996) that a polyclonal antiserum produced against spore balls of *Spongospora subterranean f.sp.subterranea* prepared from potato tubers was able to detect as little as 0.02 spore balls in an enzyme-linked immunosorbent assay (ELISA). In spiked soil samples, the antiserum detected 100 spore balls per g soil. How ever, the different spore ball contamination levels were discriminated better in ELISA tests at concentration above 2000 spore balls per g soil than at lower concentrations. In contrast, a bioassay test based baiting soils with tomato seedlings gave good discrimination of spore ball contamination levels in spiked soils containing <1000 spore balls per g soil and poor discrimination of levels in spiked soils containing >2000 spore balls per soil. Tests on a limited number of field soils suggested, ELISA may be capable of predicting disease levels on tubers grown in such soil better than the biossay. The antiserum did not react with 30 other micro-organisms tested, including many that are saprophytes or pathogens on potatoes and resting spores of the taxonomically related *Plasmodiaophora brassicae.* It detected spore balls of different cultivar origin equally well. It also detected spores from different geographial origins. An attempt to improve the sensitivity of the serological detection through concentrating spore balls from field soils by sieving was unsuccessful. Cross absorption of the antiserum with uncontaminated field soil increased the sensitivity of detection of spore balls in spiked soil samples four-fold. The ability of the antiserum to discriminate contaminated field soils from an uncontaminated soil was much improved by using the gamma-globulin fraction of cross-absorbed screen. Western blot analysis revealed that the antiserum detected a number of different proteins the most distinct of which had a molecular weight of slightly less than 6.5 kDa. A technique was developed to suppress autoflurescence of spore balls, allowing immunofluoresence studies to be carried out. Using this technique in conjunction with indirect FITC immunofluoreserence, discrete bright fluorescent spots were visualized using the specific serum. With the non-specific serum, only a very dull background fluorescence was evident.

Plant antigens prepared from healthy leaves of five selected tea varieties and fungal antigens prepared from three isolates of *Glomerella cingulata* (GC-1, 2 & 3) as well as non- pathogens of tea (*Bipolaris tetramera and Fusarium graminearum*) were cross reacted with PAb raised against *G.cingulata* (isolate GC-1). Reciprocal cross reaction with PAb raised against leaf antigens of TV-18, Teen Ali 17/1/54 and CP-1 were also made using immunodiffusion, immunoelectrophoresis as well as PTA-ELISA formats in order to detect CRA shared between tea varieties and isolates of *G. cingulata.* CRA were found only among the susceptible varieties and *G. cingulata* isolates but not detected between *G. cingulata* and resistant varieties of tea, non-pathogens and tea varieties as well as *G. cingulata* and non-pathogens. Indirect staining of antibodies using FITC indicated that in cross section of tea leaves, CRA is concentrated in epidermal cell, mesophyll tissue and vascular elements (Chakraborty *et.al,* 1996b)

Population of *Phytophtora spp.* were determined by Miller *et.al.* (1997) using ELISA in field soils used for pepper and soybean production in Ohio. Srivastava and Arora (1997) detected and quantified *Macrophomina phaseolina,* causal agent of charcoal rot disease in many crop plants following PTA-ELISA formats. Polyclonal antisera raised against soluble extracts of mycelium, the residual water insoluble mycelial materials or ribosomal proteins were evaluated for specificity and cross-reactivity with 16 common soil fungi by DAS-ELISA . Cell wall antisera exhibited strong cross reactivity with most of the fungal isolates. Ribosomal antibodies were less reactive to common soil fungi except *Fusarium oxysporum f,so.ciceri.* Mycelial antigens of *M. phaseolina* on chickpea roots were detectable with DAS-ELISA at a minimum concentration of 10ng g^{-1} at 1:100 root: buffer dilution. Quantitative estimation of *M. phaseolina* on roots was evaluated by ELISA under different temperatures and moisture conditions, and in soil amended with a potential antagonist (*Trichoderma harzianum*). A significant reduction in ELISA values was observed in *T. harzianum* amended treatments. This method proved to be useful for detection and rapid screening of *M. phaseolina* under different environmental conditionsPolyclonal antisera raised against mycelial suspension of *Fusarium oxysporum* and the root antigen of the susceptible soybean cultivar (UPSM-19) were cross reacted with root antigens of ten soybean cultivars and mycelial antigen of pathogen in order to detect cross reactive antigens (CRA) shared by the host and the pathogen. The purified immunoglobulin fractions were used for detection of CRA by

ELISA. Antigens of susceptible cultivars showed higher absorbance 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 fluorescein isothiocyanate (FITC) indicated that in cross-sections of roots of susceptible cultivar, CRA were concentrated around xylem elements, endodermis and epidermal cells, while in the resistant variety, fluorescence was concentrated mainly around epidermal cells and distributed in the cortical tissues. CRAs were also present in microconida and chlamydospores of the fungus (Chakraborty *et.al.*1997).

The initial infection process involving adhesion/recognition events between plants and fungal pathogens is essential for the establishment of pathogenesis. The basis of the specificity between *Phytophthora infestans* and potato may lie in the recognition between extracellular matrix and plasma membrane components of the penetrating structures of the fungus and host cells during the earliest stages of infection process. Laxalt *et.al.*(1998) used polyclonal antibodies (AZ and AC) obtained after immunizing rabbits with either 10^7 zoospores (Z) or germination cysts (C) of *P. infestans* to detect putative fungal components located on the surface of invasive structures. AZ recognized several bands, while AC recognized three polypeptides of approximately 87,73 and 45 kDa in western blots. Zoospore suspensions pre-incubated either with AZ of AC were unable to infect potato leaves. Since it was already known that some anti-idiotypic (anti-IT) antibodies may resemble the antigen, thus mimicking its structure, anti- IT antibodies (a-AZ and a – Ac) were generated in mice and used as inducers on potato tuber discs. In northern blots a –AZ treatments increased 1.5- fold the glyceraldehyde-3-Pdehydrogenase(GAPDH) and 1.8 fold the phenylalanine ammonia-lyase (PAL) transcript levels, while an increase of 1.8 and 1.7 fold for GAPDH and PAL, respectively, was observed when the inducer was a –AC. Both PAL and GAPDH transcript levels have previously been shown to be modulated by fungal and elicitor treatments. These results show that the anti-idiotypic antibodies are able to mimic the pathogen components, leading to the accumulation of transcripts related to defense responses in potato *P. infestans* interactions.

Immunodetection of teliospores of Karnal bunt (*Telletia indica*) of wheat using fluorescent staining test was done by Gupta *et.al.*(2000). Polyclonal antibodies were

raised against intact teliospores in Newzeland white rabbits for the immunodetection of Karnal bunt (KB) teliospores. The indirect immunofluorescence (IIF) test was developed using anti-teliospores serum and binding was monitored by goat-rabbit antibody conjugated to fluorescein (FITC) label. The standardization of IIF test was carried out by optimization of dilutions of anti-teliospores antibodies, florescent 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 2328, UP 2328,PBW 393, WH 542, as well as RR 21 (Susceptible cultivars) respectively, is an indication of the presence of similar antigenic configuration to teliospores. Again, they did not exhibit variation in the expression of teliospore associated molecular pattern (TAMP) during previous and subsequent years of infection.

Polyclonal antiserum were produced in albine white rabbits against intact teliospres. The immunoprobe was used for the development of Immunoblot binding assay for detecting infections in wheat seed samples. The antiserum reacted strongly with intact teliospores of *T.indica*, pantnagar isolate in agglutination reaction. 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 testing on nitrocellulose paper, formed a coloured imprint after the paper was assayed. The SIBA developed should not only be a better indication of teliospores load on seed but also quality of seed in terms of vigour. The developed immunodetection method apparently proves to be useful in routine monitoring of wheat lots for the presence of K.B. 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.,* 2001). 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 were 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.

Polyclonal antibody raised separately against antigen preparation from mycelia and cell wall of *G. cingulata* (isolate GC-1) were evaluated for early detection of the pathogen in tea leaf tissue by Chakraborty *et. al.* (2002). PAb raised against cell wall preparations gave better recognition from that against mycelial preparations as observed in ELISA test with antigens of tea leaves of different ages.

Biochemical changes in plants following fungal 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. One of the well documented host responses is the biochemical changes following infection. Phenols in plants occur constitutively and 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. Resistance of maize to *Helminthosporium carbonum* and subsequent changes in host phenolics and their antifungal activity have been discussed by Werder and Kern (1985). Host responses could be differentiated by changes in content of phenolic compounds. 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. However, 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. They isolated four antifungal compounds, A, B, C and D from hydrolyzed maize leaf extracts which were found to be fungitoxic to *B. zeicola* in spore germination and chromatographic bioassays.

Saxena *et al.* (1986) evaluated the changes in phenolics of resistant and susceptible wheat varieties in response to *Puccinia recondite.* Resistant varieties exhibited higher concentration of phenolics than the susceptible ones. 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).

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. Changes in phenol contents was also 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.

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). 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).

Accumulation of two caffeic acid esters after inoculation of maize with *Glomerella graminicola* 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. 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*). Seasonal changes in the phenolic constituents of jack pine seedlings (*Pinus banksiana*) in relation to the purpling phenomenon was 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 phenyl propanoids, 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

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 following inoculation with all the three test pathogens of jute, while reducing sugars, total phenols and orthodihydroxy phenols increased. 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).

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 exhange 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). 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 PAL and the isoflavanoid phytoalexins. 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.

Yoshikawa and Sugimoto (1993) identified the putative receptor like target sites for glucanase-released elicitor in soybean membranes. The elicitor molecules that function *in vivo* for phytoalexin elicitation in *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. The elicitor induced incorporation of phenylpropanoid derivatives into the cell wall and the secretion of soluble coumarin derivatives by parsley 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*. When the parsley suspension cultures were preincubated 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 PAL and 4-coumarate: coenzyme A ligase.

Resistance or virulence are modelled by multiple biochemical components of two living organisms. *Costus speciosus* a major sapogenin bearing medicinal plant was severly 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. 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 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 on 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 (Kali *et al.,* 2000).

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 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).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.

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). 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).

Six apple rootstocks [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 apple rootstock (MM115) and minimum in highly susceptible ones (MM103, 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, TAL) and oxidizing enzymes (PPO, PO) increased rapidly in resistant root stocks (MM115, 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 20th or 25th day of inoculation) in highly susceptible root stocks (MM103, 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).

Joshi *et al,*(2004) attempted to quantify biochemical changes in highly susceptible (IC 116835) and moderately resistant (IC 116903) genotypes of cluster bean against *Alternaria cucumerina* var. *cyamopsides* . The catalase activity 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.

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 (Copper *et al.*, 2004).

Association of defense enzymes with resistance in tea plants against *Exobasidium vexans* was demonstrated by Sharma and Chakraborty (2004). Results revealed significant changes in the level of enzymes mainly □-1, 3 glucanase and chitinase exhibiting antimicrobial activity. A wide variety in the activities of the enzymes involved in phenol metabolism including PAL, POX and PPO 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.* (2004a). 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.*, 2005a).