Literature Review

In general, plants respond in two different ways to pathogens. There is either no obvious interaction, or an interaction occurs and is, in the extreme cases, either incompatible or compatible. The biochemical events occurring in interactions between host or non-host plants with potential pathogens are basically similar, but their timing of appearance and both the intensities and patterns depend on their genomic as well as on environmental conditions. It has been established that different plant species, even varieties within a species may have fine differences in biochemical make-up particularly in respect of phenolics, lignins, proteins and various other metabolic as well as constitutive components as also in their enzyme components and they also differ in their responses to infection in most of these respects. Such biochemical differences in the host responses to inoculation with their potential pathogens have been studied for many host pathogen combinations, and the sum of all available data suggests that most, or all of them, are part of a typical resistance responses of plants, one often determining over the other as the major defence mechanisms. Variations may occur more at the level of timing of induction, location and relative amounts, than in terms of an all-or-none response. Immune systems in plants play a significant role in the host response to the pathogen, and though different from that of animals, is functionally similar. Plants, in general, have their own natural immunity against several diseases; this could also be induced by physical, chemical or biological agencies (Purkayastha, 1994).

A short and comprehensive review on the biochemical changes following induced resistance in plants and serological relationship between host and parasite have been presented below.

Biochemical changes following induced resistance in plants

Well-documented evidences are now available to indicate that effective resistance can be induced in plant host by prior restricted inoculation with the same pathogen or inoculation with its mildly virulent or avirulent races or other homologous or heterologous pathogen or even prior treatment with their metabolites or constitutive compounds. Many chemicals with little or no toxicity *in-vitro* against the pathogen have also been tested occasionally for plant disease control. These include metal salts, amino acids, plant growth regulators and various other

compounds with varied chemical and biological action. Initial information in this respect was reviewed by van Andel (1966) and Wain and Carter (1972). There has been increased activity based on this approach and the resultant information has been reviewed by number of workers (Sequeira, 1979; Matta, 1980; Hamilton, 1980; Goodman, 1980; Langcake, 1981, Kuc, 1987; Lazarovits, 1988; Sinha, 1989; Kessmann *et al.*, 1994; Sticher *et al.*, 1997; Hammerschmidt and Becker, 1997) and more recently by Hammerschmidt (1999).

Significant levels of success has been achieved by using chemicals of widely diverse nature without any direct toxic action against many plant diseases (Sinha, 1984). Apart from chemicals, physical agents such as X-ray (Purkayastha and Ghosh, 1983), UV (Bridge and Klarman, 1973) and biological agents (Sinha and Das, 1972, Chakraborty and Chakraborty, 1989) are also known to alter disease reaction. Numerous molecules have been implicated in mediating disease resistance. There is evidence that some products either of biotic or abiotic origin are capable of activating the host's defense reaction by accumulating secondary metabolites or "Stress" metabolites such as phytoalexin, in treated (physically or chemically) plants (Darvill and Albersheim, 1984; Purkayastha, 1986). Several elicitors of phytoalexin synthesis also induce the expression of other host plant defense responses (e.g.,proteinase inhibitor synthesis and accumulation of hydroxyproline-rich glycoproteins). In some of the recent reviews the mechanisms of induced resistances in plants have been well documented (Ouchi, 1983; Sequeira, 1983; Halverson and Stacey, 1986; Madamanchi and Kuc, 1991).

Oku (1960) presented evidence indicating that resistance of rice plants to *Cochliobolus miyabeanus* could be broken down by treatment with reducing agents such as ascorbate or glutathione. The resistance of rice plants against hyphal penetration by *C. miyabeanus* could partially be attributed to fungal oxidation product, perhaps quinones, derived from host cells or membranes.

The influence of gibberellic acid on the seedling blight of corn was noted by Wilcoxon and Sudia (1960). They observed that treatment of maize hybrid seed with 5, 10 and 20 ppm gibberellic acid enhanced the severity of seedling blight. Use of nickel chloride as foliar spray to tea plants (*Camellia sinensis*) for the control of blister blight caused by *Exobasidium vexans* was demonstrated by Venkataram

(1961). Percentage shoot infection was lower in nickel chloride than in the cuprous oxide treatment. Hale *et. al.*, (1962) reported that growth regulators (viz. Indole-3-acetic acid, napthalene acetic acid, 2,4-dichlorophenoxy acetic acid and maleic hydrazide) caused an increase in size and number of leaf spot/ plant on the susceptible inbred corn like K-44 and the resistant line K-41 when the plants were inoculated with *Helminthosporium carbonum.*

Severity of lesion development on the hypocotyls of red kidney bean increased by foliar applications of gibberellic acid when plants were grown in soil infested with *Rhizoctonia solani,* isolate Rh-5. However, when the plants were treated with gibberellic acid and grown in soil infested with two other pathogenic isolates of *R. solani*, severity of the disease was not affected. The increased virulence of Rh-5 was probably caused by root excretions resulting from the gibberellic acid treatments (Peterson *et al.*, 1963). It was speculated by Daly and Deverall (1963) that hormonal concentration in a leaf could be important in controlling the development of a pathogen. The initial establishment of the disease could be due to hormonal changes brought about by entry of pathogen. Foliar application of different concentration of IAA and GA to detached bean leaves had little effect on lesion production by *Botrytis fabae* and *Botrytis cinerea* and were ineffective in the spread of lesion by *B. cinerea* (Purkayastha and Daverall, 1965).

The effect of maleic hydrazide (MH) on wheat and barley rust were studied by Joshi (1965). The solutions of maleic hydrazide (0.02 percent) were administered to wheat roots and barley seedlings at the time of emergence. The doses, however, varied between 50-120 ml/plot (10 cm. diam). Barley plants (varieties Bolivia and Oderbrucker) treated with 0.2% MH solution (110ml/plot) showed reduction in growth and higher susceptibility of plants to *Puccinia hordeii.* The response was very poor in case of Agra local variety.

Foliar or soil application of CCC [(2-chloroethyl) trimethyl ammonium chloride] reduced the infections of bean seedlings by *Sclerotium rolfsii* (Tahori *et al.*, 1965). But Crosier and Yountburg (1967) reported that CCC (2-4 pounds of CCC/acre) was ineffective against *Tilletia foetida* on winter wheat when used alone as foliar spray. Sinha and Wood (1967) have shown that IAA reduced wilt disease of tomato caused by *Verticillium alboatrum.* On the other hand, maleic hydrazide

(300 ppm), greatly retarded growth of the plant and made them susceptible. Cycocel and napthalene acetamide gave good control of disease over a range of concentrations when applied to the soil in which the plants were growing. Of the other growth regulating substances tested, 2, 4, 5-trichlorophenoxyacetic acid increased disease at some concentrations and reduced it at others.

Chalutz and Stahmann (1969) induced pisatin formation in carrot tissue by ethylene. However, production of pisatin in pea tissues in response to ethylene treatment was less than that induced by fungi. It is possible that ethylene could induce some of the enzymes (phenyl alanine deaminase) involved in the biosynthesis of the pisatin. Carrot roots treated with IAA, 2, 4-D and 2,4,5-T also elicited coumarin accumulation. In all cases production of isocumarin was related to the amount of ethylene produced by root tissue. Foliar spray with either GA_3 or CCC (both 1 and 100 ppm) increased susceptibility of jute seedlings growing in *Macrophomina* infested soil. Maximum susceptibility was observed when treated with GA_3 but minimum in case of CCC treated plants (Purkayastha *et al.*, 1972). Under the influence of IAA and GA_3 some aspects of host parasite relationships were studied by Valken (1972). He reported that IAA increased the *Fusarium* wilt of tomato while the reverse result was obtained with GA_3.

Furrer and Staulfer (1972) demonstrated that by the application of cycocel in combination with nitrogen, yields of spring wheat was augmented; lodging and eye spot caused by *Cercosporella herpotrichoides* was reduced. Bojarezuk and Ruszkowski (1972) also noted that application of cycocel (3-4kg/ha) at the end of tillering reduced eye spot infection in wheat and rye and increased yield in both cases. The effects were more pronounced in varieties susceptible to lodging with high nitrogen fertilizer. Efficacy of cycocel against grey rot of grapevine caused by *Botrytis cinerea* was tested by Natalina and Svetvov in 1972. About 25-50% reduction in the incidence of disease was recorded after spraying vine with cycocel. But cycocel treatment increased the infection of jute caused by *Septoria nodorum.*

Artificial application of natural and synthetic chemicals could also induce disease resistance in plants. Sharma (1973) reported that application of DL-tryptophan, IAA and HCN induced resistance in some sorghum varieties to *Colletrotrichum graminicola.* Particularly, 0.12% and 0.062% of KCN (in place of

HCN) proved to be effective in inducing the resistance.The most encouraging results were obtained with 25+5, 25+10 and 50+5 ppm concentrations of DL-tryptophan and zinc respectively (zinc is known to take part in conversion of tryptophan to IAA). By the application of IAA (50 ppm) resistance to *C. graminicola* was noticed more than DL-tryptophan (25 or 50 ppm).

Sad and Rashid (1973) recorded that 16 ppm CEPHA (2-chloro ethane phosphonic acid) controlled the chocolate spot disease of potato and induced the production of small size tubers and tuber crackie (4%). Similarly, 25 ppm GA also controlled the disease but induced knobiness (12%) and sprouting (15%) of tubers in the field. On the other hand application of IAA (80-50 ppm) did not influence disease but induced the production of large size tubers. It is interesting to note that a mixture of CEPHA and IAA when sprayed 2 weeks after flowering decreased disease incidence considerably and undesirable side effects produced by CEPHA alone were not observed.

The gibberellins and tri-iodobenzoic acid decreased severity of charcoal rot disease of soybean under all experimental conditions (Oswald and Wyllie, 1973). The effects of indole acetic acid and kinetin on the development of *Verticillium* wilt of cotton was explained by Abrarov *et al.*, (1973). These compounds inhibited the spread of necrosis of leaf blades and stimulated formation of leaves and generative organs.

The role of auxins in leaf spot incidence in ragi was discussed by Vidyasekaran (1976). Young leaves of ragi (*Eleusine coracana*) was resistant to blight disease caused by *Helminthosporium tetramera* while the older leaves were highly susceptible. Young leaves contained more auxin than the older leaves. The IAA treatment inhibited spore germination and growth of the pathogen only at high concentrations.

The effect of foliar application of plant hormones on the development of anthracnose disease caused by *Colletotrichum corchorum* in two cultivars of jute (*Corchorus capsularis*) were studied by Purkayastha and Ray (1977) under identical conditions. These hormones were also tested on the growth of the pathogen *in-vitro,* Gibberellic acid (10 and 100 ppm) and indole acetic acid (10 ppm) increased disease susceptibility in both resistant and susceptible cultivars. These compounds stimulated mycelial growth of *C. corchorum* at a low (0.1 ppm) concentrations. Apparently there

was no correlation between mycelial growth and pathogenecity of fungus. Inflorescence of grapevine sprayed with 10 ppm gibberellic acid significantly reduced *Botrytis* infection (Rivera and Mavrich, 1978). Mercuric acetate caused accumulation of rishitin and lubimin in potato tuber discs. Accumulations of these terpenoids was not directly correlated to the necrotic reaction. When two cultivars of *P. vulgaris* showing different degrees of susceptibility were treated with $HgCl_2$, the yield of phytoalexin was similar in both the cultivars. However, the accumulation pattern differed when inoculated separately with 3 isolates of *Botrytis cinerea* differing in virulence (Cheema and Haard, 1978).

In the glass house, application of 2,4-D (40% butyl ester) and atrazine (72%) increased susceptibility of soybean to blight disease caused by *Sclerotium rolfsii.* Incidence was higher in plants with low or high sugar content, but lowest on those with normal sugar content. It was also noted that monosodium phosphate, zinc sulphate, 2,4-D and atrazine were mildly phytotoxic (Carlos, 1979). Some growth retardants mitigated *Verticillium* wilt and increased yield of cotton. Particularly the application of chloromegnat [(2-chloroethyl) trimethyl ammonium chloride], Pix (N,N-dimethyl piperidinium chloride) and chemagro-8728 (tributyl) slightly migrated the severity of symptoms of *V. dahliae* on cotton and reduced internal populations of the pathogen in the petioles, cotton yield was increased (10.29%) by these treatments (Erwin *et al.*, 1979).

A fungicide known as 2,2-dichloro-3,3-dimethyl cyclopropane carboxylic acid (WL 28325) has been found to activate the natural resistance of rice plants against blast disease caused by *Pyricularia oryzae.* The activity of WL-28325 is unique in that it does not itself stimulate phytoalexin production but rather increase the capacity of rice plants to synthesize more momilactones (rice phytoalexins) in response to fungal infection. The antifungal activity of rice phytoalexin may be the basis for its disease reduction properties (Cartwright *et al.*, 1980). The effect of mercuric chloride on glyceollin synthesis or degradation of glyceollin was tested by Moesta and Grisebach (1980). They observed that $HgCl_2$ produced only a slight effect on the biosynthetic activity but strongly inhibited glyceollin degradation.

The effect of foliar spray of bacitracin, chloramphenicol and GA on rhizosphere microflora of pea seedlings (*P. sativum* L.) infected with *V. dahliae* was

studied. The antibiotics increased fungus and actinomycetes counts and reduced the bacterial populations in the rhizosphere. The GA reduced all three groups of micro-organisms while 100 ppm increased actinomycetes slightly. Foliar spray also affected the percentage occurrence of particular genera of fungi in the rhizosphere, for examples, *Trichoderma* spp. were stimulated by all treatments, the maximum being with 10 ppm GA. Foliar spray however, markedly reduced disease severity (Ramarao and Isacc, 1980).

The effect of three growth substances 6-Furfuryl aminopurine (Kinetin), 6-Benzyl aminopurine (BAP) and gibberellic acid (GA_3) on the development of charcoal rot disease of soybean caused by *Macrophomina phaseolina* was studied by Chakraborty and Purkayastha (1981). Two foliar sprays with 1 or 10 ppm GA_3 at an interval of 3 days before inoculation of plants reduced the disease significantly. But the application of 10 ppm kinetin or BAP markedly augmented the disease.

Eight chemicals reported to induce phytoalexin in plants were used for wet seed treatment in an attempt to develop resistance in susceptible rice seedlings to *Drechslera oryzae*, the brown spot pathogen. While all produced appreciable effects, cysteine, thioglycollic acid, cycloheximide, sodium selenite, p-chloromercuribenzoate and lithium sulphate caused marked reduction in symptoms in rice seedlings when challenge inoculation was done at the age of 3-4 weeks. With sodium selenite and thioglycollic acid the induced effect persisted upto 8 weeks after sowing. A second treatment in the form of foliar spray with these chemicals caused sharp increases on protection, but these disappeared 2 weeks after treatment. Leaf diffusates from 2 week old seedlings in different treatments showed considerable fungitoxicity, which declined with seedling age and become practically non-existent by the end of fourth week. Inoculation with treated plants at this age resulted in moderate to marked toxicity in their diffusates. Seed treatment was found to be more effective than foliar spray treatment (Sinha and Hait, 1982). Accumulation of phytoalexin in excised cotyledons of *P. vulgaris* was detected when treated with 10^{-4}M abscissic acid or benzylaminopurine (BAP). In case of former, cotyledons were incubated both in light and in dark but in case of latter, they were kept under light only (Stoessel and Magnalato, 1983).

Furocoumarin (Phytoalexin) was induced in celery by copper sulphate (Bier and Oertelli, 1983). Capsidiol (pepper phytoalexin) production has also been induced in fruits of *Capsicum annum* by 0.1M copper sulphate, sodium nitrate and chloramphenicol (Watson and Brooks, 1984). Gibberellic acid (GA_3) induced momilactone synthesis in treated inoculated (with *Acrocylindrium oryzae*) leaf sheaths and coleoptiles. Since GA_3 is a degraded diterpene it may act as a precursor of gibberellin mediated enzyme (associated with momilactone biosynthesis) which may account for the elicitation of momilactone synthesis in rice plants (Ghosal and Purkayastha, 1984). Seed treatment of wheat with dilute concentration of nickel chloride and barium sulphate significantly induced resistance to *Drechslera sorokiniana* (Chakraborty and Sinha, 1984).

Twenty out of twenty four chemicals known to induce phytoalexin production in other plants when used as seed treatment provided effective protection to 3-week-old susceptible wheat seedlings against inoculation with *Helminthosporium sativum.* The number of lesions was very significantly reduced by most of the treatments and there was evidence for inhibition of lesion expansion in a few. Studies with twelve of the more effective chemicals showed that the protection effect persisted at significant levels even in 5 week old plants and that at this stage this inhibiting effect on lesion expression was more pronounced in most of the treatments. Different treatments led to the development of a moderate to high level of fungitoxicity in young wheat seedlings which markedly declined with age of the plant and disappeared in 5 week old plants. When inoculated at the age of 3 or 5 weeks, plants receiving most of the treatments developed appreciably higher fungitoxicity than the untreated plants (Hait and Sinha, 1986).

Chakraborty and Purkayastha, (1987) studied the effect of six metabolic inhibitors (viz. sodium iodoacetate, 2,4-dinitrophenol, sodium fluoride, sodium malonate, sodium azide and sodium molybdate) on the development of charcol rot disease of soybean (cv. Soymax). The effect of sodium azide (100 μg/ml) was found to the most significant among the metabolic inhibitors tested, in reducing the disease symptom. The reduction in disease was evidenced by minimum loss in weight of roots and minimum root rot index. The glyceollin content of soybean roots before and after disease reactions by sodium azide treatment was estimated and compared. The

production of glyceollin was maximum when plants were treated with sodium azide followed by inoculation with M.phaseolina. Sodium azide induced glyceollin synthesis even in uninoculated soybean plants.

The effect of foliar application of growth substances on the development of charcoal rot disease of soybean caused by *Macrophomina phaseolina* was tested by Chakraborty *et al.*, (1989). Among the eight growth substances (3-indole-acetic acid, 2,4-dichlorophenoxyacetic acid 2,3,5-tri-iodobenzoic acid, 2-napthoxyacetic acid, L-naphthalene acetic acid, gibberellic acid, 6-furfuryl amino purine and 6-benzyl aminopurine) examined, gibberellic acid was most successful in reducing the disease severity, followed by 3-indole acetic acid and 2,3,5-tri iodobenzoic acid. Low concentrations of these compounds stimulated while high concentrations inhibited the mycelial growth of *M. phaseolina in vitro*. Glyceollin contents of host roots before and after treatments with gibberellic acid (10mg/l.) were estimated; this compound significantly increased glyceollin production in infected roots.

Spray with $AgNO_3$ and $CuCl_2$ solution on the leaves of *Brassica juncea* and *B. napus* also caused accumulation of phytoalexin and the effect of cycloheximide suggested that its accumulation was associated with induced plant metabolism. Phytoalexin was detected in *B. juncea* and *B. napus*, 6h and 18h after challenge with $CuCl_2$ a non-specific elicitor. *B. juncea* always accumulated 4 to 10 times more phytoalexin than did *B. napus* (Rouxel *et al.*, 1989).

Purkayastha and Banerjee (1990) used six antibiotics (penicillin, cloxacillin, tetracyclin, chloramphenicol, cephaloridine and kanamycin) as foliar spray on a susceptible soybean cultivar (soymax) to induce resistance against anthracnose. Among the six antibiotics tested cloxacillin and penicillin induced maximum resistance against anthracnose. Spraying the lower surface of the first true levels of cucumber plants with 50M K_2HPO_4 induced systemic resistance to anthracnose caused by *Colletotrichum lagenarium*. Correlations were made between peroxidase and chitinase activities induced by several treatments on first leaf and the level of protection observed in leaf 2 after challenge with *C. lagenarium* (Irvan and Kuc, 1990). Reduction in barley stripe disease induced by *Helminthosporium gramineum* was observed by Mathur and Bhatnagar (1991) when seeds were immersed in ferrous sulphate solution or moistened with sulphates of manganese and cobalt before sowing.

Wet seed treatment with phytoalexin inducer chemicals and related compounds protected rice plants from the attack of both brown spot and blast diseases. Such compounds were effective at dilute concentrations, mostly non-hazardous and with little or no fungitoxic effect at the concentration employed. Many of the chemicals have equally strong effective action against both diseases; some are more effective against one than against the other. Sarkar and Sinha (1991) concluded that such chemicals may be mostly acting through an induction of general host resistance and also provide in the process a broad spectrum action effective simultaneously against a group of pathogens.

Effectiveness of 19 non-conventional (mostly non-toxic) chemicals in wet seed treatment (10^{-4} to 10^{-2}M) in controlling wilt of tomato (cv. Patharkuchi) caused by *Fusarium oxysporum* f. sp. *lycopersici* was demonstrated by Mandal and Sinha (1992). While most of the compounds could reduce wilt symptoms appreciably, cupric chloride, ferric chloride, zinc chloride, manganese sulphate, mercuric sulphate, L-cysteine, IAA and DL-methionine showed very strong protective effect. These reduced leaf symptoms by 52 to 71%, prevented mortality completely and also limited vascular colonization by the pathogen. Most of the test compounds showed little or no *in vitro* fungitoxicity at their effective concentrations and stronger protection was often achieved at lower than higher concentration. These non-conventional chemicals act in plant disease control not by toxic action but by inducing resistance in susceptible tomato plants, mediated through host tissue conditioning.

A varied group of non-conventional, non-toxic compounds such as metal salts, growth regulators, amino acids, metabolic inhibitors, and biological compounds used as seed treatment (at 10^{-6} to 10^{-8} M) were tested in controlling *Sclerotium* rot of soybean, *Fusarium* wilt of chickpea and *Sclerotium* rot and early leaf spot of peanut. The significant levels of protection were achieved with cupric chloride and ferric chloride in soybean; IAA, chitosan, cycloheximide in chickpea; IAA, chitosan in peanut against *S.rolfsii* and IAA, DL-phenylalanine, cycloheximide, barium sulfate and lithium sulfate against *Cercospora* infection (Chowdhury and Mitra, 1999; Chowdhury, 2000).

Serological relationship between host and parasite :

It is generally accepted that the cells recognize one another through pairs of complementary structures on their surfaces: a structure on one cell carries encoded biological information that the structure on the other cell can decipher. This idea represents an extension of the lock and key hypothesis. Each and every living plant has its own immune system functionally similar to that of animals. Conclusive evidences are now available to confirm the existence of phytoimmunity but unlike humoral immunity or specific target of antibodies which commonly operates in animals. A number of reviews pertaining to serological relationships between host and parasite have already been published (DeVay and Adler, 1976; Chakraborty, 1988; Hansen and Wick, 1993; Werres and Steffens, 1994).

Three species of the genus *Phytophthora* were studied serologically by gel diffusion and immunofluorescence. The type and concentration of nitrogen source, the amount of inoculum, and the age of mycelium greatly affected the antigenic efficacy of the preparations. The antigens were found to be localized in the young growing tips of the hyphae. Species specific sera were obtained and proved efficient for the identification of *P. cactorum,P. cinnamomi* and *P. erythroseptica* (Burrell *et al.*, 1966). Serodiagnostic methods for the differentiation between susceptible and resistant Egyptian cotton varieties with *Fusarium oxysporum* and *Citrus* sp. with *Phytophthora citrophthora* have been described by Abd-El-Rehim and Hashen (1970) and Abd-El-Rehim *et al.*, (1971a). Serological and immunoelectrophoretical studies on resistance and susceptible watermelon varieties to *Fusarium semitectum* also revealed that the cultivars could be differentiated by the titre or the time after which reaction occurred between antisera specific to the pathogens and seed globulins. In this case only $\alpha_2\beta$ globulin was present in the resistant varieties (Abd-El-Rehim *et al.*, 1971b).

Wimalajeewa and DeVay (1971) detected common antigenic relationship between *Zea mays* and *Ustilago maydis.* A pair of compatible haploid lines and two diploid solopathogenic lines of *U. maydis* were used in immunological studies. *Hordeum vulgare* var. "California Mariout" and *Avena sativa* var. "Victory" were taken as resistant hosts. Certain antigens were found common between corn and *U. maydis.* A strong antigenic relationship existed between the solopathogenic lines 132

and 3 day-old oat seedlings. Barley did not have any antigen in common with any of the *U. maydis* lines tested. Antigenic comparison of the four lines of *U. maydis* did not indicate any quantitative significant serological differences among them. Charudattan and DeVay (1972) compared common antigenic relationship among four cotton varieties and isolates of *Fusarium* and *Verticillium* species. One antigenic substance was common among the varieties of cotton and isolates of *Fusarium oxysporum* f. sp. *vasinfectum, F. solani* f. sp. *phaseoli, Verticillium albo-atrum* and *V. nigrescens*. Cotton varieties which were resistant or susceptible to *Fusarium* wilt as well as pathogenic and nonpathogenic isolates of *F. oxysporum* f.sp. *vasinfectum* shared the common antigens. The common antigens were not shared between cotton and nonpathogen (*F. moniliformae*). The common antigenic determinant shared by cotton and the fungal isolates does not appear to be related to the severity of wilt symptoms, but it may affect host pathogen compatibility during the process of root infection.

The presence of *Aspergillus* and *Penicillium* spp. which were predominantly isolated from stored barley grains, were confirmed by immunofluorescence, but the amounts of mycelium involved were small. *Alternaria* spp. though not isolated from 3 of the samples, mycelium were detected in grains of all the samples in small amounts (Warnock, 1973). Fluorescent antibody staining of *Fusarium culmorum* using antiserum raised against the mature hyphae of the fungus was reported by Hornok and Jagicza (1973). Both the direct and indirect methods of the fluorescent antibody technique resulted in a specific yellow-green immunofluorescence, however, indirect staining was always more intense. There was a significant difference in the intensity of the fluorescence between the mycelia of the homologous *F. culmorum* strain and those of the heterologous *F. graminearum* and *F. culmorum* strains. All the other *Fusarium* species tested showed no detectable fluorescence. The heterologous strains of *F. culmorum* and *F. graminearum* could not be distinguished. No significant difference was found between the 'culmorum' strains from different host plants. The serum specific for *F. culmorum* and *F. graminearum* is suitable to separate these species from other fungi.

Common antigen was shared by both avirulent and virulent isolates of *F. oxysporum* f.sp. *vasinfectum* with disease resistant and susceptible lines of cotton. In

all cases, the fungal isolates invaded and parasitized cortical tissues of cotton roots, but only those fungal isolates that caused disease became established in the vascular system (Kalyanasundaram *et al.*, 1978).

Rabbit antiserum rose against soluble extracts of *Phytophthora infestans* (Race-4) and tubers of "Aran Banner" and "Golden Wonder" potato cultivars showing field susceptibility and resistance respectively to late blight were used to test for the presence of common antigens between extracts of the fungus and various host and non-host plants (Palmerley and Callow, 1978). Cross reactive antigen was detected between *P. infestans* (Race-4) and potato tubers of both the field susceptible and field resistant cultivars and also between the fungus and leaves of tomato and tobacco. Common antigens were not detected between *P. infestans* (Race-4) and leaves of non-host (mungbean, pea, radish, cucumber and maize), or between potatoes and the alternative pathogen, (*Fusarium solani* var. *caeruleum*) and two non pathogens (*Ustilago maydis* and *Phytophthora cinnamoni*).

Fluorescent antibodies were used to study the survival of zoospore cysts of *Phytophthora megasperma* and *P. cinnamoni* in soil (Mac Donald and Duniway, 1979). Germinated zoospore cysts of both the species were stained by an immunofluorescent detection technique and counted. Under laboratory conditions, zoospores cysts of *P. megasperma* survived no longer than three weeks at water potentials ranging from 0-15 bars, and under field conditions they survived no longer than two weeks. Zoospore cysts of *P. cinnamoni* survived no more than 3 weeks in soil at water potential of 0 to –1 bar under either field or laboratory conditions. However, at water potentials of –5 or –15 bars, zoospore cyst of *P. cinnamoni* survived upto 6 weeks in the laboratory.

An antiserum, prepared to homogenates of washed *Epichloe typhina* mycelium grown in a liquid medium, was used in an ELISA to detect antigens of the fungus in tall fescue (*Festuca arundinacea*) tissue samples by Johnson *et al.*, (1981). ELISA could detect as low as 100 ng of freeze dried *E. typhina* mycelium/ml and could detect *E. typhina* in individual seeds of tall fescue. Of 14 fungal genera tested, including *Acremonium, Claviceps, Helminthosporium, Pythium, Rhizoctonia* and *Sclerotium* all showed reactivities less than 0.1% that of *E. typhina.*

Major cross reactive antigenic substances (CRA) common to cotton (*Gossypium hirsutum*) and certain fungal parasites of cotton roots, was isolated and purified to homogeneities from conidial cultures of *Fusarium oxysporum* f.sp. *vasinfectum.* Agar gel double diffusion tests indicated the presence of crossreactive antigen not only in *F. oxysporum* f.sp. *vasinfectum* and cotton roots and seeds but also in *Theielaviopsis basicola* (Devay *et al.*, 1981a). Indirect staining of antibodies and labelling with FITC indicated that in cross sections of roots, cross reactive antigen (CRA) was concentrated mainly around xylem elements, the endodermis and epidermis cells and was present throughout the cortex tissue. Protoplasts prepared from cross section of cotton roots also contained the CRA which was concentrated in the region of the plasmalemma. Treatment of mycelia and conidia of the pathogen with antiserum of cotton and using indirect staining with FITC indicated that the CRA was mainly present in hyphal tips and in patch like areas on conidia (DeVay *et. al.*, 1981b). Four formae speciales of *F. oxysporum* (*dianthi, mclonis, pisi, lycopersici*) and three physiological races (1,2,3) of *F. oxysporum* f.sp. *melonis* have been differentiated by Iannelli *et al.*, (1982) using agargel double diffusion technique. Three isolates of *F. oxysporum* f.sp. *dianthi* race 2 and 10 of f.sp. *lycopersici* race 1 tested against the appropriate antisera reacted the same within each races.

Gendloff *et al.*, (1983) produced antisera against both a whole cell and cell wall preparation of *Eutypa armeniacae* Rhodamine isothiocyanate (RITC) conjugated antisera were tested for reactivity with various fungi on glass slides. Specificity of the sera was improved by cross adsorption of the RITC-conjugated cell wall antiserum with *Phomopsis viticola.* Wood cross sections from concord grapevines inoculated with *E. armeniacae* and also inhabited by various other fungi were stained directly with the conjugated anti-*Eutypa* rabbit serum. In an indirect staining procedure, sections were treated with anti-*Eutypa* rabbit serum and then stained with RITC labeled goat anti-rabbit gamma globulin. Both procedures specifically stained hyphae in wood sections. Hyphae stained indirectly in woody vine sections showed a much brighter fluorescence than analogous hyphae stained by the direct method. Fungi of some species that reacted strongly with the direct method on glass slides did not always react when stained indirectly in woody vine sections in which they were growing.

The presence of *Ganoderma lucidum* in roots of betel nut was detected by the induction of fluorescent antibodies (Reddy and Ananthanarayanan, 1984). Indirect immunofluorescence microscopy was used by Dewey *et al.*, (1984) to detect the binding of species-specific antisera against *Phaseolus schweinitzii* antigens to extracellular macromolecules secreted by the fungus, to cell surface of basiodiospores, and to the cell surface and cross walls of mycelia. Common antigenic determinants were present in extra cellular culture filtrate material and walls of mycelia, chlymodospores 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. Identification of the kind of propagule most likely to be the source of field isolated of the organisms was possible by immunofluorescence and strongly suggested that the pathogen could survive saprophytically in the soil. In contrast, isolated mycelial cell wall preparations did not prove to be a suitable source of immunogenic material for these studies.

Chard *et.al.*, (1985) carried out immunofluorescence tests, involving 34 species of fungi on an anti-*Mycena galopus* serum raised against a partially purified antigenic fraction. Cross-reacting fluorescence was produced primarily by *Mycenia*, deuteromycetes and ascomycetes species. Non-*Mycena* basidiomycetes generally showed less fluorescence. Absorption of the antiserum with mycelium from cross-reacting fungi resulted in a reduction in fluorescence of cross-reacting species, mostly to an acceptable control level.

Rabbit antisera were raised against antigens of *Macrophomina phaseolina* (isolate MP_1) and roots of soybean cultivars Soymax and UPSM-19 which were susceptible and resistant respectively to charcoal rot disease. These antisera were used in agar gel double diffusion tests for the presence of common antigens between isolates of *M. phaseolina* and soybean cultivars. Immunoelectrophoretic tests revealed that four antigenic substances were common between the susceptible soybean cultivars and the fungus (Chakraborty and Purkayastha, 1983). A close relationship between lower production of glyceollin by soybean cultivars and presence of common antigen following immunodiffusion and immunoelectrophoretic tests was also described by Purkayastha and Chakraborty (1988).

Immunological comparisons of teliospore surfaces using polyclonal antisera and monoclonal antibodies indicated that *Tilletia controversa* and *Tilletia caries* were very similar (Banowetz *et al.*, 1984). Although two polysaccharide antigens were present in teliospore extracts, these components appeared to be immunologically identical in both species and no protein antigens were demonstrated by either electrophoretic or immunologic means. None of seven fluorescein-lebelled lecitins bound these teliospores, even after the spores were treated with 8M Urea to enhance exposure of potential lecitin binding sites. An antibody "double sandwich" enzyme immunoassay demonstrated quantitative differences in the numbers of certain monoclonal antibody binding sites of the two fungi, although these differences did not provide a basis for the unambiguous detection of either bunt species in contaminated wheat shipments. Specific antibodies to *Phoma exigua* var. *foveata* and var. *exigua* were isolated and immunoenzymatic techniques (double antibody sandwich ELISA and indirect ELISA) were used by Aguelon and Dunez (1984) to test for the fungus in inoculated tubers and sprouts and in stems grown from these tubers. The fungus was detected in these different tissues with var. *foveata* being more aggressive, demonstrating the applicability and sensitivity of the techniques. They also reacted with the two varieties of the fungus were not specific to their own varieties. They also reacted with *Phoma tracheiphila* but did not react with several other common potato pathogens. The ELISA technique also showed good specificity and sensitivity in detecting "Sclerotinia antigen" at a concentration as low as 10 ng/ml, from both artificially and naturally infected host plants (Walcz *et al.*, 1985).

Cross reactive antigens were detected in crude preparations and in purified preparations from mycelia of *Phytophthora infestans* Race-4 and Race 1,2,3,4,7 with antisera of potatoes cv. King Edward and cv. Pentland Dell by using an indirect ELISA technique, (Alba and DeVay, 1985). Results suggests that the fungal mycelia did not easily release cross reactive antigens into synthetic media where they grew; that most of the *P. infestans* cross reactive antigens were thermo liable and that they could be concentrated by precipitation in the presence of 40% saturated ammonium sulphate (SAS). The results also revealed an antigenic disparity when 40% SAS from *P. infestans* Race-4 mycelial preparation was assayed with antisera for King Edward and Plentland Dell.

Immunodiffusion, immunoelectrophoretic and crossed immunoelectrophoretic analysis of rice in relation to sheath rot disease revealed a serological relationship between susceptible rice cultivars and isolates of *Acrocylindrium oryzae* (Purkayastha and Ghosal, 1985). One precipitin band was observed when the antigen preparation of *A. oryzae* was cross reacted with its own antiserum or with the antisera of four susceptible rice cultivars. No precipitin band was detected between the antiserum of resistant cv. Mahsuri and antigen preparations from isolates of *A. oryzae* or between the antigens of the resistant cultivars Mashuri and Rupsail and the antiserum of *A. oryzae.* Crossed immunoelectrophoretic tests confirmed that there was a common antigen between Mahsuri and Jaya, and between Mahsuri and CR-126-42-1. The precipitin band between the antigen preparation of Jaya and *A. oryzae* was found to be similar.

Purkayastha and Banerjee (1986) determined common antigenic relationship between soybean cultivars and *Colletotrichum dematium* var. *truncata* following immunodiffusion, immunoelectrophoretic and crossed immunoelectrophoretic tests. At least one antigen was found to be common between host cultivar and the pathogen. No antigenic relationship was observed either between soybean cultivars and the non pathogen (*C. corchori*) or avirulent pathogen (*C. dematium*). Alterations in disease (charcoal rot) reaction as well as alteration in antigenic pattern were evident in soybean roots by the application of sodium azide (Chakraborty and Purkayastha, 1987).

Antigen from two isolates of *Macrophomina phaseolina* a pathogen of groundnut, four non pathogens of groundnut (viz. *Corticium sasakii, Colletotrichum lindemuthianum, C. corchori* and *Botrytis allii*), and five cultivars of *Arachis hypogea* were compared by immunodiffusion, immunoeolectrophresis and crossed immunoelectrophoretic studies for the presence of cross reactive antigens (Purkayastha and Ghosal, 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. Cross immunoeletrophoretic tests confirmed that at least one antigen was common between cv. J-11 and cv. TMV-2 and cv. Kidiri-71-1 and isolates of *M. phaseolina.*

Gerik *et al.*, (1987) detected hyphae of *Verticillium dahliae* in cotton root tissue with an indirect enzyme linked immounoassay. A soluble protein extract of *V. dahliae* was detected with an alkaline phosphatase antirabbit IgG conjugate that hydrolyzed the substrate, napthol-As-phosphate, to a product that reacted with a diazonium salt, yielding a colored precipitate outlining the fungal hyphae. The hyphae were readily observed on and in the root cortex of the host using a dissecting microscope. *Phytophthora fragariae* was detected by ELISA in roots of strawberry cv. 'Teniva'. Because of high sensitivity of ELISA, presence of fungal antigen was demonstrated before symptoms could be detected in microscopical observations (Werres and Casper, 1987).

Gleason *et al.*, (1987) detected seed borne infection of soybean by *Phomopsis longicola* using indirect ELISA and immunoblot assay. Antigen of *P. longicola* was detected by indirect ELISA in as little as 250ng of dried mycelium/ml of extract. The antiserum reacted with mycelium preparation of *Diaporthe phaseolarum* var. *sojae* showed comparatively little or no reaction when tested against 7 other seed borne fungi. Extracts of whole seeds and of seed coats produced a non specific background reaction. *P. longicola* could be detected in individual symptomless infected seeds. A single infected seed coat not be readily detected by indirect ELISA. Immunoblot assay was developed to overcome problems with nonspecific interference in indirect ELISA. Indirect ELISA absorbance values for bulked samples of seed coat halves from the same 10 seed lots correlated weakly with agar plate bioassay but strongly ($P<0.01$) with incidence of symptomatic seeds because SIBA detected only viable *P. longicola* and ELISA did not discriminate between live and dead fungus, the author concluded that SIBA should be a better indicator of pathogen activity on seeds after planting. The two types of serological assay apparently measure different aspects of the disease, however, and both may be useful for evaluating soybean seed lot quality.

Schmitthener (1988) reported the development of an agridiagnostic *Phytophthora* multiwell ELISA kit for detection of *Phytophthora* in plant tissue, which also readily detected *Phytophthora* in soil where soybeans were damaged by *P. megasperma* f.sp. *glycinea* (Pmg). Only low levels of *Phytophthora* were detected in soil stored at 3^0C. Following cold storage high levels of *Phytophthora* could be detected directly from soil, after *Phytophthora* damping off of soybean seedlings was

induced. But *Phytophthora* detection was obtained from soybean leaf discs floated on water over infested soil for 24 hours. Pmg was the only *Phytophthora* that could be detected best with an ELISA test of soil with actively rotting or from leaf disc baits with actively growing mycelium.

An indirect ELISA for quantative detection of *P. herpotrichoides* infections in wheat was presented by Unger and Wolf (1988). All tested isolates of the virulent varieties *P. herpotrichoides* var. *herpotrichoides, P. herpotrichoides* var. *acuformis* or the W-and R-type reacted on a high level in the test, while the less virulent *P. anguioides* was assessed only with 40% and the avirulent *P. aestiva* with 20% of the homologous reaction. No cross reactions occurred with extracts of 11 other species of *in-vitro* cultivated fungi nor with plant material infected with other pathogens. The infection profile throughout the leaf sheath was clearly reflected by ELISA. The examination of 24 stem base samples from the field showed that the values assessed by ELISA correlated well also with the disease indices of naturally infected plant material.

Antiserum raised against pooled mycelial suspension from five isolates (designated Pf 1, Pf 2, Pf 3, Pf 10 and Pf 11) representing five physiologic races of *Phytophthora fragariae,* used in an enzyme linked immunosorbent assay (ELISA) detected homologous soluble antigens at protein concentrations as low as 2ng/ml. Fungal antigens could also be detected in extracts of strawberry plants infected with *P. fragariae*. Root extracts prepared from the alpine strawberry *Fragaria vesca* and *F. ananassa* cv. Cambridge Favourite infected with any of the five isolates studied, produced strong reactions in ELISA. In *F. vesca,* ELISA positive material was detectable 6-8 days after inoculation before macroscopic symptoms appeared. The cultivar Red Gauntlet, which was resistant to Pf 1,2 and 3 but susceptible to Pf 10 and 11 reflected this differential response in ELISA. The absorbance produced by extracts of plants infected with virulent isolates was significantly higher than that obtained with the corresponding extracts of plants with avirulent isolates (Mohan, 1988).

Amouzou-Alladaye *et al.,* (1988) reported that the antiserum obtained by injecting rabbits with mycelial protein extracts of one strain of *Phytophthora fragariae* had a dilution end point of 1:64 in double diffusion and 1/512,000 in indirect ELISA. This serum could detect 11 different strains of *P. fragariae* in pure

culture and the pathogen in naturally infected or inoculated roots. Although the sensitivities of direct DAS and direct ELISA were comparable, the direct DAS ELISA was more specific for the detection of *P. fragariae* in strawberry roots. The antiserum failed to react with some strains of *P. cactorum* and *Pythium middletonii.* In inoculated strawberry roots, *P. fragariae* was detected reliably by ELISA several days before oospores were found and before symptoms developed.

Indirect fluorescent antibody technique was also applied for diagnosis of the clubroot of Brassicaceae caused by *Plasmodiophora brassicae.* Immunoglobulin (γ - globulin; 0.91mg/ml) was purified from antiserum against resting spores of *P. brassicae* prepared from a rabbit. Resting spores and root were stained by fluorescent antibody technique with the IgG and FITC conjugated anti rabbit IgG. Resting spores were effectively detected and also clearly differentiated from small particles of soil and tissues of plant in the reflected light fluorescence microscope. (Arie *et al.*, 1988).

The antisera raised against *Penicillium verrucosum* var. *verrucosum* were characterized by immunofluorescence and by ELISA for their reactivity with 44 strains of moulds (Fuhrmann, *et al.*, 1989). Antigenically, *P. verrucosum* var. *verrucosum* appeared to be similar to strains belonging to subgenus *furcatum*, but strongly different from *Penicillium frequentans.* Specific absorption of antibodies to antigens confirmed the existence of similar biochemical structures on *Penicillium frequentans, Aspergillus versicolor* and *A. fumigatus* .

Competitive types of two novel enzyme linked immunosorbent assay (ELISA) for *Fusarium* species were developed by Kitagawa *et al.*,(1989). Antiserum against a strain (F504) of *Fusarium* species was elicited in rabbits, and a highly specific, sensitive and accurate ELISA for the homologous strain was developed by using the antiserum with β-D-galactosidase-labelled antirabbit IgG as the secondary antibody and cell fragments of the strain attached to Amino Dylark balls as a solid phase antigen in a heterologous competitive ELISA. The modified system was a general assay for 10 strains for four *Fusarium* species with a high sensitivity. The specificity of the heterologous competitive ELISA was shown to be limited to *Fusarium* species. The potential of polyclonal antisera and monoclonal antibodies to differentiate the EAN and NAN aggressive subgroups of *Ophiostoma ulmi* was explored by Dewey *et al.*, (1989). Polyclonal antisera, when tested by ELISA, cross reacted widely with

unrelated species and failed to distinguish between the two aggressive subgroups but small quantative differences were found, particularly between antigen secreted overnight by EAN and NAN germlings. Out of 33 cell lines that secreted monoclonal antibodies positive for *O. ulmi* approximately one third were non-specific; 11 were specific either to species or subspecies. Two-cell line differentiated mycelial antigens of the aggressive isolates of *O. ulmi* from those of the non-aggressive subgroup, but non-antigens from surface washings. Only quantative differences were detected between the EAN and NAN aggressive subgroups. Almost all the monoclonal antibodies and antiserum recognized antigen present in surface washings of cultures or solid medium in cell free culture fluids, and in substances secreted overnight by germinating spores. Most of the monoclonal antibodies appeared to have potential diagnostic value; they gave readings two-fold to ten fold higher with extracts from diseased than from healthy tissue. However, one cell line that secreted antibodies specific to *O. ulmi* cross reacted strongly with extracts of healthy tissue.

Antiserum (anti PfM) rose against mycelial suspensions of *Phytophthora fragariae* isolates reacted strongly with antigens from several *Phytophthora* species. Some cross reactions with antigens from *Pythium* sp. were decreased by fractioning on an affinity column of Sepharose 4B bound to extracts of *Fragaria vesca* roots infected with *P. fragarie.* The affinity purified anti PfM retained its high cross reactivity with the various *Phytophthora* species tested. It also detected infection of rasberry and strawberry roots by some *Phytophthora* sp. (Mohan, 1989).

Phelps *et al.*, (1989) reported the development of an enzyme linked immunosorbent assay (ELISA) for isomarticin, a napthazarin toxin produced by *Fusarium solani.* A carbodrimide procedure was used to couple the heptan isomarticin to BSA for the immunogen and to alkaline phosphatase for enzyme linked assays. The resulting assay had a detection limit of 2ng/ml for isomerticin, other napthazarin toxin were detectable at less than 10ng per well in ELISA plates. The assay was specific for napthazarins. The cross reactivity with a number of phenolic compounds including the closely related napthoquinones was 3 order of magnitude less sensitive.

Glycoconjugates on the surface of zoospores and cysts of the pathogenic fungus *Phytophthora cinnamoni* have been studied by Hardhan and Suzaki (1989) using fluorescin FITC lebelled lectins for fluorescence microscopy and flow

cytometry, and ferritin and gold labelled lectins for ultrastructural analysis. Of the 3 lecitins used, only Con-A binds to the surface of the zoospores, including the flagella and water expulsion vacuole. This suggests that of accessible sachharides, glucosyl or mannosyl residues predominate on the outer surface of the zoospore plasma membrane. Labelling of zoospores in which intracellular sites are accessible indicates that the soybean agglutinin binding material is stored in vescicles that lie beneath the plasma membrane. Quantitation of soybean agglutinin labelling showed that maximum binding occurs 2-3 min. after the induction of encystment.

Watabe (1990) reported immunofluorescent antibody technique as a useful method for detecting *Phytophthora* in soil. But the autofluorescence and the nonspecific staining of soil particles interfered with the detection of the fungi in soil. However, pretreatment of the samples with gelatin-rhodammine conjugate prevented the samples from autofluorescence and nonspecific staining and therefore permitted the immunofluorescent antibody staining in soil. Stained *Phytophthora* was easily detected on the yellow-orange background.

Antisera were raised against antigens of three strains of *Myrothecium roridum,* two susceptible and one resistant cultivar of soybean for analysis or cross reactive antigens. Results of immunodiffusion revealed that common antigens were present only between the virulent strain and susceptible host cultivars, but no CRA was detected in case of resistant cultivars (UPSM-19, DS-73-16). Immunoelectrophoretic analysis showed that one common antigen was shared by susceptible host and the virulent strain (M-1). This was further confirmed by both crossed and rocket immunoelectrophoresis (Ghosh and Purkayastha, 1990). Alteration in antigenic pattern in soybean leaves were also observed after induction of resistance against *Colletotrichum dematium* var. *truncata* following treatment with cloxacillin (Purkayastha and Banerjee, 1990).

Antibodies of three isolates each of *Armillaria mellea,A .ostoyae,A .tabescena* and *Lentinula edodes* were isolated from eggs of immunized laying hens. The reactivity of each antibody preparation with all 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 the homologous species from isolates of the heterologous species. The specificity of the antibodies present in egg was dependent on time elapsed since immunization. Eggs laid 3 week after immunization with *Armillaria* species isolate possessed antibodies that were most specific for isolates of that species. The intergeneric cross-reactivity was found to be smallest with antibodies from eggs laid 5 weeks or more after immunization (Burdsall *et al.*, 1990).

A polyclonal antiserum prepared in rabbit immunized with a mycelium extract of *Phytophthora infestans*, reacted in an enzyme linked immunosorbent assay (ELISA) with mycelial extracts of two *Phytophthora* species but not with those of 10 other micro-organisms found on potato. *P. infestans* mycelium in leaf tissue was readily detected by ELISA using either the plate trapped antigen or $F(ab)_2$ antibody fragment techniques. The amount of mycelium in the leaf extracts was estimated by comparing the values obtained in ELISA with those for known concentrations of *P. infestans* mycelium (Harrison *et al.*, 1990).

Ricker *et al.*, (1991) detected water soluble antigens produced by *Botrytis cinerea* in picked and naturally infected grape juice by using enzyme immunoassay with an indirect format of antibody HRPO conjugates bound to polyclonal rabbit antibodies directed against *B. cinerea* (Anti Bc IgG). Protein-A purified Gama globulin from an early bled antiserum (803-7), which reacted primarily with low molecular weight compounds present only in extracts of *B. cinerea*, was used to specifically detect *B. cinerea* and quantify levels of infection in juice from infected grape berries. Late bled higher titer antiserum (803-19), which cross reacted with proteins and carbohydrates present in extracts from species of *Botrytis*, *Aspergillus*, *Penicillium* and *Uncinula* was used to quantify the levels of rot caused by presence of multiple fungi. Minimum detectable levels of infections, based on mixture of clean and infected juice were 0.25-5%, with (803-7)IgG, and 0.2% with (803-19)IgG. Cross reactivity of all anti Bc IgG collections were low with infected grape juice. In contrast, cross reactivity of anti Bc IgG with extracted antigens (water soluble) from sterile and reproductive strains of several fungi was negligible in early bled antiserum and increased in subsequent collections. The increase in cross reactivity in late bled antisera corresponded with an increase in the overall serum titers for anti Bc IgG to antigens from *B. cinerea*. Non specific binding of 803-19 IgG was high with extracts

from *A. niger* and an unidentified sp. of *Penicillium,* which suggested numerous epitopes common to antigens from these fungi.

In ELISA, antisera raised against *Phialophora mutabilis* reacted strongly with its homologous antigen and cross reacted strongly to moderately with six other *Phialophora* soft rot spp. (Daniel and Nilsson, 1991). With the exception of *Ceratocystis* sp. the serum reacted weakly or not at all with 11 other mold, blue and rots fungi. Extracts from *Fusarium oxysporum* and *F. oxysporum* var. *redolens* isolates were compared by means of electrophoresis and cross immunoelectrophoresis. Both fungi appeared almost identical serologically. Relative amounts of their corresponding proteins differed but quantative patterns of the proteins were nearly the same with the anti- *F. oxysporum* var. *redolens* serum; however, only one specific antigen was detected in the extract from this isolate. Ratej-Guranowska and Wolko (1991) concluded that although the obtained result indicated a strong similarity between *F. oxysporum* and *F. oxysporum* var. *redolens,* they were not sufficient for an unequivocal statement that the fungi belonged to the same species.

Sundaram *et al.,* (1991) reported that when antisera prepared against *Verticillium dahliae* were tested with crude mycelial preparations of *Verticillium* spp. using indirect ELISA, they reacted positively with 11 of 12 *V. dahliae* isolates from potatoes, cotton, soil, but negatively with one isolate from tomato. The antisera did not react with mycelial proteins of other fungi tested. Double antibody sandwich ELISA, using polyclonal antisera also detected *V. dahliae* and *V. albo-atrum* in infected roots and stems of potato.

Two commercial serological assay kits were compared by Benson (1991) to a culture plate method for detection of *Phytophthora cinnamomi* in root samples from inoculated azaleas. Both the multiwell E kit and F kit detected to *P. cinnamomi* on azalea roots beginning one week after inoculation. Agreement between immunoassay kits and culture plate results for detection of *P. cinnamomi* was most consistent beginning 3-5 weeks after inoculation. Root symptoms, but not foliar symptoms, of *Phytophthora* root rot were evident during this period. There was positive correlation between root severity in green house trails and root sample absorbance (multiwell) or meter reading (rapid assay) but not between symptom severity and immunoassay

results. The multiwell kit detected *P. cinnamomi* in root samples containing as little as 10% 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.

Methods for sampling turf grass tissue were also compared for their effectiveness in monitoring *Pythium* blight epidemics with ELISA. Samples consisted either of whole plants picked and assayed as whole plants, whole plants sectioned into lower, middle and upper starta components or leaf clipping collected with a mower. ELISA reading for mowed samples generally matched those for whole plucked samples (values 0.457 to 0.601). Fluctuations in detectable *Pythium* antigens were most pronounced on the upper most stratum compared with moderate to very little change in ELISA reading for two lower strata. Several episodes of *Pythium* antigen increase were detected by ELISA assays of mowed samples, although signs and symptoms of *Pythium* blight were not evident. However, increases in ELISA readings for *Pythium* concided with, but not generally precede the onset of blight with 2-3 days sampling interval. It was concluded by the author (Shane, 1991) that antibody aided detection is useful for verification of diagnosis and detection of general *Pythium* population fluctuations, but the current method was not satisfactory for advance detection of blight epidemics.

The sensitivity of a *Phytophthora* specific immunoassay kit was tested on 17 species of *Phytophthora* collected throughout the world, including 18 isolates each of *P. cinnamomi* and *P. cactorum* by Pscheidt *et al.*, (1992). Kits were also used in a diagnosis of plant specimen with symptom characteristic of *Phytophthora infestans.* All isolates tested produced a positive result with the immunoassay kits. The lower absorbances relative to other species were obtained from *P. cinnamomi* and *P. megasperma.* Variation in absorbance was high among isolates of *P. cinnamomi* but low among *P. cactorum.* Clinic samples produced positive results with the immunoassay as did pure cultures of *Phytophthora* sp. isolated from those samples. Cross reactions occurred with several *Pythium* sp. isolated from those with several specimens infected with *Perinospora* sp. Other samples without typical *Phytophthora* symptoms but associated with other pathogens did not produce a positive reaction

with the immunoassay. Cross reactivity with some *Pythium* species made interpretation difficult, but when kit results were combined with field histories and symptomology, the immunoassay proved to be a useful tool for diagnosis.

Isolation of *Pythium* sp. from different soils in U.K. by conventional methods revealed *P. violae* to be most common, while *P. sulcatum* was isolated less frequently. Competition ELISA using polyclonal antibodies against *P. violae* and *P. sulcatum* confirmed the results of the conventional techniques. With cavities developed on the field grown carrots, ELISA confirmed the predominance of *P. violae.* In one sample, *P. sulcatum* was also isolated from a small number of lesions and was not detected in ELISA. The competition ELISA did not indicate presence of either *Pythium* in a range of non-cavity spot lesions from which attempts at isolation were negative (Lyons and White, 1992).

A rapid serological test for detecting *Fusarium oxysporum* f.sp. *narcissi* in *Narcissus* was reported by Linfield (1993). Antiserum raised to cell wall fractions of *F. oxysporum* f. sp. *narcissi* gave better recognition than that to cytoplasmic fractions. Recognition was equally good in artificially and naturally infected bulbs. Little cross-reactivity in bulb tissue was shown by three other bulb rotting fungi. Nine isolates of *F. oxysporum* f.sp. *narcissi* from a wide geographic area gave similar results in an indirect ELISA of mycelial extracts, although some cross-reactivity was observed with two other *Fusarium* spp. and four other fungi showed little cross reactivity. Ten days after inoculation, the pathogen was detected in 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 enzyme linked immunosorbent assay and recovery of the pathogen on selective medium.

A double antibody sandwich ELISA test has been developed by Priestley and DeVay (1993), for the detection of *Pseudocercosperella herpotrichoides* using a high specific monoclonal antibody pH-10 as the capture antibody and genus specific polyclonal rabbit antisera as test antibody. The assay recognized extracts from plants both artificially and naturally infected with *P. herpotrichoides* giving at least 3 fold higher absorbance values with extracts of *P. herpotrichoides* infected tissue than with extracts from healthy tissue. The assay tested positively with all isolates of *P. herpotrichoides* including W type and R type isolator. The immunogen used was a

mycelial extracts from which high molecular weight proteins and glycoproteins were removed by SAS. The high mol. wt. fraction was shown to contain cross reactive antigens; it induced antiserum in mice that cross reacted with the other step base fungi even at high dilution.

Enzyme linked immunosorbent assay (ELISA) has also been used in the determination of fungal biomass during the early stages of infection of tuber disc of *Solanum tuberosum* by *Phytophthora infestans.* 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 discs could be achieved by 8-18 h after inoculation (Beckman *et al.*, 1994). Differences in growth between avirulent and virulent isolates of *P. infestans* on the resistant potato cv. Kennebec were quantified by 32-48h after inoculation. On the resistant host, the growth of the avirulent isolate as detected by ELISA was essentially arrested by 16 h 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. Two monoclonal antibodies and three polyclonal antisera were raised by White *et al.*, (1994) to cell wall/membrane fractions of *Pythium violae* and *Pythium sulcatum.* When screened with a collection of 40 isolates of genus *Pythium* including 20 species and the H-S group there was extensive cross-reaction. However, when the binding of the antibodies was assessed in an enzyme linked immunosorbent assay using cytoplasmic fraction antigens, the combined recognition pattern produced profiles unique to each species. In multivariety analysis, isolates of the same species tended to group together, while the affinity for fungi other than members of the genus *Phytophthora* was low.

Brill *et al.*, (1994) analyses two ELISA formats and antigen preparations against *Phomopsis longicolla.* The PAbs were purified to IgG 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 specifity 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 anti *P. longicolla* activity after 3 immunizations, and the activity become constant against most members of complex at the same time. Reactivity to some cultures of *P. longicolla* was in detectable following the 4th and 5th immunization, where as reactivity of all other cultures of the complex remained high.

Soluble protein extracts of chlamydospores and mycelium of *Thielaviopsis basicola* infecting *Gossypium hirsutum* were used to raise polyclonal mouse ascites antibodies. In a fungal capture ELISA, using the purified and biotin labelled IgG fraction both brown and grey cultural types of *T. basicola* were detected, while negligible cross reactivity was observed with other common soil borne fungi. The minimum detection limit of ELISA was between 1 and 20 ng of *T. basicola* protein depending on the assay. *T. basicola* could be detected in cotton roots two days after inoculation. At the time, initial symptoms were apparent. The antibody also was used to observe *T. basicola* on cotton roots with immunofluorescence microscopy. (Holtz *et al.*, 1994).

Jamaux and Spire (1994), developed a serological test that allows the early detection of infection of young petals of *Sclerotinia sclerotiorum*, an important pathogen of rapeseed. Soluble mycelial extracts of *S. sclerotiorum* were used to produce the first generation antiserum. This was not specific for *S. sclerotiorum* in DAS-ELISA and allowed the screening of cross reacting fungal species such as *Botrytis cinerea*, a pathogen commonly present on rapseed petals. Use of polyclonal anti-*B. cinerea* serum enabled the absorption by serial cycles, of *S. sclerotiorum* antigens common to *B. cinerea*. Residual antigens were used as immunogens for the production of the second generation antisera (S1 and S2) which were then tested by DAS-ELISA. Cross reactions with some unrelated fungi slightly increased. *S. sclerotiorum* and *B. cinerea* were distinguishable using antiserum S2.

Antigens obtained from tea varieties, isolates of *B. carbonum* and non pathogens of tea (*Bipolaris tetramera* and *Bipolaris setarae*), were compared by immunodiffusion and immunoelectrophoresis, in order to detect cross-reactive antigens (CRA) shared by the host and the parasite (Chakraborty and Saha, 1994). 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 the isolates of

B. carbonum and resistant varieties (TV-16,25 and 26), non pathogens and tea varieties, as well as non-pathogens and isolates of *B. carbonum.* When three antisera preparations (T 18A, T 26A and BC 1A) at 1:125 dilution, were tested against homologous and heterologous antigens (5 and 25µg/protein/ml), indirect ELISA could readily detect cross reactive antigens in semipurified mycelial preparations at concentrations ranging from 5-25µg/ml with antiserum BCTA at 1:125 dilution. Antigenic preparations from *B. carbonum* (isolates BCI) exhibited higher absorbance value when cross reacted with antiserum of susceptible variety (TV-18) than the resistant one (TV-26). Antigen preparations from pathogen *B. carbonum* showed greater absorbance value than from the non-pathogen, *B. tetramera* in its cross reaction with antiserum of TV-18. Indirect staining of the antibodies raised against *B. carbonum* using fluorescein isothiocyanate (FITC) indicated that in cross sections of tea leaves (TV-18), the CRA concentrated mainly around epidermal cells. Treatment of mycelia and conidia of *B. carbonum* with antisera of leaves (TV-18) and indirect staining with FITC indicated the presence of CRA in the young growing hyphal tips and conidia.

Leaf and fungal antigens from 12 varieties of tea and from mycelia of three isolates of *Pestalotiopsis theae,* causal agent of grey blight disease were prepared. These antigens were compared with antisera raised against mycelial suspension of *P. theae* (Pt-2) and leaf antigens of Teenali – 17/1/54 and CP-1 by immunodiffusion tests (Chakraborty *et al.,* 1995) to detect crossreactive antigens (CRA) shared by the host and the parasite. CRA were found among the susceptible varieties and the pathogen (Pt-1,2 and 3). Such antigens were not detected between isolates of *P. theae* and resistant varieties. Antiserum preparation from leaf antigens of two varieties (Teenali 17/1/54 and CP-1) and mycelial antigen of *P. theae* each at a diluation of 1:125 were tested against homologous and heterologous antigens (25µg protein/ml). Results showed that indirect ELISA could readily detect cross reactive antigens both in crude and semi purified mycelial antigen obtained from *P. theae.* Higher absorbance values were obtained in reaction with Teenali 17/1/54 antiserum than when reacted with antiserum of CP-1 thus showing an antigenic disparity. When mycelial extracts of non-pathogen *Bipolaris tetramera* was tested in ELISA using antisera of CP-1, Teenali-17/1/54 and *P. theae,* no such cross reactivity was detected.

Using fluorescein isothiocyanate (FITC) and antibodies raised against *P. theae*, Chakraborty *et al.*, (1995) reported that CRA was present in the young hyphal tips of the mycelia, setulae and appendages of conidia of *P. theae* and in the epidermal and mesophyll tissue of cross sections of tea leaves.

An immunoassay for the detection of *Phomopsis* was used to detect levels of the mycotoxin in the epidermal peels from resistant and susceptible lupin cultivars asymptomatically infected with *Diaporthe toxica.* Quantifiable levels (>6.25μg/kg) of *Phomopsis* were detected in susceptible lupin cultivars but not in very resistant breeding lines or in the controls. These differences reflect the difference in resistance observed in the microscopical assay and mature plants in the field (Williamson *et al.*, 1995).

Polyclonal antiserum was raised against mycelial extract of *Pestalotiopsis theae* and immunoglobulin fractions were purified by ammonium sulfate fraction and chromatography of DEAE sephadex. In ELISA antiserum dilution upto 1:16000 detected homologous antigen at 5 mg/L concentrations and at 1:125 antiserum dilution fungal antigens could be detected at concentration as low as 25 μg/L. In 15 varieties of tea tested, from Darjeeling, UPASI and Tocklai Research Stations, absorbance values of infected leaf extracts were significantly higher than those of healthy extracts at 40 mg/L concentration in indirect ELISA.ELISA positive material was detected in tea leaves as early as 12h after inoculation with *P. theae.* At serum dilution upto 1:125, pathogen could be detected in infected leaf extracts upto 2mg/L concentration. The results demonstrated that ELISA can be used for early detection of *P. theae* in leaf tissues even at very low level of infection (Chakraborty *et al.*, 1996).

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

sensitivity in the dipstick assay in the indirect ELISA and indirect immunofluorescence, for all which, these was a limit of detection of $1:10^2$. The indirect ELISA was successful only after the substitution of alkaline phosphatase by protein-A conjugated Horse radish peroxidase. Of the assay 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.*

Polyclonal antisera were raised against *Fusarium oxysporum* and soybean roots (cv.UPSM-19). Cross-reactive antigens (CRA) shared by *F.oxysporum* and *Glycine max* were detected by indirect ELISA and their cellular location in root tissues were determined by immunofluorescence test. CRA were concentrated mainly around epidermal cells and distributed in the cortical tissues. CRA were also present in microconidia, macroconidia and chlamydospores of the fungus (Chakraborty *et.al.*, 1997). Immunological formats for detection of *Sphaerostilbe repens,* causal agent of violet root rot disease of tea, as well as biocontrol agents such as *Trichoderma harzianum* and *Trichoderma viride* from soil has been developed by Chakraborty *et.al.* (2000).