LITERATURE REVIEW

Resistance or immunity of a plant against pests or pathogens is related to multiple factors. Disease resistance can also be described on several levels such as non-host resistance, parasite - and race-specific resistance, plant age - and organ-specific resistance, and acquired resistance. In fact, if one considers the multitude of microorganisms to which plants are being continuously exposed in nature, the significance of specificity becomes more apparent (Chakraborty, 1988). The physiological/biochemical basis of resistance of plants to fungal and bacterial pathogens has been associated with both preformed and infection induced antimicrobial compounds. Phenolics are significant components of the host response which involve the isolation, identification from substantially large amount of tissues following inoculation. Keeping this in mind the following review is presenting briefly the observation of previous workers in concord with the present line of investigation. Two major objects such as (A) Biochemical defense strategies of plants against pests and pathogens and (B) Efficacy of plant extract in plant defense response have been discussed in the following pages.

(A) Biochemical defense strategies of plants against pests and pathogens

Defense response of a plant depends on the speed and extent of phenols. Some occur constitutively and are thought to function as preformed inhibitors associated with non-host resistance (Millar and Higgins, 1970; Schonbeck and Schlosser, 1976; Mansfield 1983; Stoessal, 1983). Others which are formed in response to ingress of pathogens and their appearance is considered as part of an active defence response. Since the phenolic intermediates have a role in the active expression of resistance, an underlying problem in ascertaining that such secondary metabolites are of primary (rather than secondary) importance has been the localization and timing of the host response (Nicholson and Hammerschmidt, 1992).

Tea, one of the important plantation crops of North East India provides a stable microclimate and a continuous supply of food for rapid build up of more than 300 insects. Despite crop loss, pest infestation also adversely affects the quality of processed tea. Damage by sucking pest like stripes and mites resulted in dull appearance of tea. Tea made from flushworm infested shoots have low levels of extractable solids and high crude fibre content. Liquors obtained from such tea are 'flat' and the presence excreta of the larvae perhaps responsible for the deterioration in quality. The serve infestation of flushworm, pink mite, strips and tea mosquito bug adversely affected flavour and decrease in polyphenolic contents. An increase in polyphenol oxidase activity and total polyphenols was associated with high pigment formation in these teas. The tea mosquito bug *Helopeltis theivora* Waterhouse, popularly known as 'tea mosquitoes' is one of he major pest causing extensive damage in Darjeeling tea plantation. The nymphs and adults of *Helopeltis* are the active sucker of sap from buds, young leaves, tender stems and shoots. They feed at early morning, late evening and night hours. On sunny and worm day, *Helopeltis* takes shelter in lower layer of bush canopy. The insect injects toxic saliva through their needle like rostrum which causes necrosis of the tissues and turn brown first then black and they dry up subsequently. Eggs are laid in tissue of tender stem, mid-rib and petiole of leaves. It was reported that a single first instar nymph of *H. theivora* could make as many as eighty feeding lesions in 24 hours. In order to determine how the tea plant reacts to the attack by the insect, change in activities of antioxidant enzymes - peroxidase, ascorbate peroxidase as well as defense related enzymes - phenyl alanine ammonia lyase, chitinase and β-1,3 glucanase was determined in both healthy and infested tea leaves (Chakraborty *et. al.*, 2005). Protein, carbohydrate, chlorophyll, total phenol and free amino acid contents were found to be reduced in the *H. theivora* infested leaves, while a slight increase in ortho-dihydroxy phenol content and a sharp increase in proline content were noticed in infested leaves (Chakraborty and Chakraborty, 2003). In addition biochemical response of tea to

attack by *H. theivora* with special reference to oxidative enzymes and flavonoid flavor components was determined by Chakraborty and Chakraborty (2005).

Phenols are considered as one of the significant components of the host response following fungal infection. Paschenko (1978) demonstrated the role of phenols in resistance of *Nicotiana didebta* to *Peronospora tabacina.* From a study of the effects of pyrogallol, pyrocatechol and hydroquinone and aqueous extracts from leaf tissues of *N. didebta* and leaf washing on conidial growth, no direct relationship was found between their quantity and the resistance of mature plants to *P. tabacina.* Pyrocatechol and hydroquinone showed extremely high fungitoxicity in relation to *P. tabacina.* Spore growth was more strongly inhibited by extracts from tissue of receptive cultivars. Pyrogallol somewhat stimulated conidial growth. Polyphenoloxidase of resistance cultivars were highly activated during infection. In potato tubers chlorogenic acid was reported to accumulate slower following inoculation with *P. infestants* than in non-inoculated controls, regardless of cultivar resistance (Gans, 1978). In contrast, in some susceptible cultivars chlorogenic acid accumulates at an accelerated rate after inoculation (Henderson and Friend, 1979). The differentiation of the responses of plants to pathogens based on host and non-host interactions also has been argued by Health (1980).

Friend (1981) showed that the accumulation of chlorogenic acid might represent a general rise in phenolic biosynthesis. Chlorogenic acid act as a reservoir for the caffeoyl moiety that, as an activated phenylpropanoid, could be shunted to the synthesis of other phenolics possibly involved in containment of the pathogen. Such synthesis can ultimately result in the accumulation of compounds with sufficient toxicity to be involved in resistance. When carrot root slice is infected with *Botrytis cinerea*, the infection leads to the production of inhibitors such as 6-methoxymellein, p-hydroxybenzoic acid and falcarinol (Harding and Heale, 1981). According to Mayama *et. at.*, (1981) oat produces nitrogen containing phenolic phytoalexins, the

avenalumins, and these compounds accumulated only in incompatible host pathogen interactions.

UV-absorbance and autofluorescence spectra of the avenalumins were used microspectrophotometry to reveal the presence of intense fluorescence only in cells immediately associated with the infection site (Mayama and Tani 1982) where as rapid accumulation of phenols may result in the effective isolation of the pathogen (or non-pathogen) at the original site of ingress (Legrand, 1983; Ride, 1983). For most plants it are low molecular weight phenols, especially the phenyl propanoid, that are involved in the initial response to stress. In potato, phenols accumulate as an initial response to infection (Hammerschmidt, 1984; Hachler and Hohl, 1984). The accumulation of polymerized phenols also occurs as a rapid response to infection. Farmer (1985) and Bolwell *et. al.*, (1985) took hydroxycinnamic acids and their derivatives to reveal the contribution of the discoloration and autofluorescence of host tissues at the site of infection. In maize leaves change in phenolics after inoculation with *Bipolaris zeicola* and their antifungal activity was demonstrated by Werdes and Kern (1985). Maize inbreds Prl (resistant) and Pr (susceptible) to *B. zeicola* race 1 were inoculated and phenolic material was extracted from maize leaf tissue. The components were than analyzed and resistance was studied with respect to phenol metabolism and accumulation of fungitoxic compounds. Host responses could be differentiated by changes in content of phenolic compounds. The pattern of changes of total phenolic content (hydrolyzed and unhydrolyzed ethylacetate soluble phenols) of resistant and susceptible inbreds did not differ much between 0 hr. and 96 hr. after inoculation. However, phenolics content in the resistant inbred increased between 96 and 120 hr. after inoculation to a level two to three times higher than that of susceptible and non-infected control in breds. They isolated four antifungal compounds, A, B, C and D from hydrolyzed maize leaf extracts. All four compounds were fungitoxic to *B. zeicola* in spore germination and chromatographic bioassays. Compounds A and B were inhibitory to *B. zeicola* only in high concentrations. The

investigators suggested a role of the phenol metabolism in the resistance of maize to *B. zeicola* based on different content of total phenolics in resistance and susceptible inbreds. The compounds C and D were supposed to play a role in the resistance mechanism as fungitoxic component.

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

According to Cuypers *et. al.*, (1988) the temporal and spatial differences in the accumulation of phenylalanine ammonia-lyase (PAL) mRNA occurred as a response to infection which was rapidly elevated in interactions involving an incompatible race of fungus, where as a significantly different profile of mRNA accumulation occurred in interactions involving a compatible race The kinds of phenolic compounds that accumulate prior to the active defence response as well as their origin has been addressed by Matern *et. al., (*1988) using parsley leaves with *P. megasperma* f. sp. *glycinea* (Pmg) or treatment of parsley cell suspensions with a Pmg elicitor results in the accumulation of substantial concentrations of coumarin phytoalexins as well as esterification of phenylpropanoids, in particular ferulic acid, to cell walls. Treatment of parsley cells with the Pmg. elicitor cause the synthesis of the coumarin phytoalexins isopimpinellin, psoralen, bergapten, xanthotoxin and graveolone. The healthy leaves of *Morinda tomentosa* contained the two methoxyflavonols 4'-OMe Kaempferol and 3', 4'- di OMe quercentin, and the four phenolic acids-vanillic,

syringic, gentisic and ferulic. The *Colletotrichum gloeosporoides* infected leaves contained the hydroxyflavonols kaempferol and quercetin along with four phenolic acids found in healthy leaves. The diffusates of both the pathogen and non-pathogen (*F. solani*) treated leaves contained quercetin and kaempferol (Abraham and Daniel, 1988).

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

Phytoalexins represent one component of a battery of induced defence mechanisms used by plants, including the important formation of physical barrier to invasion by alteration plant cell wall, the transient generation of antimicrobial active oxygen species (such as H_2O_2) which are generated by the oxidative burst, and release of biologically active lipids such as a result of lipid peroxidation (Ride, 1986; Graham and Graham, 1991; Croft *et. al.*, 1993; Levine *et. al.*, 1994; Low and Merida, 1996; Bestwick *et. al.*,1997). It is important to recognize that phytoalexins accumulation may be part of co-ordinated defence strategies, in which any one factor may alone be unable to account for restriction of the potential pathogen. There are some

interactions in which the speed of accumulation of the inhibitors and their high level of toxicity argue strongly that they are the principal cause of restriction of microbial growth. Such examples are the accumulation of phaseollin in *Phaseolus vulgaris* hypocotyls and wyerone derivatives in cotyledons of *Vicia faba* (Mansfield *et. al.*, 1980; Bailey, 1982).

Total phenol increased after infection in green and ripe tomato fruits in course of rotting due to *Sclerotium* rolfsii (Prasada *et. al.*, (1988). There is often a greater increase in phenolic biosynthesis in resistant host species than in susceptible host and it is sometimes postulated that the inscrease in phenolic compounds is part of the resistance mechanism. Some of these compounds are toxic to pathogenic and nonpathogenic fungi and have been considered to play an important role in disease resistance (Vidyasekharan, 1988). Changes in phenol contents was also demonstrated by Oke (1988) in young, matured, healthy and *Cassicola corynospora* and *Colletotrichum nicotianae* infected leaves of tobacco. After infection the quantity of total pehnols and orthodihydroxyphenol increased in both stem and leaves of susceptible and resistant varieties. Tore and Tossi (1989) investigated the changes in phenolic and nitrogen metabolism in healthy and infected with *(Thielaviopsis basicola)* tobacco roots and leaves. The chlorogenic acid content increased in infected root and leaves compared with the control beginning on the 8th day after inoculation. Polyphenol content in sweet cherry bark was drastically changed after infection by *Cercospora personii* (Bayer, 1989). Infected tissue and closely neighbouring areas were characterised by the appearance of phenolic aglycons which inhibited growth of both the pathogen. Mechanically wounded bark tissue showed different phenolic patterns than infected ones.

Glycoproteins were extracted from isolated cell walls of *Phytopthora megasperma* f. sp. *glycinea* (formerly *P. megasperma* var. *sajae*) with 0.1 N NaOH at 0°C and elicited glyceollin in soybean hypocotyles with the same specificity as the fungus races from which they were obtained. Fraction of the crude extracts on DEAE

Bio-Gel and Bio-Gel A-5m columns showed that specific elicitor activity of glycoproteins was not diminished by boiling at 100^0C or pronase treatment, but was destroyed by periodate, thus indicating that the carbohydrate proteins are important for activity. The glycoproteins were the only concanavalin A reactive species detected in the crude cell wall extracts, and fluorescein labeled concanavalin A was hepten-specifically bound to living hyphae of the fungus and to native but not NaOH-extracted isolated cell walls. Therefore it was concluded that the glycoproteins are present at the surface of fungal cell wall. Tunicamycin, which inhibit the glycoprotein of eukaryote glycoproteins, was a potent inhibitor of mycelial growth of the fungus. The data supported the hypothesis that race specificity in the soybean *P. megasperma* f. sp. *glycinea* system may be determined by specific plant recognition of fungus surface glycoproteins. (Keen *et. al.*, 1980)

Quantitative changes in phenolic compound at different time intervals on barley varieties inoculated with *Puccinia hordei* detected by Etenbarian (1989). Luthra (1989) determined the levels of total phenol in sorghum leaves, resistant and susceptible to *Ramulispora sorghicola* at 15 days interval after 25 days of sowing. Resistant varieties exhibited high phenol content in comparison to susceptible ones at all stages of growth. Phenolic compounds inhibitory to the germination of spores of *Colletotrichum graminicola* were shown to leach from necrotic lesions on corn leaves caused by the fungus. Primary components of the phenolic mixture were identified as esters and glycosides of p-coumaric and ferulic acids as well as the free compound themselves. Spores of *C. graminicola* produced in acervuli of infected leaves were shown to be surrounded by a mucilaginous matrix as in the case when the fungus is cultured *in vitro*. It is suggested that the mucilage protects spores from the inhibitory effects of the phenols by the presence of proline rich proteins that have been shown to have a high binding affinity for a variety of phenols (Nicholson *et. al.*, 1989). The relatively non-specific disruptive effects on cells that result from wounding lead almost immediately to a variety of physiological changes, including oxidation of secondary metabolites. The accumulation of these esters preceded the onset of visible

necrosis of infection sites, the concentration of the compounds fell substantially after the onset of necrosis both of which strengthen the argument for their involvement in the browing response (Bostock and Stermer, 1989). Toxic phenylpropanoids, such as ferulic acid, can form rapidly without the involvement of the traditionally accepted route of phenylpropnoid synthesis and conversion to CoA esters (Hahlbrock and Scheel, 1989). It has long been recognized that responses are characterized by the early accumulation of phenolic compounds at the infection site and that limited development of the pathogen occurs as a result of rapid (hypersensitive) cell death (Fernandez and Heath, 1989). Baker *et. al.*, (1989) examined specific race interaction with clones of resistant and susceptible genotypes and they found greater accumulation of phenolic compounds in resistant reaction than in susceptible reaction. They suggested that accumulation of phenolics might play a role in natural and induced interaction involving *Colletotrichum trifolii* and *Medicago sativa.*

Biochemical changes in the pearl millet shoots infected with downy mildew pathogen (*Sclerospora graminicola*) (Kumar *et. al.*, (1990) The estimation revealed that the total phenol and free amino acids content were found to be low both in diseased shoot and roots of pearl millet (*Pennisetum glaucum*). In maize there is a marked accumulation of two caffeic acid esters after inoculation with *Glomerella graminicola* or *C. heterostrophus* in both compatible and incompatible combination (Lyons *et. al.*, 1990). One compound was identified as caffeoyl glucose, whereas the other was a caffeoyl ester of an unknown organic acid moiety. Although neither compound was fungitoxic, a pattern of rapid accumulation followed by a sharp decrease in the amount of both compounds in the tissue suggested that they may serve as a pool of phenols required for diversion to other products. Mansfield (1990) has proposed that cell death results from irreversible membrane damage that may occur in response to pathogen recognition or as a result of activated host response.

Niemann *et. al.*, (1991) analysed the low molecular weight phenols, such as benzoic acids and the phenylpropanoids, are formed in the initial response to infection. Early after infection, low molecular weight phenols accumulate in both

incompatible (resistant) and compatible (susceptible) interactions. Whether these compounds, are significant in the ultimate host response presents a perplexing problem. Bruzzese and Hasan (1991) demonstrated that accumulation of phenols at the infection site occurred as early as 3 hr. after inoculation, indicating an association of phenols with the initial stages of the response. The contents of phenols, O-dihydroxyphenols and peroxidase activity in healthy and *Curvularia andrepogonis* infected leaves of *Java citronella (Cymbopogan winterianus)* were determined by Alam *et. al.*, (1991). As a result of infection the content of phenols and peroxidase increased two and fourfold, respectively in necrotic lesions compared to healthy leaves. . It has been suggested by Permulla and Heath (1991) that the accumulation of phenolics as an initial response to infection may reflect a general increase in host metabolism as well as an accumulation of relatively non-toxic secondary metabolites, which could ultimately serve as precursors for compounds essential to expression of resistance. In the interaction of potato tubers with *Verticillium dahliae*, hypersensitive browning and suberization are characteristic of the initial events in resistance rather than production and accumulation of phytoalexins (Vaughn and Lulai 1991). The *Fusarium* sp infected leaves of *Trianthema portulacastrum* contained 6,7, dimethoxy-3, 5, 4'- trihydroxy flavone, vanillic acid, p-hydroxybenzoic acid, quercetin and ferulic acid. By using drop diffusate technique it was found that the pathogen induces the formation of quercetin and ferulic acid (Darshika and Daniel, 1992). Changes in carbohydrates, amino acid and phenolic contents in jute plant on inoculation with Macrophomina phaseolina, *Colletotrichum corchori* and *Lasiodiplodia theobromae* were studied by Shabuddin and Anwar (1992). Total sugars, non-reducing sugars, starch and total free amino acids were found to decrease on inoculation with all the three test pathogens of jute, while reducing sugars, total phenols and orthodihydric phenols increased.

Chakraborty *et. al.*, (1994) reported that among fourteen varieties of tea tested separately against *Glomerella cingulata, Pestalotiopsis theae* and *Bipolaris carbonum,* TV-18 and TV-26 were highly susceptible and resistant respectively to *G.*

cingulata and *B. carbonum.* While TV-23 and CP-1 were found to be highly susceptible and resistant to *P. theae.* Twelve separate phenolics were detected on thin layer chromatograms after extraction from healthy tea leaves and some were identified as gallic acid catechol, caffeic acid and p-coumaric acid. Total phenol level decrease by 4.5, 1.2 and 8.5% in the susceptible varieties TV-18, TV-9 and TV-17 respectively after inoculation with *B. carbonum*, whereas in case of resistant varieties TV-26, TV-25 and TV-16 total phenol level increased by 11.1, 5.7 and 12.2% respectively after inoculation. Similar pattern was observed for O-dihydroxy phenol content in healthy and inoculated leaves of resistant and susceptible varieties. The healthy leaves of *Tectona grandis* contained two flavones: 4'-O Me-apigenin and luteolin. The phenolic acids present were syringic, sinapic, vanillic, melilotic and gentisic acid. The other constituents of the leaves were quinones (lepachol and tectaquinon), proanthocyanidins, iridoids, alkoloids and tannins. The infected leaves did not contain any flovone but a flovonol 3', 4'- dimethoxyquercetin instead and phenolic acid such as ferulic, vanillic, melilotic and gentisic acids. They contained the same quinones as at healthy leaves as well as proanthocyanidins, iridoids, alkoloids and tannins. There was no significant chemical differences between the diffusate of control and treated leaves when the healthy leaves were treated with the spore suspension of *Curvularia clavata.* But when the leaves were treated with a non-pathogen *Fusarium solani,* the diffusate contained p-hydroxybenzoic acid. Mycelial growth, spore germination and germ tube growth of *F. solani* and *C. clavata* is strongly inhibited by P-hydroxybenzoic acid (Daniel, 1995).

One of the best and longest-studied defense responses of plants to the infection is the induced accumulation of antimicrobial, low-molecular-weight secondary metabolites known as phytoalexins. Since the phytoalexins hypothesis was first proposed in 1940, a role for the compound in defense has been revealed through several experimental approaches. Support has come, for example, though studies on the rate of phytoalexins in relation to cessation of pathogen development, quantification of phytoalexins at the infection site, and the relationship of pathogen

virulence to the phytoalexins tolerance. Evidence in support of phytoalexins in resistance as well some recent advances in phytoalexins biosynthesis are reviewed. Criteria for evaluating a role for phytoalexins in disease resistance are also discussed (Hammerschmidt, 1999).

Two antifungal compounds isolated from healthy and *Bipolaris carbonum* infected tea leaves exhibited clear inhibition zones at Rf 0.8 and 0.65, respectively in a chromatographic bioassay. On the basis of their colour reaction on TLC and UV-spectra these were identified to be catechin and pyrocatechol, respectively. Resistant varieties accumulated 439-510 μg/g fresh weight tissue of catechol in comparison to 187-212 μg/g fresh weight tissue in susceptible varieties after inoculation with *B. carbonum.* Low concentration of this compound was also detected in healthy leaf tissues (Chakraborty and Saha, 1994, 1995). Phenolic contents in pea genotypes in relation to powdery mildew disease was studied by Sharma *et. al.,* (1998). Guleria *et. al.,* (2001) demonstrated increased levels of peroxidase, polyphenol oxidase, phenylalanine ammonia lyase and phenols in salicyclic acid sprayed leaves in comparison to untreated control plants. The multicomponent phytoalexin response that is found in many plants is clearly demonstrated by bioassay carried out on thin layer chromatograms. After chromatography, the TLC plate was sprayed with suspension of spores of *Clasporium herbarum* in nutrient solution. White zone of inhibition occurred where the dark green fungus failed to grow. No activity was detected in healthy leaves using the assay technique.

Complex drug cerbiden has been studied in vitro for the antifungal activity of its components. It has been established that the spectrum of antifungal effect and activity of cerbiden, with respect to fungi conditionally pathogenic for people, is determined by antibiotic compounds--aromatic carbohydrate phenylheptatryin and sesquiterpene phenol cernusol. They process a spectrum of antifungal activity analogous to cerbiden and activity close to Candida spp., some basidial yeast, dermatophytes, a number of Mucorales. (Stoessl, 1982)

In some host parasite interactions phenolics have been associated with phytoalexin accumulation (Mansfield *et. al.*, 1974; Langcake and Pryce, 1976; Langcake and Macarthy, 1979; Holliday *et. al.*, 1981; Pierce and Ersenberg, 1987; Baker *et. al.*, 1989). Phytoalexin accumulation is believed to be an important early defence response in several plant pathogen interactions. A lot of work has been done and several comprehensive reviews have appeared on phytoalexins and their role in disease resistance (Daniel and Purkayastha 1995; Hammerschmidt, 1999). A fundamental aspect of the definition of phytoalexins is that they are synthesized from remote precursors. Thus, simple labeling studies have demonstrated that the amino acid phenylalanine is used for the synthesis of a complex isoflavonoid such as phaseollin .Activation of defence responses usually lead to a massive, albeit transient and local diversion of normal metabolism into synthesis of groups of secondary products. The interaction between primary and secondary metabolism leading to biosynthesis of chemically diverse phytoalexins. The long term goal of their research is to define target for genetic manipulation to enhance disease resistance in plants. Phenylpropanoids include several classes of phytoalexin including furanocoumarins, flavonoids, isoflavonoids and stilbenes, all of which are ultimately derive from *p*-coumarate. For certain compounds such phytoalexin from common metabolites, whereas synthesis of glyceollin requires the co-ordinate activity of a multienzyme pathway. as the stilbenes resveratrol, a single enzymic step is required to produce the (Dixon *et. al.*, 1992; Nicholson and Hammerschmidt, 1992).

Purkayastha, (1995) analysed phytoalexins constitute a chemically heterogeneous 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 50 years, it is now clear that more than one phytoalexin could occur in a single host species of which one may be dominated 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. During incompatible host-parasite interaction, phytoalexin is synthesized rapidly and accumulates at the infection site. In contrast in the compatible host parasite interaction the plant also synthesis the phytoalexin but relatively slowly and in reduced concentration. The degree of stimulation of phytoalexin biosynthesis depends on several factors such as quantity of elicitors, presence or absence of receptors in the host cell membrane, if present, strong or weak response of receptor, duration of treatment, and environmental conditions. Some selected observations in this line of research have been incorporated in the following paragraphs. An elicitor used with great success in biochemical analysis of alfalfa is derived by heat treatment of yeast cell walls; plants are unlikely to be exposed to the complex product under natural conditions (Dixon *et. al.*, 1995). Having used such elicitors to unravel the fascinating biochemistry underlying biosynthesis it will, therefore be necessary to extend experiments to live pathogens and whole plants to determine if the same control operate within infected tissue. A glucan was isolated from the cell wall extracts of *Fusarium oxysporum* f. sp. *lycopersici* (Anderson, 1980) and a polypeptide (monilicolin A), from mycelia of *Monilina fructicola* (Cruckshank and Perrin, 1968). Both compounds elicited phaseollin production. An elicitor of phaseollin was isolated from the mycelial walls and culture filtrates of *Colletotrichum lindomuthianum*, which was identified as a polysaccharide. The molecular weight varied between 1 million and 5 million Da, and consisted predominantly of 3-and 4- linked glycosyl residue (Anderson and Albersheim, 1975). An amount equivalent to 100 ng of glucose elicited a similar response in the bean tissue.

Daniels and Hadwiger, (1976) isolated *Fusarium solani* which differed in their pathogenicity also should differential pisatin-eliciting potential. It was confirmed when their culture filtrates were tested on pea There was a difference in the concentration of elicitor in the culture filtrates of isolates. The elicitor was fairly heat-stable and also stable in freezing, but eliciting activity was reduced significantly by

pronase digestion. This strongly suggests that some of the activities were due to proteinaceous components.

Sojae by Ebel *et. al.*, (1976) isolated an elicitor of glyceollin from the mycelial wall of *Phytophthora megasperma* var. This elicitor stimulated the activity of phenylalanine ammonialyase and also induced glyceollin production in soybean cell cultures. They concluded that the action of elicitors is not species or variety specific but is a part of the general defence response of plants. Shiraishi *et. al.*, (1978) detected both elicitor and suppressor of pisatin in the pycnospore germination fluid of *Mycosphaerella pinoides.*

In order to examine the control of biosynthesis pathway leading to phytoalexin production in detail, it has been necessary to use artificial model systems and in particular the use of cell culture which produce phytoalexins after treatment with various elicitors. Experiments with cell cultures have shown the induction of phenylpropanoid synthesis is typically the results of increased transcription of gene encoding the corresponding biosynthetic enzymes (Kneusel *et. al.*, 1989; Dixon and Paiva, 1995). The regulation system of phaseollin synthesis in cell suspension cultures of dwarf French bean *(Phaseolus vulgaris)* was studied by Dixon and Christopher (1979). Considerable amount of phaseollin accumulated when french bean was treated with an elicitor from the cell wall of *C. lindemuthianum.* But the elicitors isolated from the cell walls of *P. sojae* and *Botrytis cinerea* were less effective.

Bruegger and Keen, (1979) extracted elicitors from the cell walls of *Saccharomyces crevisiae* and were identified as structural glucans. These are able to stimulate glyceollin accumulation in soybean. Specific elicitors of glyceollin were also detected in the cellular envelops of incompatible races of *Pseudomonas syringe* pv. *glycinea.* However, elicitor activity could not be detected in lipopolysaccharide preparation of exopolysaccharide fraction, or the culture fluids of various races of *P. glycinea.* elicitors were solubilized with sodium dodecyl sulfate and then preparations from five bacterial races expecting one had similar specificity for elicitation of

glyceollin in cotyledons of two soybean *(Glycine max)* cultivars These observations suggests that elicitors are not always race specific. Glycoproteins were extracted from isolated cell walls of *Phytophthora sojae* with 0.1 N NaOH at 0°C and elicited glyceollin in soybean hypocotyls with the same specificity as the fungus races from which they were obtained (Keen and Legrand, 1980). Fraction of the crude extracts on DEAE Bio-Gel and Bio-Gel A-5 m columns showed that specific elicitor activity was associated with the presence of high molecular weight glycoproteins detected by SDS gel electrophoresis. The glycoproteins appeared to contain only glucose and mannose as neutral sugar. The elicitor activity of the glycoproteins was not diminished by boiling at 100°C or pronase treatment, but was destroyed by periodate, thus indicating that the carbohydrate portions are important for activity. The glycoproteins were the only concanavalin A reactive species detected in the crude cell wall extracts, and fluorescein labelled concanavalin A was hapten-specifically bound to living hyphae of the fungus.

Elicitor activity of fresh mycelial wall extract of *Myrothecium roridum* was reported by Purkayastha and Ghosh (1983). Spores were suspended in mycelial wall extract, drops placed on leaf surfaces of soybean and incubated for 48 hr. The results of bioassay test revealed that the spores suspended in mycelial wall extract were inhibitorier than the spores suspended in mycelial wall extract were inhibitorier than the spores suspended in sterile distilled water and incubated on leaf surfaces for a similar period. Mycelial wall extract induced greater production of glyceollin in soybean leaves. Yomoto *et. al.*, (1986) demonstrated that pisatin could be induced in pea leaves by elicitors from *Mycosphaerella pinoides, M. melonis* and *M lingulicola.* Accumulation of pisatin increased after removal of epidermis and application of elicitors from germination fluid of the fungus.

Tepper and Anderson (1986) reported a carbohydrate rich extracellular component from a race of *C. lindemuthianum* showed a high level of phytoalexin activity on a resistant cultivar "Dark Red" of kidney bean but not on the susceptible cultivar "Great Northern." Other extracellular components were also recognised as

elicitors by both cultivars. It is noteworthy that the two cultivars of *Pheseolus vulgaris* displayed a differential response to extracellular components. These observations support the hypothesis that both general and specific mechanisms exist in race cultivar interaction.

Gusine *et. al.*, (1990) reported that the metabolites and viable cells of *Pseudomonas corrugata* from liquid culture medium elicited biosynthesis of the phytoalexin medicarpin in ladino white clover *(Trifolium repens)* leaflets and callus. The biologically active elicitors components were soluble in 80% ethanol. They were partially purified by removing components greater then 3,500 Da by dialysis and fractionating by preparative reversed phase HPLC. None of the four fractions separated by HPLC elicited appreciable quantities of medicarpin in callus, but fraction 1 combined with fraction 4 elicited high concentrations of medicarpin. Any combination of fractions 2, 3 and 4 synergistically elicited medicarpin in callus. Elicitor activity was concentration - dependent. The active fractions were acidic in solution, but their elicitor activity was not dependent on low *p*H. Fraction 1 contained primarily uncharacterized reducing carbohydrate and phosphate. Fractions 2 and 3 were composed primarily of two related, unidentified fluorescent compounds and fraction 4 contained another unidentified fluorescent compound *Phytophthora*, the phytopathogenic fungi subspecies elicit hypersensitive-like necrosis on their nonhost tobacco *(Nicotina tabacum)*, with the exception of the tobacco pathogen *Phytophthora nicotianae*. In culture, these fungi except *P. nicotianae* secrete proteins, called elicitins, that cause these remote leaf necrosis and are responsible for the incompatible reaction. These proteins protect tobacco against invasion by the agent of the tobacco black shank, *P. nicotianae*, which is unable to produce such an elicitor. Cryptogein secreted by *P. cryptogea,* has been purified, sequenced and characterized by terce-Laforgue (1992) as an elicitin, a novel family of 10k da holoproteins. The secretion of cryptogein began later than its synthesis and stopped earlier, simultaneously with mycelium growth, when the nitrogen source in the culture medium was nearly exhausted. Electrophoretic patterns of total protein from

mycelium extracts and N-terminal sequence analysis showed that cryptogein accumulated in the mycelium in its natural form. Cryptogein was synthesized as a preprotein. Fifteen isolates of *Phytophthora parasitica*, nine from tobacco (causing black shank disease) and six from other host plants were compared by root inoculation with regard to their pathogenicity to young tobacco plants. A progressive invasion of the aerial parts over 1 week was observed only with the black shank isolates, while the non-tobacco isolates induced leaf necrosis within 2 days. Similar necrosis occured when the roots of tobacco plants were dipped in diluted culture filtrates from non-tobacco isolates, but not in those from tobacco isolates. The necrosis inducing filtrates were shown contain a c 10K Da protein band which was not present in the other filtrates. This protein (named parasiticein) was purified by ion exchange chromatography to homogeneity in SDS-PAGE and reverse phase HPLC. Parasiticein was serologically related to cryptogein, a member of the elicitin family of proteinaceous elicitors. Like the other elicitins, parasiticein induced necrosis in tobacco plants and protected them against black shank. It most closely resembled little leaf necrosis. Ricci *et. al.*, (1992) suggested that the absence of parasiticein production by the black shank isolates might be a factor involved in their specific pathogenicity to tobacco.

Coleman *et. al.*, (1992) showed that a glycoprotein elicitor of phytoalexin accumulation in leaves of *Phaseolus vulgaris* produced well before lysis in the medium of cultures of *Colletotrichum lindemuthianum* was purified to homogeneity. The glycoprotein was a monomer of M.W.28k Da with a pI of 4.25. The glycosyl side chains which accounted for 43% of the weight of the holoprotein, were composed principally of galactose, mannose and rhamnose exhibited a minimum degree of polymerization of eight and were apparently O-linked to abundant serine and/or threonine residues of the peptide backbone. In a *P. vulgaris* leaf infection bioassay the purified glycoprotein had activity easily detectable at nanomolar concentrations and inducing browning of the treated tissue and the accumulation of both phenylalanine ammonia-lyase and the isoflavanoid phytoalexins phaseollinisoflavin.

For these three linked defence responses, sub optimal concentrations of the glycoprotein induced respectively 4.2,7.6 and 9.7 fold more activity in the cultivar resistant to race delta (cv. Kievit) than in a cultivar susceptible to that race (cv. Pinto). Protein integrity was not required for elicitor activity and glycosyl side-chains isolated from the protein were shown to be active elicitor. The effects of an elicitor (CG-elicitor) from *Colletotrichum graminicola* was studied by Ransom *et. al.*, (1992). Roots of sorghum (*Sorghum bicolor*) accumulated 3-deoxyanthocyanidin phytoalexins in response to CG elicitor. Elicitation of the phytoalexins prior to treatment with the elicitor did not prevent infection and development of milo disease symptoms in susceptible seedlings inoculated with conidia of *Periconia circinata.* However, treatment of roots with the CG elicitor enhanced the synthesis of 16k Da proteins in both resistant and susceptible genotypes without expression of disease symptoms. Cruciferous has long been known to contain a family of secondary metabolites termed as glucosinolates, which are sulphur containing glucosides (Fenwick *et. al.*, 1983). Following cellular damage, glucosinolates undergo hydrolysis catalysed by the enzyme myrosinase to produce glucose, sulphate and a variety of low molecular weight products possessing diverse chemical and biological properties. Some of the degradation products, notably isothiocyanates and oxazolidine-2-thiones have been shown to possess antifungal activity as well as acting a stimuli for feeding and egg deposition in insects (Mithen *et. al.*, 1986; Chew, 1988; Mithen 1992; Mari *et. al.*, 1993).

According to Toyoda *et. al.*, (1992) the effects of the elicitor and the suppressor from a pea pathogen, *Mycospharella pinodes,* on polyphosphoinositide metabolism in pea plasma membranes were examined *in vitro* by Lipid phosphorylation in the isolated pea plasma membrane was drastically stimulated by the elicitor, but markedly inhibited by the suppressor. A similar inhibitory effect was observed by the treatment with orthovanadate or K-252a that blocked pisatin production induced by the elicitor. Neomycin, an aminoglycoside antibiotic that interacts with the

polyphosphoinositide metabolism, also affected the lipid phosphorylation *in vitro* and blocked the elicitor induced accumulation of pisatin *in vivo*. Rapid changes of poly-phosphoinositide metobolism in pea plasma membranes in one of indispensable process during the elicitation of defence responses. Cell walls of germ tubes from wheat stem rust (*Puccinia graminis* f. sp. *tritici*) contain a glycoprotein with a molecular mass of about 67 KD referred to as the Pgt elicitor. This glycoprotein induces a hypersensitive-like response in wheat leaves. In elicitor active intercellular washing fluid (IWF) from compatible wheat stem rust interactions, several elicitor-active glycoproteins were detected. One of these glycoproteins had an electrophoretic mobility identical to the Pgt elicitor. This IWF glycoprotein exhibited elicitor activity upon elution from SDS gels. It was recognized by anti Pgt elicitor antiserum suggesting partial structural identity between Pgt and IWF elicitors. As with Pgt elicitors, the elicitor activity of the IWF glycoprotein residues in the carbohydrate moiety because periodate, but not trypsin or pronase destroyed activity. These results suggest that the Pgt elicitor is released from hyphal cell walls into the wheat apoplast during stem rust infection Beissmann *et. al.*, (1992).

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

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

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

An interesting interaction phenomenon was noticed by Kumar *et. al.*, (1995) that resistance or virulence are modelled by multiple biochemical components of two living organisms. *Costus speciosus* a major sapogenin bearing medicinal plant was severely affected by *Drechslera rostrata* causing leaf blight disease. The HPLC analysis indicated the accumulation of glyceollin II and III as potent phytoalexins by *C. speciosus* in response of nonpathogenic *D. longirostrata.* Further the presence of a

polysaccharide elicitor, or mycelial wall component seems to be detrimental cause of phytoalexin accumulation. The same elicitor was also present in mycelial wall of pathogenic *D. rostrata* but in much lower concentration. Additionally it was associated with another polysaccharide component with different identity. The bioassay method of elicitor preparation was expressed in terms of antimicrobial activity mediated through glyceollins. It was determined to be 88.6% in incompatible which was considerably low (13.7%) in pathogenic reaction. During the pathogenesis of *D. rostrata* the susceptibility was not only exercised with low concentration of elicitor but also being mediated with the association of additional carbohydrate component of mycelial wall hence expressing the involvement of multiple biochemical components to regulate susceptibility.

The inductions of phytoalexin production by nonspecific elicitors (which include proteins, glycoproteins, various types of oligosaccharides, and unsaturated fatty acids) are more difficult to assign. (Hahn, 1996). A race specific elicitor has been isolated from *Uromyces vigna*. This elicitor can induce phytoalexin production in cowpea resistant to this race of the pathogen based on hypersensitive response (HR)-like symptoms induced by treatment of resistant cowpea leaves with the elicitor (D'Silva and Heath, 1997). The relative roles of glyceollin, lignin and the hypersensitive response (HR) in pathogen containment and restriction were investigated in soybean cultivars that were inoculated with *Phytophthora sojae*. Incompatible interactions in leaves and hypocotyls were characterized by HR, phenolic and lignin deposition and glyceollin accumulation. The uncoupling of glyceollin synthesis from the HR and phenolic and lignin deposition by ABA treatment showed that glyceollin is a major factor in restriction of the pathogen during these interactions (Mohr and Cahill, 2001). The presence of phenolic acids in cell walls- esterified p-coumaric acid and ferulic acids bound to cell wall polysaccharides are widespread in gramineae. Cell wall bound phenolics in resistance to rice blast disease was demonstrated by Kumar *et. al.*, (1997).

To understand the coordination functions of the different classes of defense related gene expression in plant defense resistance, the expression pattern of pathogenesis related protein (PR) genes and genes involved in antooxidation and the production of secondary metabolites were examined by Kong moonkyung *et. al.*, (1998). Northern blot analysis showed that PR genes such as β-1, 3 glucanase and chitinase were strongly induced in tobacco leaves upon salicylic acid treatment. Phenylalnine ammonia lyase (PAL), involved in phenylpropanoid biosynthesis was mildly induced during latter strategies of normal hypersensitive response (HR) or after salicylic acid treatment. However in acute HR they were strongly expressed during early stage. The expression of the antioxidative genes, anionic peroxidase and ascorbate peroxidase were inversely expressed following salicylic acid treatment. Differential expression of 3 groups of gene involved in plant defense responses were discussed in relation to different signal transduction pathway.

Zhang *et. al.*, (1998) demonstrated that a boicontrol agent for field compost maize, suppressive to several disease caused by soil born pathogens, induced systemic acquired resistance (SAR) in cucumber against anthracnose caused by *Colletrotrichum orbicular* and in Arabidopsis against bacterial speck caused by *P. maculocola.* A peat mix conducive to soil born disease did not induce SAR. The population size of *P. syringae* Pv. *maculicola* was significantly lower in leaves of Arabidopsis plants grown in the compost mix compared with those grown in the peat mix. Autoclaving destroyed the SAR-inducing effect of the compost mix, and inoculation of the autoclaved mix with nonautoclaved compost mix. Topical spray with salicylic acid (SA) induced the severity of bacterial spick on plants in the peat mix but did not further reduce the severity of symptoms on plants in the compost mix.

The mechanism of pathway of plant systemic acquired resistance are reviewed by Cai-Xin Zhay *et. al.*, (1999). After a plant is inoculated with a necrotizing pathogen or treated with some chemicals, the uninoculated or untreated parts of the plant demonstrate resistance to the infection of second pathogens. This is plant systemic acquired resistance (SAR). SAR is systemic, long lasting resistance to wide range of

pathogens. The inoculated or untreated parts immediately produce a systemic signal and induce the expression of SAR gene. Salicylic acid (SA) is one of the signal molecules inducing SAR. They react more rapidly and efficiently to a challenge infection wide range of pathogens. A series of SAR mutant were selected, several genes encoding components of SAR signal transduction pathway were cloned, and their function were analyzed.

Hill *et. al.*, (1999) dealt with identification of disease response genes expressed in *Gossypixm hirsutum* upon infection with the wilt pathogen caused *Verticillium dahliae* which cause disease known as verticillium wilt. To begin to understand the molecular mechanism of the disease response in cotton cultivars that display superior wilt tolerance. Two signaling pathways, one involving salicylic acid and another involving jasmonic acid participate in the expression of plant resistance to pathogens and insect herbivores. In this study Thailer *et. al.*, (1999) shown the stimulation of systemic acquired resistance in field grown tomato plants with the salicylate mimic, benzothiadiazole, attenuated the jasmonate induced expression of the anti-herbivore defense related enzyme polyphenol oxidase (catechol oxidase) and compromise host plant resistance to larvae of the beet annyworm, *Spodoptera exigua.* Conversely, treatment of plant with jasmonic acid at concentration that induce resistance to inset reduced pathogenesis related protein gene expression induced by benzothiadiazole and partially reversed the protective effects of benzothia-diazole against bacterial speck disease by *Pseudomonas syringae* Pv. *tamato.* It was concluded that the effective utilization of induced plant resistance to the multiple pest typically encountered in agricultural wilt require understanding potential signaling conflicts in the plant defense responses.

A multi-component coordinated defense response in rice plants against fungal attack was demonstrated by Bera *et. al.*, (1999). Some selective defense components such as momilactone A (a rice phytoalexin) β-1,3 glucanase and exo-chitinase (both pathogenesis related proteins) and phenylalnine ammonia lyase (PAL) were employed as biochemical parameter for evaluating the degree of resistance of rice

plants to *R. solani* the causal agent of sheath blight disease. A systemic fungicide which reduces disease significantly also concomitantly activated biosynthesis of momilacton A, induced PR-proteins and increased PAL activity. Treatment of rice leaf sheath with PR protein inhibitor increased disease but inhibited β-1,3 glucanase and exo-chitinase activity in treated plants. Similarly amino oxyacetic acid (PAL-inhibitor) enhanced disease intensity and inhibited PAL activity in plants treated with amino oxyacetic acid and inoculated with *R. solani*.

Experiment by Caroline *et. al.*, (2000) have shown that, plant developed an enhanced defensive capacity against a broad spectrum of plant pathogens after colonization of the roots by selected strains of nonpathogenic bio-control bacteria. In *Arabidopsi thaliana*, this induced systemic resistance (SR) functions independently of the salicylic acid but requires an intact response to the plant hormones jasmonic acid (JA) and ethylene.

The growth coffee orange rust fungus (*Hemileia vastatrix* Berk and Br.) isolated and the sequence of response is induced in leaves if resistant *Coffee arabica* L. and *C. congesis* Froehner as well as on a susceptible *C. arobica* were investigated cytologically and biochemically by Silva *et. al.*, (2000). The first signs of incompatibility detected 2 days after inoculation, were cytologically expressed by hypersensitive host cell death (HR), host cell wall autofluorescence and haustoria encasement with callose and β-1,4 glucans. Biochemically two peak of phenylalnine ammonia lyase (PAL) activity were detected by 2 and 5 days after inoculation. The hypertrophy of the host cells in the infection area were also observed around 12 days after inoculation corresponding macroscopically to the infection site.

Soybean phenylpropanoid defense responses to the wall glucan elicitor(WGE) from *Phytophthora sojae* include the accumulation of phenolic polymers and glyceolline in cells immediately proximal to the point of treatment and accumulation of conjugate of the isoflavones, daidzein and genistein, in distal cells. It is demonstrated by Park *et. al.*, (2002) that the WGE is indeed highly effective in protecting cell distal to the point of treatment from infection by *P. sojae*.

Mycolaminaran, jasmonic acid (JA), methyle jasmonate and ethylene precursor, 1-amino-cyclopropane carboxylic acid (ACC) are also effective, while salicylic acid (SA) is not. Methyle jasmonate, WGE and mycolaminaran are most effective, resulting in early complete protection against the pathogen even in the universally susceptible line.

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

In winter cereals, low temperature hardening plant age and genotype are known to influence the expression of resistance to snow mould diseases. A study was undertaken by Gaudet *et. al.*, (2003) to determine the effect of genotype,plant age and duration of cold hardening on the temporal expression of the PR-protein and other defense related protein. The results demonstrate that the temporal expression of cold induced, plant defense related transcripts in winter wheat is differently regulate among genotypes and during different plant development stages, and are the first to implicate lipid transfer protein in the expression of genotypic based snow mould resistance in wheat. Potential plant defense signaling pathway involved in snow mould resistance induced at low temperature during natural acclimation of winter wheat.

A study was carried out by Chakraborty *et. al.*, (2004) on the association of defense enzymes with resistance in tea plants triggered by *Exobasidium vexans* revealed significantly changes in the level of enzymes mainly β-1, 3 glucanase and chitinase exhibiting antimicrobial activity. A wide variety in the activities of the

enzymes involved in phenol metabolism including phenylalanine ammonia lyase, peroxidase and polyphenol oxidase were seen in compatible and incompatible interactions. The possibility of including resistance in susceptible varieties of tea was worked out following inoculation with salicylic acid and results established its potential in immunizing tea plants which was confirmed by immunoassays and localization of chitinase was established in tea leaf tissues after induction of resistance by employing polyclonal antibodies raised against chitinase and labeled with FITC.

Multicomponent coordinated responses of tea plants under biotic stress with special reference to a pest (*Helopeltis theivora*) and a fungal pathogen (*Exobasidium vexans* causing blister blight disease of tea) have been demonstrated by Chakraborty *et. al.*, (2004). Involvement of defense enzymes mainly chitinase, β-1,3-glucanase, peroxidase and phenylalanine ammonia lyase in developing an immune response in tea plants against pests and *E. vexans* were evident in naturally growing healthy plants as inherent immunity. Induction of these enzymes was noticed when plants showed hypersensitive responses. Various elicitors, mycopesticide bio-formulations like Metabass and Salicylic acid, Hexaconazole were studied, that seem to work in activating defense networks. However, resistant tea varieties after challenge inoculation with *E. vexans* revealed four new protein bands of ca. 61, 42, 22 and 14 kDa when probed with PAb of chitinase. Immunocytochemical localization of defense enzyme in tea leaves following induction of resistance was visualized as apple green fluorescence when FITC was coupled with PAb of chitinase. Activities of POD and PPO were significantly high in compatible interaction whereas CHT, βGLU and PAL were found to be high always in resistant reactions. Time course accumulation of CHT, βGLU and POD in tea plants triggered by *E. vexans* showed a general increase in POD activity after 48 h post-inoculation whereas activities of CHT and βGLU increased within 24 h of inoculation. Systemic accumulation of these enzymes was also evident. Salicylic acid (SA), a known to SAR inducer, elicited defense responses by accumulation CHT and βGLU as early as 24 h of post-treatment

whereas increased in activity of POD was noticed after 48 h. the accumulation of defense enzymes in tea plants in response to SA treatment suggests its role in the cellular protection mechanism which was also confirmed (Chakraborty *et.al.*, 2005).

(B) Efficacy of plant extract in plant defense response

The literature on pest control is dominated by report on chemical control. Broad spectrum pesticides offered powerful incentives in the form of excellent pest control, increased yield and reliable economic returns, but they have significant limitations. However, there are welcome efforts to adopt non-chemical strategies and evolve integrated pest management system.

Integrated plant disease management was proposed in the mid seventies and this programme should be considered as a holistic approach keeping in view the agroecological system and the overall situation of agricultural production. It includes the rotational application of cultural, biological and chemical control methods, as well as the coordination and integration of various procedures for the purpose of controlling the damage due to disease.

In the recent years there has been an increased interest in the use of eco-friendly technologies for plant disease control. Botanical pesticides are considered as potential alternatives to chemical agents, which can be hazardous to human and animal health (Mukhopadhaya, 1996). Many plant-extracts have been found to possess antifungal properties. Botanicals, however, are yet to be exploited as anti-infective or anti-infestive agents on a chemical scale. The present investigation reports on evaluation of plant extracts against fungal contamination. Fungal pathogens cause severe damage to the forest nursery stocks at seedling stages resulting in considerable loss in plantation activity. Hence, an experiment was carried out to manage the disease with bio-agents, natural products, and emission for the sustainable management of foliar diseases. The presence of antifungal compounds in higher plants has long been recognized as an important factor to disease control.

Such compounds being biodegradable and selective in their toxicity are considered valuable for controlling some plant diseases (Singh and Dwivedi, 1987). In this context, a variety of plant product having antibacterial and antifungal properties, in different parts of the plants have been reported.

Plant extracts of a *Dibymocarpus oblonga* and *Piper nigrum* were tested on sclerotia forming pathogens such as *Sclerotium rolfsii* and *Rhizoctonia solani* and observed fungitonic properties (Choudhury and Sen, 1981). The fungicidal activity of the benzene extract of *P. nigrum* was inhibitorier on mycelial growth than on sclerotia germination. The essential oils of *Caesulia oxillaris* (compositae) 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 inhibited by *H. oryzae*.

Antifungal substance with methanol, acetone and ethylether from the epidermis and periderm region of *Morus alba* were extracted and tested against *Biolaris leersiae* and other phytopathogenic fungi by (Shirata and Takashashi, 1982). Each acetone extract from root and shoot showed 3-7 active substance, the amount and number varying the different cultivars. They concluded that this antifungal substance in epidermis of root may be prohibitions and one of the resistance factors to pathogenic soil fungi. A preformed antifungal compound (1-acetoxy 2-hydroxy–4–oxo–heneicosa – 12, 15diene) was isolated from peels of unripe avocado fruits which inhibited the vegetative growth of *Colletotrichum gloesporioides* and totally inhibited the spore germination (Prusky *et. al.*, 1982). Coxon *et. al.*, (1982) took two compounds (2, 5–dihydroxy-4-methoxy–9 and 10-dihydrophenanthrene) were also isolated by from the peel of yums (*Dioscorea rotundata*) which inhibited *Cladosporium cladosporiodes* and variety of yam soft rot pathogens.

Pan *et. al.*, (1983) tested antifungal activity of some naturally occurring coumarin compound isolated from *Limonia acidissima* against *Drechslera oryzae, Fusarium solani, Alternaria solani, Sclerotium rolfsii,* and *S. hydrophyllum*. They reported that

the compounds possessed low to high inhibitory activity towards spore germination and *D. oryzae* was highly sensitive to all 4 coumarins. 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 up to 120^0C. The oil of *Ageratun honstoniarium* was found to possess broad mycotoxic spectrum exhibitory strongest toxicity against *Fusarium lateritium* f. sp. *cajani* (Tripathi *et. al.*, 1981).

Effect of some plant extracts and oils on inoculum density of *Crysipha polygoni* on different nodal leaves of *Pisum sativum* was reported by Singh and Pathak (1984). Among the various extracts (garlic bulb, garlic oil, neem leaf and ginger extracts) tested, best results were observed with ginger extracts and oil which reduced the disease (powderymildew of pea) intensity and increased the seed yielding capacity. Leaf extracts of *Anagallis arvensis, Caesalpinia pulcherima, Psidium guajava* etc. were totally fungitoxic to *Ustilago maydis* and *U. nuda* essential oils from *Ocimum basillicum* and their components showed different inhibitory effect against *Fusarium oxysporum* f.sp. *vasinfectum* and *Rhizopus nigricans* which was depended on the percentage of main components: lineol, linaleol, methyl chavical and eugenol. Effect of 49 indigenous plants on 11 phytopathogenic fungi belonging to the genes *Phytophthora ceratocystis, Phoma* etc. were studied by *Chesne et. al.*, (1984).

Tripathi *et. al.*, (1985) isolated oil from the leaves of *Ocimum qratissium* 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 *Ocimum* sp. five oils of coconut groundnut, pure vegetable and liquid paraflin were tested against spore germination of *Rhizopus oryzae, Curularia lenata, Phoma sorghina* and *Fusarium equiseti* by Adisa (1985). Post-harvest tomato fruit could not be controlled in Nigeria by dipping in 75% palmkernel oil and storing at 15^0C. Pan *et. al.*, (1985) reported that some naturally occurring flavonoids isolated from *Pangaria globra* showed antifungal activity

against some test fungi. Of these Pongenethyl ether was found to be most promising. Leaves and petal extracts of *Camellia japonica* and *C. grandhamiana* showed antifungal properties and inhibited the growth of hyphae of many fungi when mixed potato – sucrose liquid medium. Almost no condium of *Pyricularia oryzae* or *Cochlioblus miyabeamos* germinated, germtubes or conidia swelled resembling balloons and no normal hypae of *Pestatotie longiseta, Gloeosporium theae-sienensis* and *Botrytis cinerea* grew. Two triterpenoid saponins as the antifungal compounds from aqueous extracts of *Camellia* leaf was isolated which was designated as "Camellidin –I" and "Camellidin –II". Camellidin showed antifungal activity against *Pestalotia longiseta* and *Cochliobolus miyabeanus* (Nagata *et. al.*,1985).

The leaf extracts of *Eucalyptus oceidentalis* and *E. brockwayi* exhibited strong fungitoxicity against the mycelial growth of *Pestalotiopsis mangiferae* in Potato Dextrose Agar (PDA) medium (EL-Sayed *et. al.*, 1985). Levey *et. al.*, (1986) isolated a medicagenic acid from alfalfa roots, which exhibited the mycelial growth of *Aspergillus niger*, *Sclerotium roulfsii* and *Fusarium oxysporum* f,sp..*Lycopercisi*. The leaf extract of *Polyalthia longifolia* in neutral phosphate buffer were also inhibitory against mycelial growth of *Rhizoctonia solani Sclerotium rolfsii* and *Sclerotium oryzae* than in distilled water. The inhibitory principle of this leaf extract was also thermostable (Naidu and John, 1986).

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

Late leaf spot (*Phaeoisariopsis personata*) and rust (*Puccinia archidis*) of groundnut were partly controlled by using plant extracts of *Tridex procumbens*, *Pongamia glabra, Lawsonia alaba* along with carbendazim plusmaneozed and

N.C.P.75. Simultaneously, the yield also increased (Ghewande, 1987) Vapours of aqueous extracts of rhizome of *Alpinia carinata* showed strong activity against *Rhizoctonia solani.* They also isolated the essential oil from the rhizome 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 aurens* (Kishore *et. al.,* 1987).

Helle and Briner (1988) isolated phenylpropanoid glucoside isolated from *Plantago major* which inhibited bacterial growth. Minimal inhibition concentration value has been evaluated for 7 plant pathogenic bacteria and for *E. coli* (ML.30) and *Staphyllococcus aurens* (502A) after preliminary investigation following immunodiffusion test. Gourinath and Monoharachary (1988) also tested the effect of latex collected from different host plants e.g. *Calotropis Ipomoea, Carica* etc. on conidial germination and mycelial dry weight of 4 pathogenic fungi viz .*Curvularia Iunata, Fusarium solani, Cylindrocarpan lichencola* etc. Among 100 species in 54 families of plant tested, leaf extracts from *Allium cepa, Allium sativum, Malus sieboldii Reysontria japonica* and *Rheum coreanum* were inhibitory on mycelial growth of *Phytophthora* sp. However, *A. sativum* and *Malus sieboldi* were strongly inhibitory (Paik *et. al.,* 1989).

Antibacterial screening of the crude extracts of leaves and stem barks of *Cassia alata* and 2 pure compound isolated from leaves was done against 15 pathogenic bacteria by Choudhury *et. al.*, (1989). All the crude extracts and pure compounds were found to be active against both gram positive and negative bacteria, but the menthol extracts were relatively active against wide range of bacteria methanol extracts of the stem barks and kaempfrol showed MIC value against *Shigella dysenteriae* , and *Staphyllococcus aurens* .

Taking 46 plant species by (Peshney and Moghe, 1990) evaluated crude leaf extract of quite a few of them indicated presence of inhibitor of tobacco mosaic virus. The inhibitor was also present in different parts of *Polianthus tuberosa, Capsicum annum* and *Abrus precatorius* which showed 90-100% infectivity inhibition *in vitro.*

Inhibitors from these plants were systemic in nature when tested on *Chenopodium quinoa* as locaol lesion and *Capsicum annuum* cv. 'Jwala' as systemic host for the virus. Chillies were symptomless for period of 30 days with single pre-inoculation treatment with crude leaf extract of these plant species Kumar and Tripathi (1991) screened leaf juices of 18 different species, out of these only *Eupatorium cannabinum* exhibited complete toxicity against *Pythium debaryanum, Fusarium oxysporum, Rhizoctonia solani* and *Sclerotium rolfsii* shade drying of the leaves had no adverse effect, while oven drying produced an adversed effect on the fungitoxicity of the leaves of *E. cannabinum.* The crude leaf juice of *E. cannabinum* successfully inhibited (*F. oxysporum* infection of *Pisum sativum*) seeding.

Chauhan and Singh (1991) reported *in vitro* effect of garlic, onion bulb, ginger, tulsi leaves extract on germination of zoospores of *Phytophthora drechsleri* f.sq. *cajani* has been observed. Extracts inhibited spore germination at 5000 and 10000 ppm. For garlic and onion and controlled *Phytophthora* blight of pegion pea in the field. Dubey and Dwivedi (1991) reported that distilled water extract (1:1w/v) of *Acacia arabica, Allium cepa and A. sativum* inhibited the growth of *Macrophomina phaseolina* in PDA culture. Mangamma and Sreeramulu (1991) examined several plant species to explore the possible production of antibacterial compounds by plants. Of these garlic bulb extract (*Allium sativum* L.) was toxic to *Lycopersicon esculentum* and *Xanthomonas compestris*, the leaf spot pathogen of tomato. Three concentrations of extracts were prepared 10g/100ml, 20g/100ml and 30g/ml. Among these 30g/100ml concentration had larger inhibitation zone when the aqueous extracts were assayed against *X. campestris* by paper disc method on potato dextrose agar medium. Garlic extracts were also reported to be inhibitory to fungal pathogen of rice namely *Pyricularia oryzae, Drechslara oryzae* and *Corticium sasatiu.*

Effects of antiblue and biocide at various concentration and times of immersion on the growth of fungi were studied by Masuka (1991). Antiblue (active ingredient, sodium pentachloride) was the better chemical providing complete control at the lowest concentration of 1.5%. Biocide a chlorinated derivative and local substituted

for antiblue provided 55% control at 4 and 8% concentration and 74% control, at the high concentration (12%). The time of immersion did not have a significant effect on the incidence of stain and mould.

Four phenolic compounds viz. apigenin, apigenin-7-0-glucoside, echinacin, *echinaticin*, were isolated by Singh *et. al.*, (1991) from the whole plant of *Echinops echinatus*. The latter 2 compound were isolated for the first time. All these compound were assayed against germination of conidia of *Alternaria tenuissina* which incite leaf blight of *Cajanas cajan*. All showed high efficacy against the pathogen at concentration ranging from 25-150μg ml^{-1}. This compound has been suggested to use as a control measure against *Alternaria* blight of Pigeon pea. Leaf extract of five plant species *Capsicum annum, Acacia arbica, Datura metel, Azadirachta indica* and *Spinacia oleraceae* were tested *in vitro* on to *Chenopodium amaranticolor* at 3 (three) concentration) (1:10, 1:100 and 1:1000). Among them *D. metel* at 1:1000 dilution of sap produced maximum inhibition (Sawant, 1992). Aqueous, ethyl acetate and methanol extracts of *Funaria hygrometrica,* a bryophyte, were tested against a few phytopathogen and human pathogens (*Xanthomonas oryzae, X. campestris, Klebsiella, Protens, Salmonella, Fusarium oxysporum* f. sp. *lycopersisi* and *Rhizoctonia solani*). Organic (Ethanol, methanol) extract showed antimicrobial activity whereas aqueous extract did not show any inhibitory activity (Gnanagura, 1992).

Sarvamangala (1993) reported antifungal activity of leaf extracts of *Azadirachta indica, Calotropis gigantica, Catharanthus, Eucalyptus* sp., *Parthenium lysterophorus, Pongamia pinnata* was tested against *Cerotelium fici* and *Cercospora moricola* causing leaf rust and leaf spot disease in mulberry, respectively. *A. indica* was more effective in inhibiting spore germination of *C. fici* by 91.2% where as extracts of *Eucalyptus* and *C. gigantica* proved highly toxic to inhibit the conidial germination of *C. moricola.* Under field condition they showed promising result.

Gohil and Vala (1995) investigated different phytoextracts on sugar cane wilt pathogen (*Fusarium moniliforme*).The extract of the following plants were evaluated. Almond (*Allamanda cathartica* L.), Ardusi (*Adhatoda vasaca* Ness), Piludi (*Salvadora persica*), Popti (*Physalis minima* L.), Ratanjogia (*Jatropha curcas* L.), Sargava (*Moringa oleifera Lam*), Saru (*Casuarina equisetifolia* L.), Soap-nut (*Sapindus trifoliata* L.), Sweet neem (*Myrraya Koenigii*), Tulsi (*Ocimum sanctum* L.) and Trumeric (*Curcuma longa* L.). The extracts were prepared in cold distilled water (1:1). Among 33 phytoextract studied the extracts of garlic, soap-nut were found to be inhibitory against *F. moniliforme*. Spores of *F. oxysporum* f.sp. *ciceri* (race-1) was significantly inhibited by the root exudates of the wilt-resistant chick pea cultivars (CPSI and WR 315) in comparison to untreated spores and spires treated with root exudates from susceptible cultivars (Stevensen *et. al.*, (1995). The effect was concentration dependent, such that exudates from 1gm of root in 2ml. of water almost completely inhibited spore germination, whereas exudates form 1gm of root in 20ml. water did not do so. Ethyl acetate fraction of root exudates of CPSI and WR315 strongly inhibited germination and hyphae growth.

Different plant extracts against 3 major diseases of Mulberry proposed by Biswas and Das (1995). They reported that 10% alcoholic water extracts of fresh plant parts from 20 different species were studied on the development of powdery mildew (*Phyllactinia corylea* (Pers.) Karst.). Leaf spot (*Pseudocercospora mori*) and leaf rust (*Cerotelium fici*) diseases in mulberry during 1992-1993 and 1993-1994. The pooled data of these two years revealed that the extracts of *Azadirachta indica, Launea coromandelica* and *Oxalis cormiculata* which significantly minimized 2 disease, namely, powdery mildew and leaf rust, while those of *Calosia argentia* and *Eupatorium odoratum* reduced leaf rust diseases. Extracts from several other species exhibited ability to reduce either leaf rust or powdery mildew. Ansari (1995) tested antifungal activity of Ajwain (*Trachispermum ammi*), Lemon grass (*Cymbopogon citrates*), Tulsi (*Oscimum*), *Mentha, Rauwolfia, Lawsonia inermis, Vitex trifolia*

against *Rhizoctonia solani,* causal organism of sheath blight of rice. Extract from seed of ajwain and leaves of tulsi showed fungicidal activity and others were fungistatic. The spray of ajwain and tulsi leaf extracts to plants at 1:20 dilution reduced the disease by 72-25% and 69.58 respectively.

Kiregard, (1996) pointed out that superior growth of wheat following *Brassica* crops compared to that following non-Brassica crops may be due to the suppression of soil borne fungal pathogen by volatile isothiocyanates (ITCS) released in the soil during hydrolysis of glucosinolates contained in *Brassica* tissue. Investigation was made on the effects of volatile compounds released from the root, shoot and seed meal tissue of canola (*Brassica napus*) and Indian mustard (*Brassica juncea*) on the mycelial growth of soilborne pathogen of cereal *Gaeumannomyces graminis* var. *tritici, Rhizoctonia solani, Fusarium graminearum, Pythium irregulae* and *Bipolaris sorokiniana.* Three isolates of each species, originally collected from the roots of wheat (*Triticum aestivum*) and barley grass (*Hordeum leposinum*) in South Australia. The root and shoot tissue of both *Brassica* species were more suppressive of flowering than maturity and matured tissue were generally more suppressive than canola. The degree of fungal suppression by various *Brassica* tissues was related to the concentration and type of isothiocyanulate released, which varied with *Brassica* species, tissue age and tissue type. There were significant differences in the sensitivity of the fungal species and among isolates of each species, *Rhizoctonia* and *Fusarium* were generally the most sensitive to the volatiles released whereas *Pythium* and *Bipolaris* was found to be the less sensitive.

The efficacy of light anti-viral substance was tested against summer squash mosaic disease by Sandhu *et. al.,* (1996). All the antiviral products used were found inhibitory against the mosaic disease. The extract of Sorghum was much superior to the other treatments in controlling the mosaic disease followed by Thuja at both

chelldonium used. The least disease control was in plants sprayed with *Bougainvellea* extract. All the treatments increased the yield of the plants as compared to control.

The effect of aqueous leaf extracts of *Azadirachta indica, Pinus roxburghii* and *Targetes erecta* and water soluble fraction of mustard oil cake on lignin content of barley leaves in relation with incidence and development of leaf stripe disease were reported by Varshney and Sharma (1996). Concentrated (100gm of plant material/ 250ml. distilled water) and diluted (1:1000 and 1:500) extracts/fraction were used. A conventional systemic fungicide carbendazim (trade name Bavistin) used in control of leaf stripe disease of barley was also tested. As compared to control, lignin content was higher in *A. indica* and *T. erecta* treated leaves. On the contrary, treatment wilt *P. roxburghii* and mustard oil cake fraction reduced lignin content of barley leaves. A direct correlation between lignin content and incidence of leaf stripe disease was also found.

Foliar application of leaf extracts of *Azadirachta indica* and *Dryopteris filix-mas* on tea plants affecting resistance to *Pestalotiopsis theae* has been elucidated by Deb and Chakraborty (1998). There is also evidence that leaf extracts of *Lantana camara* could induce resistance in tea plants against brown blight pathogen *Glomerella cingulata* causing brown blight disease. (Chakraborty and Chakraborty, 1998).

Singh and Majumdar (2001) took an attempt to develop botanical fungicides for suppression of *Alternaria* fruit rot of pomegranate, water, ethanol and acetone extracts of leaves of neem, datura and tulsi and rhizome and bulb extracts of ginger, turmeric, garlic and onion were tested *in vitro* by poison food method at 5, 10, 15 and 20% concentrations. The five extracts found effective *in vitro* were evaluated on pomegranate fruits as pre and post inoculation treatments. All the plant extracts at 20% concentration resulted in significant disease reduction, but maximum reduction was observed with garlic extract followed by turmeric.

Paul and Sharma (2002) investigated the effects of aqueous leaf extracts of neem in inducing resistance against leaf stripe pathogen of barley (*Drechslera gsaminia*). They also recorded phylloplane micoflora of treated and untreated leaf to observe the changes, if any brought about by the treatment of leaves by neem extract. There is also evidence Imran *et. al.*, (2002) that aqueous extracts of *Argemone mexicana* L. greatly suppressed the growth of root infecting fungi *Rhizoctonia solani* and *Fusarium solani,* and at low concentrations also promoted the growth of the tomato plants.

Soil application of Karanj (Pongamia glabra) and groundnut (*Arachis hypogaea*) takes and foliar spray of karanj and subabul (*Leucaena leucocephala*) leaf extracts were evaluated separately (10 and 5%) and in integration (5 and 2.5%) against web blight of urb and mung bean caused by *Thanatephorus cucumeris*. Soil application of karanj cake (2.5%) with spraying of karanj leaf extract (2.5%) followed by karanj cake (2.5%) application with. Spraying subabul leaf extract (2.5%) and only soil application of karanj cake (5%) showed the best performance as they increased seed germination and grain yield and mung bean and decreased seedling mortality and disease intensity. Karanj leaf extract showed superiority over subabul leaf extract in all respect. Groundnut cake at 10% dose inhibited the seed germination and caused maximum mortality where as at low dose (5 and 2.5%) its performance was satisfactory, but it was inferior to karanj cake. In general, lower dose of soil cake showed superiority over higher dose (Dubey, 2002).

Accumulation of total and O-dihydroxy phenols in three maize varieties (MalaN, Ganga-5 and VL-42) infected with *Helminthosporium maydis* and *H. turcicum* was recorded as compared to their healthy counterparts. Reactions of these varieties to both pathogens varied significantly in terns of accumulation of phenolics. Ganga-5 showed three fold increases in phenolics content due to infection by *H. maydis* while double amount of total phenol was recorded in VL-42. *H. turcicum* induced

maximum amount of phenolics in variety VL-42 followed by Ganga-5 and Malan. An increase in the activity of peroxidase, polyphenol oxidase, IAA-oxidase were noticed in all the three varieties of maize under infection of *H. maydis* and *H. turcicum*. The results have suggested that the accumulation of phenolics were higher in resistant varieties like 'Ganga-5' and 'VL-42' as compared to susceptible Malan. Corresponding increase in the activity of oxidative enzyme suggested active metabolic reaction of the host to the pathogenesis and their possible role in a increased level of phenolics. (Sukhwal and Purohit 2003)

The chemical composition of ethanol extracts from a Brazilian (Et-Br) and a Bulgarian (Et-Blg) propolis, were found to have microbicidal activity against a number of bactria and fungi such as *Candida albicans, Sporothrix schenckii* and *Paracoccidioides brasiliensis* (Salomao *et. al.*, (2004). Ether and chloroform extracts and oils of *Curcuma longa* have antifungal effects. Crude ethanol extract also possesses antifungal activity. Turmeric ail is also active against *Aspergillus flavus, A. parasiticus, Fusarium moniliforme* and *Penicillium digitatum* Salomao, *et. al.*, (2004).

Meena *et. al.*, (2004) reported that aqueous bulb extract 1% (w/v) of *Allium sativum* and leaf extract of *Acacia nilotica* caused significant reduction in mycelial growth of *Alternaria brassicae.* Application of bulb extract of *Allium sativum* in mustard plant resulted in highest seed yield at Sewar in 2001-2002. Application of bulb extract of *Allium sativum* at 45 and 75 d.a.s. resulted in lowest blight severity on leaves and pods and also resulted in highest seed yield.

Plant extract from *Aloe barbadensis* provided a total inhibition of *Phaecidiopycnis pili* (Fuckle) Weindlmayr. Extracts from *Tagetes minuta, Mentha piperita* and *Pelargonium graveolens* were significantly effective. Post-harvest treatment of pears in tea extracts had a significant effect in reducing stem end rot by *P. piri.* Plant extracts from *A. barbadensis* was superior to rest of the test botanicals both as pre and post inoculation treatment (Sharma *et. al.*, 2004). Pears treated either

at 6 h before or after inoculation provided better protection and further delay in dip treatment or inoculation resulted in marked increase in rotting. Test plant extracts proved better as protectant rather than eradicants.

Zope and Thrimurty (2004) reported that botanical pesticides Neemazal (0.3%), Wanis (0.3%), Achook (0.5%), Neem gold (0.3%) increase the percent seed germination of rice varieties, Mahamaya, IR 36 and Chapti. Significant increase in root and shoot lengths were induced (except Wanis 0.5%) by these botanicals. The seedling vigour index also increased in Neemzal (0.3%), Achook (0.5%), Wanis (0.3%), and Neem gold (0.3%) and in control fungicide carbendazim (0.1%) treatment.

Walnut (*Juglance regia* L.) hull extract was evaluated dry walnut kernels viz., *Aspergillus flavus*, *Penicillium citrinum*, *Cladosporium cladosporiodes*, *Alternaria alternata*, *Fusarium maniliforme* and *Curvularia lunata* adopting. Poisoned food technique using potato dextrose agar medium at 25 ± 2^0C. Mycelial growth assessed in terms of colony diameter after 21 days of incubation in darkness was totally incubated in *C. cladosporiodes*, *F. maniliforme*, and *C. lunata* by walnut exreact at 1500 ppm and in the other three species At 2000 ppm. Mean conidial germination of *A. alternata* assessed by adopting hanging drop method at ambient temperature after 12 h was at least at 15000 ppm (1%), and at 1000 ppm (8%) in walnut extract, when compared to check (70%). Effect of three concentration of walnut hull extract 1000, 1500 and 2000 ppm, boric acid 500 ppm and sulphuric acid 1000 ppm in preventing kernel rot under artificial inoculation with *A. flavus* as test fungus was evaluated as pre and post treatment applications at 25 ± 2^0C. A similar and significant effect of walnut extract (2000 ppm) and sulphuric acid (1000 ppm) was observed in preventing rot symptoms, when applied at pre inoculation stage with 4% kernels developing rot compared to 84% in check; the other best treatment was walnut extract applied at 1500 ppm. In case of post application treatment (2000 ppm), walnut extract and

sulphuric acid (1000 ppm) recorded just 7% rot compared to 77% incidence in check (Wattal and Puttoo, 2004).

Aqueous extract of mustard cake (5%), neem cake (1%), pine needles (5%), deodar needles (3%) and neem oil (3%) respectively, led to reduce *in vitro* germination of sclerotia of test pathogen *Sclerotium rolfsii* Sacc. causing seedling blight disease in apple nurseries as compared to control have been reported by Sonali and Gupta (2004). Combination of mustard cake (5%) with neem oil (3%), neem cake (1%) with deodar needles (3%) and neem oil (3%); and mustard cake (5%) with neem cake (1%), pine needles (5%) and neem oil (3%) resulted total inhibition of sclerotial germination.

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

A number of plant species (*Azadirachta indica, Lantana camera, Dryopteris filix-mas*, *Echhornia* sp.) have been reported to possess some natural substances in their leaves which were toxic to foliar fungal pathogens (*Pestalotiopsis theae,*

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