REVIEW OF LITERATURE

Resistance of plants to microorganisms appear to be the rule, susceptibility being the exception. Disease resistance in plants depends on multiple defense mechanisms which include preformed defense barriers such as the cuticle, the cell wall or constitutive antimicrobial compounds as well as defenses triggerred by the invader (Horsfall and Cowling, 1980). One of the inducible defense mechanisms include the accumulation of low molecular weight antimicrobial compounds which are known as phytoalexins. Available evidence also indicates that resistance to disease in many cases is the result of activation of more than one biochemical defense mechanism (Ebel and Grisebach, 1988). Purpose of this review is to present briefly the observations of previous workers in concord with the present line of investigation. However, this review is selective rather than comprehensive. A few aspects such as, (a) preformed and inducible defenses in plant ; (b) plant disease alteration by chemical treatment and (c) common antigenic relationship between host and parasite, have been discussed in the following paragraphs.

(a) Preformed and inducible defense in plant

Certain organs of many plant species harbour secondary metabolites which represent a variety of chemical structutes (Bell and Charlwood, 1980). Their role is mostly unknown. However, in plant tissues various types of preformed inhibitory chemicals such as saponins, unsaturated lactones, cyanogenic

glycosides, oils and phenolic compounds are present in relatively large quantities. Such compounds are called preformed, in contrast to those which are post infectionally synthesized, such as phytoalexins. Preformed substances are considered to be important as antimicrobial barrier. Their occurrence, distribution and possible functions have been reviewed by Schonbeck and Schlosser (1976) and Schlosser (1980, 1988).

Choudhuri and Sen (1981) tested the plant extracts of Dibymocarpus oblonga and Piper nigrum on sclerotia forming pathogens such as Sclerotium rolfsii and Rhizoctonia solani and observed fungitoxic properties. The fungicidal activity of the benzene extract of P. nigrum was more inhibitory on mycelial growth than on sclerotia germination. The essential oils of Caesulia axillaris and Hyptis sauveolens exhibited strong fungitoxicity against the test organism, Helminthosporium oryzae. The oil showed a broad fungitoxic spectrum besides superiority over 8 synthetic fungicides and prevented the appearance of leaf spot disease of paddy initiated by H. oryzae (Pandey et al., 1982 a & b).

Prusky et al. (1982) isolated a preformed antifungal compound [1 - acetoxy 2 - hydroxy -4-oxo heneicoss - 12, 15 diene] from peels of unripe avocado fruits, which inhibited the vegetative growth of Colletotrichum gleosporioides and totally inhibited the spore germination. Two compounds (2,5-dihydroxy-4-methoxy-9, 10-dihydrophenanthrene) were isolated

by Coxon et al. (1982) from the peels of yams (Dioscorea rotundata) which showed antifungal activity towards both Cladosporium cladosporioides and a variety of yam soft rot pathogens.

Antifungal substances were also extracted with methanol, acetone, and ethylether from the epidermis and periderm region of Morus alba and tested against Bipolaris leersiae and other phytopathogenic fungi. Each acetone extract from root and shoot showed 3-7 active substances, the amount and number varying in different cultivars. Shirata and Takahashi (1982) concluded that these antifungal substances in epidermis of root may be prohibitins and one of the resistance factors to pathogenic soil fungi.

Tripathi et. al. (1983) observed that the seed extracts of Iberis amara showed fungicidal activity against Helminthosporium oryzae at the minimum inhibitory concentration (2%) and exhibited a broad range of activity and non-phytotoxicity. The fungitoxic principle of the seed was thermostable upto 120°C. During mycotoxic evaluation of 12 essential oils by Pandey et.al. (1983) the oil of Aegeratum houstonium was found to possess broad mycotoxic spectrum, exhibiting strongest toxicity against Fusarium lateritium f. sp. cajani. This oil was more active than the prevalent synthetic fungicides and was non-phytotoxic to the host plant (Cajanus cajan).

Antifungal activity of some naturally occurring

coumarin compounds [Luvangetin, Crenulatin and Suberenol isolated from Limonia acidissima] were tested against 3 spore forming [Drechslera oryzae, Fusarium solani and Alternaria solani] and 2 sclerotial [Sclerotium rolfsii and S. hydrophyllum] plant pathogens by Pan et.al. (1983). They reported that the compounds possessed low to high inhibitory activity towards spore germination and D. oryzae was highly sensitive to all the 4 compounds.

It was shown by Dubey et.al. (1983) that the leaves of Chenopodium ambrosioides exhibited strong fungitoxicity against mycelial growth of Rhizoctonia solani causing damping off diseases of some seedlings. They also noted that leaves of C.ambrosioides exhibited a broad range of antifungal activity and did not show any phytotoxicity on the germination and seedling growth of Phaseolus aureus. Antifungal activity of higher plants was also studied by Abraham et.al. (1983). Among 39 indigenous plants tested on 4 phytopathogenic fungi belonging to the genera Fomes, Cytospora, Pestalotiopsis, positive results were achived with 10 crude extracts, some of which were purified. Mycelial growth and conidial germination of Cryphonectria cubensis were inhibited by phenolic compounds of Eucalyptus spp. including caffeic, p-coumaric, ferulic, gallic, protocatechuic, gentisic and benzoic acid (Alfenas et al, 1983).

Effect of some plant extracts and oils on inoculum density of Erysiphe polygoni on different nodal leaves of Pisum sativum was reported by Singh et.al. (1984). According to them,

among garlic bulb extract, garlic oil, neem leaf extracts and ginger extract best results were observed in ginger extracts and oil which significantly reduced the disease intensity, eg., powdery mildew of pea and increased the yielding capacity of the plant in comparison to controls. Leaf extracts of 10 plants such as Anagallis arvensis, Caesalpinia pulcherrima, Psidium guayava etc. were totally fungitoxic against Ustilago maydis and U. nuda. Some of these were selectively effective while others were partially inhibitory or stimulatory (Singh and Pathak 1984).

Essential oils from Ocimum basilicum and their components showed different inhibition effects against Fusarium oxysporum f.sp. vasinfectum and Rhizopus nigricans which dependended on the percentage of main components : lineol, linaleol, methyl chavicol and eugenol (Reuveni et.al. 1984). Effect of 49 indigeneous plants on 11 phytopathogenic fungi belonging to the genera Phytophthora, Ceratocystis, Phoma etc. were studied by Chesne et.al. (1984). They observed positive results in most cases.

Hamaya et al. (1984) found characteristic antifungal components in camellia (Camellia japonica), wabisuke group and C. granthaniana plants. The active substances were easily extracted from leaves and petals of the plants to ten volumes of water by homogenization or autoclaving for 5 minutes at 120°C. On the potato-sucrose liquid medium mixed with the same volume of the extract, normal conidial germination or growth

of hyphae of many fungi were inhibited. Almost no conidium germinated in Pyricularia oryzae and Cochliobolus miyabeanus, germ tubes or conidia themselves swelled resembling balloons and no normal hypha grew in Pestalotia longiseta, Gleosporium theae-sinensis, Diaporthe citri, Botrytis cinerea etc. and growth of hyphae was worse in Alternaria kikuchiana and Alternaria mali. Two triterpenoid saponins were isolated as the antifungal compounds from aqueous extract of camellia leaf and given designations Camellidin I and Camellidin II. Their molecular formulas were $C_{55}H_{86}O_{25}$ (MW = 1146) and $C_{53}H_{84}O_{24}$ (MW = 1104) respectively, the activity was stronger in the former. It was presumed that these saponins were concerned in the resistance of camellia and the closely related plants against the fungal infection.

Nagata et.al. (1985) isolated two triterpenoid saponins from an aqueous or a methanolic extract of camellia (Camellia japonica) leaf. They had an antifungal activity charecterized by abnormal germination of conidia. These saponins were composed of 3β-hydroxy- 18β-acetoxy-28-norolean-12-en-16-one or 3β-, 18β, dihydroxy -28 - norolean-12-en-16-one as aglycon, and D-glucoronic acid, D-glucose and two moles of D-galactose as the sugar moiety. The authors have named these new saponins "Camellidin" which might have value for studies in the fields of phytopathology and biochemistry.

Kumar (1985) observed that the leaf extracts and leaf leachates of young and old leaves of moderately resistant,

moderately susceptible and susceptible varieties of potato did not show any major differences, except for the presence of adipic acid which inhibited conidial germination of Alternaria solani about 41.7%. Garlic clove juice (GCI) inhibited spore germination and mycelial growth of Fusarium oxysporum f.sp. niveum, the causal pathogen of watermelon wilt. The antifungal activity of GCI was probably due to its antifungal contents (El Shami et.al. 1985).

It was shown by El Sayed et.al. (1985) that the leaf extract of Eucalyptus occidentalis and E. brockwayi exhibited strong fungitoxicity against the mycelial growth of Pestatiopsis magniferae (causing leaf spot of Eucalyptus camaldulensin) in PDA medium. Pan et.al. (1985) reported that some naturally occurring flavonoids isolated from Pongamia glabra showed antifungal activity against some test fungi. Of these, pongaglabolmethyl ether was found to be most promising.

Tripathi et.al. (1985) isolated oil from the leaves of Ocimum gratissimum which showed fungitoxicity against Alternaria alternata, Colletotrichum capsici and Sclerotium rolfsii. They also reported that eugenol was the major fungitoxic principle that was isolated from the oil of Ocimum sp. Five oils of coconut, groundnut, palmkernel, pure vegetable and liquid paraffin were tested against spore germination of Rhizopus oryzae, Curvularia lunata, Phoma sorghina (soft rot pathogen of tomato fruit) and Fusarium equiseti (dry rot pathogen) by Adisa (1985). He suggested that post-harvest

tomato fruit rot could be controlled in Nigeria by dipping in 75% palmkernel oil and storing at 15°C.

Asthana et.al. (1986) observed that the leaf extracts of some higher plants showed antifungal properties against the test organism Aspergillus flavus and the essential oil of Ocimum adscendens exhibited the strongest fungitoxicity. They reported that the oil showed thermostable properties. Presence of antifungal compounds in the peel of mango (Mangifera indica) was detected by Droby et.al. (1986). According to them the antifungal agent consisted of a mixture of 5-(12-cis-heptadecenyl) and 5-pentadecylresorcinol. The ED_{50} of the compound for inhibition of germ tube growth of germinated conidia of Alternaria alternata was 120 $\mu g\ ml^{-1}$. They concluded that the mixture of the 5-substituted resorcinols are involved in the latency of A. alternata infection in unripe mango fruits.

Renu et.al. (1986) also isolated essential oil from the leaves of Aegle marmelos which inhibited the mycelial growth of the test fungus Rhizoctonia solani. They observed that the oil did not exhibit any deleterious effect on seed germination and seedling growth of the host plant Vigna radiata. Essential oil from the fruits of Trachyspermum ammi exhibited toxicity at 800 ppm. against Aspergillus flavus and A. niger. The oil showed thermostatic properties and killed the fungi within 50 seconds. The oil also protected the seeds of Arachis hypogea from fungal infection (Tripathi et.al. 1986).

Robson and Strobel (1986) reported that when liquid cultures of Alternaria helianthi were supplemented with aqueous extracts of leaf tissue of its host plant (Sunflower), pronounced effects on both growth and production of toxin deoxyradicinin were appearent. Very low levels of leaf extract stimulated toxin production but did not significantly affect growth, while higher levels markedly stimulated mycelial growth but suppressed toxin production. Leaf extracts of some medicinal plants such as Adhatoda vasica , Andrographis peniculata and Ocimum sanctum were used by Prasad and Ojha (1986) to control Fusarium equiseti, F. semitectum and Curvularia lunata causing post harvest decay of parwal and bottle gourd fruits.

The leaf extract of Polyalthia longifolia also were inhibitory against mycelial growth of Rhizoctonia solani, Sclerotium oryzae and in neutral phosphate buffer was more active than in distilled water. The inhibitory principle of this leaf extract was also thermostable (Naidu and John 1986). Levy et.al. (1986) isolated a medicagenic acid 3 - O -β -D-glucopyranosid from alfalfa roots, which inhibited the mycelial growth of Aspergillus niger, Sclerotium rolfsii and Fusarium oxysporum f.sp. lycopersici.

Although several economically important Theaceae plants such as the tea plants, Camellia sinensis, are damaged by the fungi Pestalotia longiseta, Gloeosporium theae-sinensis etc., C. japonica from the same family suffers no infection by

these fungi. Nishino et.al. (1986) have shown two triterpenoid saponins named Camellidin-I and Camellidin-II, with antifungal properties, present only in C. japonica among many components including other saponins.

The antifungal activity of the leaf extract of Lawsonia inermis on Drechslera oryzae was tested at 1 : 40 dilution (ED_{50} concentration) by measuring the growth, protein, DNA, RNA synthesis and oxygen uptake. The oxygen uptake was inhibited more than the other metabolic processes like proteins, DNA and RNA synthesis. The antifungal factor contained in leaf was identified as 2-hydroxy-1,4 naphthoquinone (Lawsoni). Under in vivo condition, foliar spray of the leaf extract effectively controlled disease than the seed treatment (Natarajan and Lalithakumari, 1987).

Late leaf spot (Phaeoisariopsis personata) and rust (Puccinia arachidis) of groundnut were partly controlled by using the plant extracts of Tridax procumbens, Pongamia glabra, Lawsonia alba along with carbendazim plusmancozeb and N.C.P. 75. Simultaneously, the yields also increased (Ghewande 1987). It was reported by Kishore et.al. (1987) that the vapours of aqueous extracts of rhizomes of Alpinia carinata showed strong fumigant activity against Rhizoctonia solani. They also isolated the essential oil from the rhizomes of A. carinata which possessed fungicidal activity and broad fungitoxic spectrum.

It did not show any phytotoxicity on seed germination and seedling growth of the host plant Phaseolus aureus.

Gourinath and Manoharachary (1988) tested the effect of latex collected from different host plants such as Calotropis, Ipomoea, Carica etc. on conidial germination and mycelial dry weight of four pathogenic fungi viz. Curvularia lunata, Fusarium solani, Cylindrocarpon lichencola etc. They reported that latex showed 100% inhibition on conidial germination.

Toda et.al. (1989) has shown that extracts of Japanese green tea leaves inhibited the growth of various bacteria causing diarrheal diseases. All tea samples tested by them showed antibacterial activity against Staphylococcus aureus, S. epidermidis, Vibrio cholerae 01, V. cholerae non 01. V.parahaemolyticus, V.mimicus, Campylobacter jejuni and Plesiomonas shigelloides, Noneof the tea samples had any effect on the growth of V. fluvialis, Acromonas sobria, A. hydrophila, Pseudomonas aeruginosa, Salmonella enteritidis, enteroinvasive Escherischia coli, enterohemorrhagic E. coli, enteropathogenic E. coli, enterotoxigenic E. coli, Enterobacter cloacae or Yersinia enterocolitica. Salmonella and Shigella showed different susceptibilities depending on the kind of Japanese green tea. Japanese green tea also showed bactericidal activity over S. aureus, V. parahaemolyticus and even enteropathogenic E.coli which was not sensitive when tested by cup method. The

bactericidal activity was shown even at the drinking concentration in daily life.

Antibacterial activity of tea catechin to Streptococcus mutans have been demonstrated by Kawamura and Takeo (1989). Catechin fraction A (CF-A) containing (-) epicatechin and (-) epigallocatechin, fraction B (CF-B) containing (-) epicatechin gallate and (-) epigallocatechin gallate, CF-A, and CF-B mixture (CF-mix), and (-) epigallocatechin gallate (EGCg) were prepared from green tea and the antibacterial activity of catechins on the S. mutans was investigated. Although the antibacterial and sterilization effects of CF-B were mild compared with those of chlorohexidine gluconate, it may be useful as a natural antibacterial reagent for a dentifrice or a mouth wash liquid etc. due to its reasonable antibacterial activity to S. mutans.

Tomas - Berberan et.al. (1990) identified antibacterial (anti-gram positive) phloroglucinol and acetophenon derivatives from the aerial parts of Helichrysum decumbens, H. stoechas and H. italicum. Phloroglucinol derivative showed eight times more antibacterial activity (MIC 12.5 µg/ml) than the acetophenon derivative, but the latter was also active against gram negative bacteria (Escherichia coli). Both types of compounds showed antifungal activity against different fungi (Penicillium sp., Cladosporium herbarum and Phythophthora

capsicii) but proved to be inactive against Aspergillus species at MIC of 100 µg/ml. These results confirm that the co-occurrence of acidic phenolic hydroxyls and lipophilic residues is an important chemical feature for the expression of antifungal activity.

Chakraborty et al. (1991) tested antifungal activity of aqueous extract of Cymbopogon pendulus, Cannabis sativa and Lantana camara, against Colletotrichum camelliae, Alternaria solani and Curvularia lunata by spore germination, poisoned food and agar-cup bioassay methods. All the three extracts inhibited the test fungi, but the extracts of Cannabis sativa was the most effective. The ED_{50} value of this extract was determined against all the three test fungi.

Plant resistance to pathogens based on preformed inhibitory substances is easy to conceive, but despite extensive studies by several workers, there are only a few well documented examples. Preformed resistance is often based on inadequate evidence ; mainly the demonstration that a crude extract of a plant resistant to a pathogen contains material which reduces the growth of the pathogen in vitro. Hence, it should not be overlooked that preformed chemical barriers might not be the sole determinants of incompatibility. Other factors, such as post infectionally synthesized phytoalexins, might equally contribute to the overall defence in a host-parasite system.

Phytoalexin accumulation is believed to be an important early defense response in several plant pathogen interaction. So far, it has been demonstrated in more than 100 plant species representing 23 families. A lot of work has been done and several comprehensive reviews have appeared on phytoalexins and their role in disease resistance (Cruickshank, 1963, 1977, 1980 ; Kuc, 1966, 1972, 1976 ; Deverall, 1972, 1976, 1977 ; Ingham, 1972, 1973 ; Purkayastha, 1973, 1976, 1985, 1986 ; Bailey and Deverall, 1983 ; Harborne and Ingham, 1978 ; VanEtten and Pueppke 1976 ; VanEtten et al, 1982, 1989 ; Wood, 1982 ; Ward, 1986 ; Paxton 1988 ; Ebel and Grisebach, 1988 ; Keen and Bruegger, 1977 ; Keen, 1981, 1982, 1990).

Phytoalexins constitute a chemically heterogenous group of substances belonging to various classes of natural products which include isoflavonoids, sesquiterpenoids, polyacetylenes and stilbenoids. Many phytoalexins are absent in healthy, unchallenged plants. It was originally believed that phytoalexins were host specific. With the evidences accumulated so far, concerning the wide spread occurrence, isolation and characterization of phytoalexins during the past several years, it is now clear that more than one phytoalexin could occur in a single host species of which one may be dominant. Again, similar phytoalexins may also occur in different host species. Plant organs including roots, stem, leaves and fruits have been shown to respond to infection with the formation of phytoalexins. Among plant pathogens, fungi, some bacteria and

viruses are capable of inducing phytoalexin production in plants, but involvement of the last two groups of organisms seems to be quite negligible in comparison with the large group of fungi. During incompatible host-parasite interaction, phytoalexin is synthesized rapidly and accumulates at the infection site. In contrast, in the compatible host-parasite extraction the plant also synthesises the phytoalexin but relatively slowly and in reduced concentration.

Since it is not possible to incorporate all the work done so far in the line of phytoalexin research in this brief resume, some selected observations have incorporated in the following paragraphs.

Muller and Borger, (1940) first demonstrated the production of post infectional antifungal compounds. When they inoculated the potato tuber with an avirulent race of Phyto-phthora infestans, an inhibitory compound or phytoalexin was formed in the tuber. A phytoalexin like antifungal substance was related to the resistance of rice plants against Helminthesporium oryzae (Oku and Nakanishi, 1962). The production pf phytoalexin by leaf and endocarp tissues of broad bean (Vicia faba) was first demonstrated by Purkayastha and Deverall (1964, 1965), which led to the isolation and identification of wyerone acid, (Deverall, 1967, ; Deverall and Vessey, 1969 ; Letcher, 1970).

An antifungal compound - hydroxyphaseollin was first

detected in Phytophthora megasperma - infected soybean cultivar by Keen (1971). Later, Burden and Bailey (1975) also isolated this phytoalexin from infected soybean and identified it as glyceollin instead of hydroxyphaseollin.

Purkayastha and Ray (1975) noted the accumulation of an antifungal compound (Phytoalexin) in jute leaves after infection by Colletotrichum corchorum. Leaf diffusates of JRC 321 (moderately resistant cultivar) contained more antifungal substance than that of JRC 412 (susceptible cultivar). Differential antifungal activities of resistant and susceptible rice cultivars (to Helminthosporium oryzae) were also reported (Purkayastha and Chattopadhyay, 1975 ; Johnson et al. (1976) explained the possible role of phytoalexin in the resistance of sugarbeet (Beta vulgaris) to Cercospora beticola. Highly resistant cultivars appeared to produce greater quantities of betavulgarin in the lesions than the more susceptible ones. Three phytoalexins viz. phaseollin, phaseollidin and phaseollin isoflavone were detected in the resistant bean plants 48 hour after inoculation with Uromyces phaseoli but not in the non-inoculated plants or in the inoculated susceptible variety (El-Naghy and Heitefuss, 1976). Several corn inbreds resistant and susceptible to Helminthosporium turcicum were tested for phytoalexin production. All the resistant lines produced antifungal or phytoalexin like compounds three days after inoculation. The quantity of antifungal compound and time required

for maximum production differed with the inbreds, but a correlation could be established between disease rating and production of antifungal substances. (Obi et.al. 1980).

A series of crosses were made between isolates of Nectria haematococca mating population VI that differed in sensitivity to the pea (Pisum sativum) phytoalexin, pisatin by Tegtmeir and VanEtten (1982). The projeny were tested for sensitivity to pisatin, ability to demethylate pisatin, and virulence on pea. Of the three projeny analysed, all of the moderately or highly virulent projeny were tolerent to pisatin and able to demethylate it. Therefore, either pisatin tolerance and demethylating ability are required for virulence on pea or genes for pisatin tolerance and demethylating ability are closely linked to genes for virulence. The results of this study indirectly support the hypothesis that pisatin accumulation is an active mechanism of resistance in pea.

Purkayastha and Chakraborty (1983) correlated the resistance of soybean cultivars to charcoal rot disease caused by Macrophomina phaseolina with greater production of phytoalexin (glyceollin). Glyceollin content of resistant soybean cvs. UPSM - 19 and DS-73-16 were 400 - 421 µg/g fresh wt. while of susceptible cvs. Soymax and R-184 were 265 and 279 µg/g fresh wt. respectively. In another study Purkayastha et.al.(1983) demonstrated that a phytoalexin (momilactone A) was associated

with the resistance of rice plants to sheath rot disease caused by Acrocylindrium oryzae. Coleoptiles of tall cultivars (resistant to A. oryzae) contained greater amount of momilactone A (12.91 - 21.36 µg/g fresh wt.) while the semidwarf cultivars contained relatively lower amount (5.58 - 8.14 µg/g fresh wt.). Difference in momilactone A content of leaf sheaths of tall cv. Mahasuri (19.36 µg/g fresh wt.) and semidwarf cv. Jaya (8.64 µg/g fresh wt.) was also significant.

The expression of resistance and susceptibility to inoculation with zoospores of Phytophthora megasperma f. sp. glycinea race 1 was determined by Bhattacharyya and Ward (1986) in roots, hypocotyls and cotyledons of etiolated and green seedlings and leaves of soybean cvs. Harosoy and Harosoy 63. In each case higher concentration of glyceollins accumulated in resistant than in susceptible reactions, the difference being greatest in hypocotyls and smallest in roots. The relative proportions of glyceollins I, II and III varied with the organ, exposure of seedlings to light, the interaction type and the incubation period. Glyceollin I was relatively the most abundant isomer in roots and to a lesser extent in hypocotyls. Glyceollin III was relatively the most abundant isomer in leaves.

Subsequently, Bhattacharyya and Ward (1987) studied the biosynthesis and metabolism of glyceollin I in soybean

hypocotyls following wounding or in cubation with Phytophthora megasperma f. sp. glycinea. They pointed out that the stimulus of wounding or infection induces a metabolic pathway in which glyceollin I is not an end product. The accumulation of higher levels of glyceollin I in resistant than in susceptible responses appeared to be due to earlier initiation and subsequently higher rate of biosynthesis in the former. The interaction of soybean, cv. Horosoy 63 with Phytophthora megasperma f. sp. glycinea race 1 (incompatible) and race 4 (compatible) and of the near isogenic cv. Horosoy with these races (compatible) was also examined by light and electron microscopy in hypocotyl tissues fixed 2, 3, 5 and 7 h after inoculation. The extent and rate of colonization was similar in both incompatible and compatible interactions. Hyphae reached the first layer of the cortex by 2 h and the third layer by 3 h. However, there were major differences in host cell responses. (Ward et al, 1989). The time course of accumulation of two phytoalexins, the terpenoid rishitin and the polyacetylene cis-tatradeda-6-ene-1, 3 diyne-5, 8-diol, was determined in near isogenic susceptible and resistant tomato lines inoculated with either Verticillium albo-atrum or Fusarium oxysporum f.sp. lycopersici. Resistant cultivar contained more rishitin than the susceptible cultivar after 3 days or inoculation with F. oxysporum f. sp. lycopersici (Elgersma and Liem, 1989). A phytoalexin, brassilexin has been isolated from Brassica juncea and Brassica napus. Its

accumulation was related to resistance to Leptosphaeria maculans, a fungus which causes the black leg disease of crucifers (Kollmann et.al. 1989).

(b) Plant disease alteration by chemical treatment

Chemicals of widely diverse nature without any direct toxic action have been used against many plant diseases and significant levels of success has been acheived (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 reactions by accumulating secondary metabolites or "stress" metabolites such as phytoalexin, in treated (physically or chemically) plants (Darwill and Albersheim, 1984 ; Purkayastha, 1986). Several elicitors or phytoalexin synthesis also induce the expression of other host plant defense responses (eg, 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).

The differential effect of maleic hydrazide on the growth of leaf and stem rust of wheat was studied by Samborski et. al. (1960). It was concluded that this metabolic inhibition could induce susceptibility in the resistant Khalpi variety of wheat. Similarly, OKu (1960) presented evidence to indicate 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 Wilcoxson and Sudia (1960). They observed that treatment of maize hybrid seeds with, 5, 10 and 20 ppm gibberellic acid enhanced the severity of seedling blight. Foliar spray with 50 ppm of potassium gibberellate augmented desease intensity in red kidney beans inculated with California isolates Rh-5 or Rhizoctonia solani (Peterson et.al. 1961).

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, naphthalene acetic acid, 2,4-dichlorophenoxy acetic acid and maleic hydr azode) caused an increase in size and number of leaf spots/plant on the susceptible inbred corn line 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 gibberllic acid and grown in soil infested with two other pathogenic isolates of R. solani, severity of the diseases 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 the entry of the pathogen. Sequeira (1963) also reported that diseased plants contained altered concentrations of hormones, the causes underlying these changes being unknown.

Foliar application of different concentrations of IAA and GA to detached bean leaves had little effect on lesion

productions by Botrytis fabae and Botrytis cinerea and were ineffective in the spread of lesions by B. cinerea (Purkayastha and Deverall, 1965).

The effect of maleic hydrazide (MH) on wheat and barley rust was 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/pot (10cm diam.). Barley plants (varieties Bolivia and Oderbrucker) treated with 0.2% MH solution (110 ml/pot) showed reduction in growth and higher susceptibility of plants to Puccinia hordei. 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) has shown that IAA reduced wilt disease of tomato caused by Verticillium albo-atrum. On the other hand Maleic hydrazide (300 ppm), greatly retarded growth of the plant and made them susceptible. Cytocel and naphthalene 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, 2,4,6, trichlorophenoxyacetic acid, 2,3,5-tri-iodeobenzoic acid and 2,4 - dichlorophenoxyacetic acid increased disease at some concentrations and reduced it at others.

Chalutz and Stahmann (1969) induced pisatin formation in pea pods by $CuCl_2$ and isocoumarin 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 (phenylalanine deaminase) involved in the biosynthesis of pisatin. Carrot roots treated with IAA, 2,4-D and 2,4,5-T also elicited coumarin accumulation. In all cases, production of isocoumarin was related to the amount of ethylene produced by the root tissue. Foliar spray with ether 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 relationship were studied by Volken (1972). He reported that IAA increased the Fusarium wilt of tomato while the reverse result was obtained with GA_3.

Furrer and Stawlfer (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 (3-4 Kg/hac.) 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.

Saad and Rashid (1973) recorded that 16 ppm CEPHA (2-chloro-ethale 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 (8-50 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.

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 Colletotrichum 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 the 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).

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) were resistant to the 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 anthrac nose 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 of both resistant and susceptible cultivars. These compound stimulated mycelial growth of C. corchorum at a low (0.1 ppm) concentrations. Apparently there was no correlation between mycelial growth and pathogenicity of the fungus. Inflorescences of grapevine sprayed with 10 ppm gibberellic acid significantly reduced Botrytis infection (Rivera and Mavrich, 1978). 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 increases the capacity of rice plants to synthesize more momilactones (rice phytoalexins) in response to fungal infection. The antifungal activity of rice phytoalexin may be basis for its disease production properties (Cartwright et. al, 1977 ; Cartwright et. al, 1980).

Mercuric acetate caused accumulation of rishitin and lubimin in potato tuber discs. Accumulation of these terpenoids

was not directly correlated to the necrotic reaction (Cheema and Haard, 1978). 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, applications 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 mono-sodium 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-chlorethyl) trimethyl ammonium chloride_7, Pix (N,N- dimethyl piperidinium chloride) and chemagro 8728 /¯tributyl7slightly mitigated the severity of symptoms of V. dahliae on cotton and reduced internal populations of the pathogen in the petioles, Cotton yeild was increased (10.29%) by these treatments (Erwin et al. 1979).

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 effects of foliar spray of bacitracin, chloramphenicol and GA on the rhizosphere microflora of pea seedlings (P.sativum L.) infected with V. dahliae were studied. The antibiotics increased fungus and actionomycetes counts and reduced the bacterial populations in the rhizosphere. Ten ppm GA reduced all three groups of micro-organisms while 100 ppm increased actinomycetes slightly. Foliar sprays also affected the percentage occurrence of particular genera of fungi in the rhizosphere, for example, Trichoderma spp. were stimulated by all treatments, the maximum being with 10 ppm GA. Foliar spray however, markedly reduced disease severity (Ramarao and Isaac, 1980).

The effect of three growth substances 6-Furfuryl aminopurine (Kinetin), 6-Benzyl aminopurine (BAP) and Gibbrellic 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 production 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 inoculated 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 from of foliar spray with these chemicals caused sharp increases in protection, but this disappeared 2 weeks after treatment. Leaf diffusates from 2-week-old seedlings in different treatment showed considerable fungitoxicity, which declined with seedling age and became practically non-existent by the end of fourth week. Inoculation of treated plants at this age resulted in miderate to marked toxicity in their diffusates. Seed treatment was found to be more effective than toliar spray treatment (Sinha and Hait, 1982). Accumulation of phytoalexin in excised cotyledons of P. vulgaris was detected when treated with 10^{-4} M abscisic 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 Magnatato, 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 annuum by 0.1 M copper sulphate, sodium nitrate and chloramphenicol (Watson and Brooks, 1984). Gibberellic acid (GA_3) induced momilactone synthesis in healthy dark grown rice coleptiles as well as leaf sheaths and markedly stimulated momilactone biosynthesis 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 nickle 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 in most of these treatments and there was evidence for inhibition of lesion expansion in a few. Studies with twelve of the more effective chemicals showed that the protective effect persisted at significant levels even in 5-week-old plants and that at this stage this inhibiting effect on lesion expansion was more pronounced in most of the treatments. Different treatments led to the development of a more 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 iodacetate, 2,4 - dinitrophenol, sodium-fluoride, sodium malonate, sodium azide and sodium molybdate) on the development of charcoal rot disease of soybean (cv. Soymax). The effect of sodium azide (100 µg/ml) was found to be the most significant among the metabolic inhibitors tested, in reducing 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 treatments 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. Accumulation of phytoalexin in $CuCl_2$ and $AgNO_3$ treated leaves of Brassica juncea was also reported by Rouxel et al. (1989).

The effects 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-triido-benzoic acid, 2-naphthoxy-acetic acid, L-naphthaleneacetic acid, gibberellic acid, 6-furfuryl aminopurine, and 6-benzyl aminopurine) examined, gibberellic acid was most successful in reducing the disease sevGrity, followed by 3-indole acetic acid and 2,3,5-triiodobenzoic 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 treatment with gibberellic acid (10 mg/L) were estimated ; this compound significantly increased glyceollin production in infected roots.

Purkayastha and Banerjee (1990) used six antibiotics (Penicillin, cloxacillin, tetracyclin, chloramphenicol, cephaloridine, 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 leaf (Leaf 1) of cucumber plants with 50 mM 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 leaf 1 and the level of protection observed in leaf 2 after challenge with C.legenarium (Irving and Kuc,1990).

(c) Common antigenic relationship between host and parasite

Common antigens are antigenic determinants in various biological materials that cause formation of antibodies with similar antigen specificity. Common antigens usually show precipitin reaction in the agar gel. The discovery of the precipitin reaction arose from the demonstration of the precipitates formed when cell-free filtrates by typhoid cultures were mixed with corresponding antiserum. Precipitation reactions in which antigens and antibodies diffuse through and react in semisolid matrices (i.e., agar gel) have become essential tools in biochemical analysis (Chausen, 1969). These techniques generally fall into three distinct categories viz., simple diffusion, double diffusion and immunoelectrophoresis. Early in this century Bechhold (1905) discovered that immunochemical reaction could also be performed in gels. The gel diffusion method was further developed by Arrhenius (1907) who showed that it could fractionate antigen mixtures such as tetanus and diphtheria toxins in complex with their corresponding antibodies. Qualitative method for identification of antigens and antibodies were introduced by Nicolle et al. (1926). Subsequently, Petrie (1932) further developed the gel method as a means of identification of bacterial antigens. In 1933, Maegraith suggested gel diffusion as a microanalytical tool for demonstration of antigenic composition of unknown samples. The basic work of Oudin (1952) was extended and modified by

Ouchterlony (1958). Relation of common antigens of host and pathogen (Melampsora lini and Linum usitatissimum) with host's susceptibility was pointed out by Doubly et al. (1960). They found specific antigens in each of the four races M. lini and four rust-differentiating varieties of L. usitatissimum. Arirulence and virulence were related to resistance and susceptibility through the specific rust antigens. A race was virulent to varieties containing its specific rust antigen as minor constituent, and avirulent to varieties lacking that antigen. Relation of antigens in selected host parasite systems of L. usitatissimum and M.lini was also noted by Petermann (1967).

Protein extracts of resistant (Jersey Queen) and susceptible (Early Jersey wake) cabbage seedlings (both uninoculated and inoculated with Fusarium oxysporum f. conglutinans) were subjected to electrophoretic and immunochemical analysis. By electrophoresis in starch gel, 4 components were separated but no significant differences was observed in uninoculated or inoculated resistant seedling extracts. In contrast, immunochemical analysis with rabbit antisera revealed up to 7 components in extracts of infected susceptible cabbage, compared with 4 components in healthy susceptible and healthy and inoculated resistant plants. The additional components detected in infected susceptible cabbage were not original fungus protein. They may have been formed either by the fungus after infection or more likely by the infected plant cell. On the basis of

different immunological experiment, it is suggested that these components are not merely breakdown products but are antigenic substances, probably proteins, that differ from the normally present substances in the healthy plant (Heitefuss et al. 1960).

The relationship between antigenic compounds produced by sweet potato in response to black rot infection and the magnitude of disease resistance was pointed out by Uritani and Stahmann (1961). Tissue extracts of healthy sliced and black rotted (Ceratocystis fimbriata) sweet potato roots of several Japanese varieties showed immunochemical precipitation lines with antisera towards corresponding extracts from an American variety (Sunnyside). Antigenic components designated A and C were distributed in tissue extracts of all varieties. B and D were produced in response to the infection. The amount produced in several Japanese varieties was correlated with the degree of resistance [i.e., Norin Na. 10 (highly resistant) Norin No. 1 and Okimarsari (resistant) Norin Nos. 4 and 5 (susceptible)]. B and D seemed to be present in healthy root tissue in very small amounts and increased in response to simple injury or slicing but to a much lesser extent than after infection.

Wyllie and DeVay (1970) compared the immunological responses of isolates of Verticillium albo-atrum and V. nigrescens pathogenic to cotton. On the basis of antigenic pattern Verticillium species were distinctly differentiated

from one another. Non-defoliating strain of V.albo-atrum (SS4) was shown to differ antigenically from the defolating strain (T 9). It appeared to be more closely related serologically to the mildly virulent V. nigrescens isolates than was the defoliating T 9 isolates.

Serodiagnostic methods for the differentiation between resistant and susceptible varieties of cotton infected with Fusarium oxysporum and Citrus spp. with Phytophthora citrophthora have been described by Abd-El. Rehim and Hashan (1970) and Abd-El-Rehim et.al. (1971a). Serological and immuno-electrophoretical investigation on water-melon varieties, resistant and susceptible 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 pathogen and seed globulins. It was noted that a_2b globulin fraction was present only in resistant varieties. (Abd-El-Rehim et.al. 1971 b).

Common antigenic relationship between Ustilago maydis and Zea mays was shown by Wimalajeewa and DeVay (1971). A pair of compatible haploid lines and two diploid solopathogenic lines of U.maydis (highly virulent on the inbred corn selection B-164) were used in serological studies. Hordeum vulgare var. "California Mariout" and Avena sativa var. "Victory" were taken as resistant hosts. Corn and all U. maydis lines shared certain antigens in common. 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 used did not indicate any qualitative significant serological difference among them.

Charudattan and DeVay (1972) compared common antigens among four varieties of cotton (Gossypium hirsutum) and isolates of Fusarium and Verticillium species. Atleast one antigenic substance was common among the varieties of cotton and isolates of F. oxysporum f. sp. vasinfectum, F. solani f. sp. phaseoli, V. albo-atrum and V. nigrescens. Cotton varieties which were resistant or susceptible to Fusarium wilt shared the common antigen with both pathogenic and non-pathogenic isolates of F. oxysporum f. sp. vasinfectum, However, the common antigen was not shared between cotton and non pathogenic isolate of F. moniliformae. In gel-diffusion tests five to eight precipitin bands were observed in homologous reactions, of these only one or two bands were common in heterologous reactions between the fungal and the cotton preparations. The common antigenic determinant 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.

Antigenic affinity among the saline soluble proteins of the soil borne fungus Ophiobolus graminis and wheat

(Triticum aestivum) and oat (Avena sativa) was noted by Abbott (1973). Single precipitin band in immunodiffusion test was formed when antisera of the wheat and oat roots were allowed to diffuse with the antigens of O. graminis.

The presence and possible significance of cross reactive antigens in Rhizobium-legume association was described by Charudattan and Hubbell (1973). They compared the soluble antigens of three Rhizobium species with those of eight legumes representing compatible and noncompatible hosts following by agar gel double diffusion tests. Cross reactive antigens were found between all the legume hosts and the three rhizobia. These common antigens among hosts and bacteria were not related to the specificity of compatible Rhizobium - legume associations. The cross reactive antigens were absent between rhizobium and eight non-legume plants tested, but present between five out of eleven gram negative phytopathogenic bacteria and legumes.

Rabbit antisera were raised against soluble extracts of Phytophthora infestans (race 4) and tubers of "Arran Banner" and "Golden wonder" potato cultivars showing field susceptibility and field resistance respectively to late blight. Their antisera were then 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 reaction 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-hosts (mung-bean, pea, radish, Pelargonium, cucumber and maize), nor between potatoes and the alternative pathogen, (Fusarium solani var. caeruleum and two non-pathogens (Ustilago maydis and Phytophthora cinnamoni).

Common antigen was also shared by both avirulent and virulent isolates of F. oxysporum f. sp. vasinfectum with disease resistant and susceptible line 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).

Charudattan and DeVay (1981) isolated, purified to homogenity and partially characterised a major cross reactive antigenic substance (CRA) from conidial cultures of Fusarium oxysporum f. sp. vasinfectum common to roots of cotton (Gossypium hirsutum).

The CRA migrated as a single band in polyacrylamide or agar gel electrophoresis and sedimented as a single band during analytical ultracentrifugation. It was antigenic in rabbits and was a protein carbohydrate complex. The tissue and cellular location of the CRA and their possible role in

host-parasite compatibility has been subsequently described by DeVay et al (1981).

Immunodiffusion test indicated the presence of CRA not only in F. oxysporum f. sp. vasinfectum and cotton roots and seed but also in Thielaviopsis brassicola. Indirect staining of antibodies using fluorescein isothiocyanate (FITC) indicated that in cross sections of roots, cut near or just below the root hair zone, the CRA was concentrated mainly arround xylem elements, the endodermis and epidermal cells and was present throughout the cortex tissue. Protoplast prepared from cross sections of young cotton roots also contained the CRA which was concentrated in the region of plasmalemma. Treatment of conidia and mycelia of F. oxysporum f. sp. vasinfectum 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.

Serological relationship between Macrophomina phaseolina and soybean cultivars was detected by Chakraborty and Purkayastha (1983). Rabbit antisera were raised against antigens of M. 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 tested against both homologous and heterologous antigens following agar gel double diffusion technique. Strong precipition reactions occurred when antiserum of M. phaseolina was

reacted against its own antigens and antigens of susceptible soybean cultivars (Soymax, R-184). No such precipitation reaction was observed in the case of resistant cultivars (UPSM-19 and DS-73-16). Reciprocal cross reactions between antiserum of the resistant cultivar and antigens of three isolates of M. phaseolina also failed to develop even weak precipition bands. Immunoelectrophoretic tests revealed that four antigenic substances were common between the susceptible soybean cultivars and isolates of M. phaseolina but no common antigens were detected between resistant cultivars and the fungus. Purkayastha and Chakraborty (1983) further detected that in susceptible soybean plants (cvs. soymax, R-184) a close relationship exists between lower production of phytoalexin (glyceollin) and presence of common antigens. The production of glyceollin was much higher in resistant soybean cultivars (cvs. UPSM-19 and DS - 73-16) where common antigens were absent.

Common antigens were also detected in extracts of urediniospores of Hemileia vastatrix and in leaf and root extracts of Coffee plant. An antigenic disparity was observed between coffee plants of physiologic group D and E. Common antigens shared between coffee plants and urediniospores of H. vastatrix and their possible involvement in such interaction were discussed by Alba et.al. (1983).

Immunodiffusion, immunoelectrophoretic and cross immunoelectrophoretic analysis of rice antigens and their serological relationship between Acrocylindrium oryzae was determined by Purkayastha and Ghosal (1985). One precipition band was observed when the antigen of A. oryzae was cross reacted with its own antiserum or against the antisera of four susceptible rice cultivars (Jaya, Ratna, IR-8, CR-126-42-1). No precipitin band was detected between the antiserum of the resistant cv. Mahsuri and antigen preparation from three isolates of A. oryzae or between the antigens of resistant cvs. Mah suri and Rupsail and the antiserum of A. oryzae. Bhattacharyya and Purkayastha (1985) also detected common antigenic relationship between susceptible jute cultivar (JRC 212) and Colletotrichum corchori.

Heide and Smedegard-peterson (1985) prepared rabbit antisera against soluble antigens extracted from barley (Hordeum vulgare) and Erysiphe graminis f. sp. hordei. Antigens extracted from four near isogenic barley lines were cross reacted with the antisera of E. graminis f. sp. hordei which shared immunologically identical antigens.

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 for potatoes cv. King Edward and cv. Pentland Dell by using an indirect enzyme linked immunosorbent assay (Alba and

DeVay 1985). They suggested that the fungal mycelia do not easily release cross reactive antigens (CRA) into synthetic media where they grow and most P. infestans CRA are thermolabile and can be concentrated by precipitation in the presence of 40% saturated ammonium sulphate (SAS). An antigenic disparity was noticed when 40% SAS from P. infestans Race 4 mycelia preparation was assayed with antisera for cvs. King Edward and Pentland Dell. The occurrence of CRA in P. infestans mycelium and their involvement in such interactions were discussed.

Purkayastha and Banerjee (1986) ascertained the common antigenic relationship between soybean cultivars and a pathogenic strain of Colletrichum 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 nonpathogen (C. corchori) or avirulent pathogen (C. dematium).

Antigens obtained from two isolates of Macrophomina phaseolina, a pathogen of groundnut, four non-pathogens of groundnut (viz., Corticium sasaki), Colletotrichum lindemuthianum, C. corchori and Botrytis alii) and five cultivars of Arachis hypogea were compared by immunodiffusion,

immunoelectrophoretic and cross immunoelectrophoretic techniques for the presence 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-pathogen and M. phaseolina isolates. Cross immunoelectrophoretic tests confirmed that atleast one antigen was common between cv. J-11 and cv. TMV-2, cv. Kadiri-71-1 and cv. TMV-2, and cv. Kadiri-71-1 and isolates of M. phaseolina (Purkayastha and Ghosal, 1987). Changes in antigenic patterns were detected in susceptible cultivar after chemical induction of resistance in soybean to Macrophomina by Chakraborty and Purkayastha (1987). Sodium azide (100 µg/ml) altered antigenic patterns in susceptible cultiver soymax and reduced charcoal rot disease of soybean. Ghosal and Purkayastha (1987) also indicated that common antigenic relationship between susceptible rice cultivar Jaya and Sarocladium oryzae could be altered by the application of gibberellic acid (100 µg/ml) and sodium azide (100 µg/ml). These chemicals reduced sheath rot disease of rice.

Evaluation of antisera raised against pooled mycelial suspensions from five isolates (Pf-1, Pf-2, Pf-3, Pf-10 and Pf-11) representing five physiologic races of Phytophthora fragariae for detecting the red core disease of strawberries by enzyme linked immunosorbant assay (ELISA)

was done by Mohan (1988). Root extracts prepared from 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, (Resistant to Pf-1,2 and 3 but susceptible to Pf-10 and 11) reflected 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 inoculated with avirulent isolates. The root extracts of the cultivars Hapil, Ostara and Providence (Susceptible to all the five isolates) were also positive in ELISA. The ELISA test proved valuable in screening certified strawberry stock.

Cross reactivity of antiserum raised against Phytophthora fragariae with other Phytophthora as a genus detecting antiserum has also been discussed by Mohan (1989). Antiserum of P. fragariae isolates (Anti PfM) reacted strongly with antigens from several Phytophthora species. Some cross reactions with antigens from Phythium species was decreased by fractionating on an affinity column of sepharose 4B bound to extracts of Fragaria vesca roots infected with P. fragariae. The affinity purified anti-PfM retained its high cross reactivity with the various Phytophthora species.

Anti-PfM could not be made specific for P. fragariae because it was raised against components shown to be antigenically similar in all Phytophthora species tested. However, immunoblotting with the affinity purified anti-PfM produced distinct patterns for P. fragariae, P. erythroseptica and P. cactorum.

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

anti-FO antibody to use in a heterologous ELISA for general detection of ten Fusarium species with high sensitivity.

A polyclonal antiserum which was prepared by immunising rabbit with a mycelial extract of Phytophthora infestans, reacted in an enzyme linked immunosorbent assay with mycelial extracts of two Phytophthora species but not with those of ten other microorganisms found on potato. P. infestans mycelium in patato leaf tissue was readily detected by ELISA using either the plate trapped antigen or F (ab')2 antibody fragment techniques (Harrison et. al., 1990). Amount of mycelium in leaf extracts was estimated by comparing the values obtained in ELISA with those for known concentrations of P. infestans mycelium.

Similarly Mazarei and Kerr (1990) reported a rapid and convenient serological method to distinguish the two very closely related plant pathogenic bacteria, Pseudomonas syringae pv. pisi and Pseudomonas syringae pv. syringae associated with peas. Polyclonal antisera against P. syringae pv. syringae and pv. pisi have a high level of specificity against their homologous antigens after cross absorbtion. By using antisera to glutaraldehyde-fixed bacteria cells both in Ouchterlony gel double diffusion and in indirect ELISA following cross absorbtion, the two pathovars can be easily differentiated, using whole untreated, sonicated or

heat killed bacterial cells as test antigens. Antiserum to P. syringae pv. pisi has considerable potential to detect pea seed. Cross reactive antigens shared by soybean cultivars and the different strains of Myrothecium roridum (M-1, ITCC-1143 ; ITCC-1409) were analyzed by Ghosh and Purkayastha (1990). Results of immunodiffusion revealed that common antigens were present only between virulent strain of M. roridum (M-1) and susceptible host cultivars (DS-74-24-2 and PK-327). No cross reactive antigen was detected in case of resistant cultivars (UPSM-19 and DS-73-16). Immunoelectrophoretic analysis showed that common antigen was shared by susceptible hosts and the virulent strain which was further confirmed by both crossed and rocket immunoelectrophoresis.

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

between resistant cultivars (UPS M-19) and the pathogen, while the results of indirect ELISA indicated the presence of common antigen between the two at a very low level. They compared antigenic patterns of untreated and cloxacillin treated soybean leaves which induced resistance of soybean against anthracnose disease, Disappearance of one antigen from cloxacillin treated leaves of susceptible soybean cv. "Soymax" was correlated with alteration of disease reaction.

A number of reviews pertaining to common antigens and host parasite interaction have been published by different workers during last four decades (Damian, 1964 ; DeVay et, al. 1967 ; DeVay et.al., 1972 ; Purkayastha, 1973 ; DeVay and Adler, 1976 ; Damian, 1979 ; Chakraborty, 1988 ; Purkayastha, 1989).