Literature Review

A main factor limiting the yield and commercial value of horticultural crops is their susceptibility to diseases. Brinjal plants are no exception. A number of diseases caused by several pathogens have been reported to occur in brinjal. To control diseases of plants effectively, it is necessary to understand different aspects of host-parasite interactions. Recent advances in plant pathology have paved the way for the development of innovative techniques to manage crop diseases. Molecular biology of pathogenesis and induced systemic resistance has been recognised as the present era of plant pathology (Vidyasekharan, 1988). Besides, biological control and use of botanicals for control of diseases have also gained importance due to the recent global awareness on negetive effect of chemical fungicides and considerable research activity is focussed on this area. At the onset of the present study, it was considered to review the works of the previous workers in a selective manner. The observations are presented briefly in the following paragraphs. For convenience, the observations have been divided into several subtopics which are as follows:

- Diseases of brinjal.
- Diseases caused by *Colletotrichum gloeosporioides.*
- Studies on growth and physiology of the pathogens.
- Antigenic relationship in host and pathogen.
- Plant disease alteration by chemical treatment.
- Disease control by fungicides.
- Disease control by antagonistic organisms.
- Disease control by botanicals.

Diseases of brinjal

With the increase of population, market demand of brinjal is increasing rapidly leading to an increase in cultivation of the crop. This in turn has led to an increase in disease problems associated with the crop. Like many other vegetables, brinjal is also subjected to attack by many fungi, bacteria, viruses and nematodes. Some common fungal diseases of brinjal are damping-off (caused by *Pythium* sp., *Fusarium* sp., *Rhizoctonia solani* etc.), phomopsis blight (caused by *Phomopsis* vexans), anthracnose (caused by *Colletotrichum gloeosporioides*), fruit rot (caused by *Pythium* spp.), southern blight (caused by *Sclerotium rolfsii*), early blight (caused by *Alternaria solani*) etc. Fungal diseases are considered as a major factor that adversely affect successful cultivation of the crop.

Anthracnose disease caused by *Colletotrichum gloeosporioides* (Penzig) Sacchardo is one of the most common fungal diseases of brinjal. The pathogen attacks leaves and fruits and produces typical anthracnose lesions. Characteristic symptoms are sunken black lesions and blackening of the inner tissues of the affected areas. Fernandes *et al.* (2002) reported 34 *C. gloesoporioides* isolates obtained from naturally infected garden-egg, sweet peeper and egg-plant fruits. Wijesekara *et al.* (2005) isolated twenty *Colletotrichum* isolates from different crops comprising of five species *C. capsici, C. dematium, C. falcatum, C. gloeosporioides* and *C. lindemuthianum* from different geographical locations of India. They isolated *C. gloeosporioides* from brinjal plant in Solan region.

Several other pathogens are reported to attack brinjal plants throughout the world causing various types of diseases. Singh (1992) reported that *Phomopsis vexans* produced symptoms like damping off, leaf blight, stem canker, collar rot and fruit rot in brinjal. Panwer *et al.* (1970) described phomopsis leaf blight and fruit root of brinjal caused by *P. vexans* as a serious disease causing 10-25% loss in marketable fruits.

Bletsos *et al.* (1997) reported that *Verticillium* wilt caused by *Verticillium dahliae* Kleb. caused an estimated yield reduction of up to 50%. According to him, it is one of the most destructive disease of eggplant. Symptoms of this disease are yellow bronze wilted areas, mainly between the leaf veins and vascular discoloration. Bueno *et al.* (2000) also conducted studies on *Verticillium* wilt caused by *Verticillium dahliae* on egg plant. Kennet *et al.* (1970) and Kishi (1974) reported that *Fusarium oxysporum* f. sp. *melongenae* induces vascular wilt disease in egg-plant that led to major yield losses in Asian countries. As the symptoms are often confused with those of *Verticillium* wilt, the incidence of *Fusarium* disease is probably underestimated (Stravato *et al.*, 1993).

Elad *et al.* (1993) reported an egg-plant disease caused by *Botrytis cinerea* causing reduction in yield of the crop. During field survey of brinjal crop in Bareilly region in 1996-97, Pandey and Pandey (2001) observed that some fungi were parasitizing either on leaves or fruits. Those fungi are *Cladosporium fulvum, Helminthosporium speciferum, Trichothecium roseum, Fusarium solani, Alternaria tenuis, Choanephora cucurbitarum* and *Curvularia lunata.*

Wilson *et al.* (1996) tested potential aecial hosts of *Puccinia substriata* var. *indica* for resistance and susceptibility to better understand their potential role in

epidemics of pearl millet rusts. They evaluated 31 accessions of *S. melongena,* each collected from a different country, and accessions of 27 other *Solanum* species. Resistance or susceptibility was determined from a natural infection in an isolated field location and inoculations in the greenhouse. All accessions of *S. melongena* were susceptible, except PI 413784 from Burkina Faso and PI 401533 from the Ivory Coast.

Zapata *et al.* (2001) isolated *Rhizoctonia solani* and *Fusarium solani*, causal agents of wilting, root-rot and basal canker from affected brinjal plants. They performed pathogenicity test separately for each fungus. From the results of these tests, they concluded that *F. solani* is the causal agent of eggplant root-rot and wilt, whereas *R. solani* causes basal canker only. Jadon *et al.* (2005a) reported *Scleroyium rolfsii,* causal organism of collar rot of brinjal as one of the limiting factor in its successful cultivation.

Other than fungi, some nematode, bacteria, MLO and viruses were also reported. Mittal and Goswami (2001) observed a severe infestation of *Melodogyne incognita* root-knot nematode in association with of fungus *Fusarium solani* on brinjal during a survey of vegetable field carried out around New Delhi in August, 1999.

The bacteria *Pseudomonas solanacearum* Smit. has been reported to cause wilt in brinjal (Sharma *et al.,* 1995). Chaudhary and Sharma (2000) screened nine genotypes of brinjal (*S. melongena* L.) for the incidence of bacterial wilt and attack of fruit and shoot borer in mid hills of Himachal Pradesh, India. They observed Arka keshav, Arka neelkanth, Arka nidhi and SM 6-6 to be resistant to bacterial wilt.

Boiteux *et al.* (1994) reported a disease of brinjal associated with a mycoplasma like organism (MLO). Symptoms characterized by teratological changes in the flowering structure, reduction and malformation of leaves, proliferation of lateral buds and an overall plant starting. Gupta *et al.* (1992) reported that a strain of tobacco mosaic virus causing necrotic mosaic disease on brinjal.

Diseases caused by *Colletotrichum gloeosporioides*

The genus *Colletotrichum* was established in 1831 as a group of fungus with hyaline, curved, fusiform conidia and setose aecrvuli (Jeffries *et al.,* 1990). *C. gloeosporioides* can be segregated into many physiologically biologically or genetically differentiated types (Wheeler and McGahen, 1952; Simmonds, 1965). *C. gloeosporioides* is reported as an economically important pathogen that cause

substantial yield loss by affecting both fruit and vegetative parts in many plant species. When multiple hosts such as mango, avocado, coffee, papaya and citrus are grown in close proximity, huge losses are reported (Freeman and Shabi, 1996; Freeman *et al.*, 1998). However some others (Alahakoon *et al.*, 1994; Freeman *et al.;* 1996 and Adaskaveg and Hartin, 1997) observed that *C. gloeosporioides* isolates obtained from a specific host were more pathogenic on that crop than on other.

Gorter (1982) reported 22 hosts of *C. gloeosporioides* in South Africa including sisal, coffee, walnut, grapevine and several ornamental plants. Disease caused by *C. gloeosporioides* include anthracnose, die-back, root rot, leaf spot, blossom rot and seedling blight on a wide range of crop including avocado, almond, peach (Freeman *et al.*, 1998), peppers (Manandhar *et al.*, 1995a), papaya (Dickman, 1994), mango (Ploetz, 1994), citrus (Timmer *et al.*, 1994), rubber trees (Brown and Soepena, 1994), passion fruit (Jeffries *et al.*, 1990) and strawberry (Denoyes and Bandry, 1995).

Tebeest (1988) reported several species of *Aeschynomene, Lathyrus, Lupinus*, *Vicia faba* and *Pisum sativum* cultivars susceptible to *C. gloeosporioides* f. sp. *aeschynomene* and lesions developed on leaflets, petioles and stems. Chakraborty and Jones (1993) reported that anthracnose on *Stylosanthes* spp. results in a reduction in dry matter yield and seed production, seriously limiting utilization of these pasture legumes. Alahakoon and Brown (1994) isolated *C. gloeos*porioides isolates from 23 different fruit crops in Srilanka and recommended for protection of the seedling from major sources of the pathogen.

Fruit rot disease of Jack-fruit caused by *C. gloeosporioides* in Bangladesh has been reported (Basak, 1995). Black spot of basil incited by *C. gloeosporioides* has been reported in Italy by Gullino *et al.* (1995). Amusa and Alabi (1996) observed that *C. gloeosperioides* isolated from infected leaves and pods of *Gliricidia sepium* induced necrotic lesions on leaves of cassava, melon, cowpea, soybean, yam, tomato and pepper. Susceptibility of these plant to *Colletotrichum* sp. from *G. sepium* therefore poses a threat to further usage of *G. sepium* in alley cropping. Seedling blight and damping off of papaya caused by *C. gloeosporioides* in Hawaii has been reported (Uchida *et ai.*, 1996).

Pathogenicity of *C. gloeosporioides* have been studied by experimental infection of the pathogen in different crops by several workers. While evaluating seven methods to inoculate brinjal fruits by four isolates of *C. gloeosporioides,* Madeira

and Reifschneider (1987) suggested that sub epidermal injection of 0.1 ml of conidial suspension utilizing a hypodermic syringe was most effective.

Boland *et al.* (1995) assessed infected stems of *Stylosanthes scabra* over two consecutive dry seasons to determine if *C. gloeosporioides* survived as conidia on lesions formed during the previous wet season or as hyphae within infected tissue and found that it can survive and could produce an epidemic once favorable conditions for dispersal and infection occurred.

Fransisco-Neto *et al.* (1995) studied the influence of leaf age, leaf surface wounding before inoculation and light during the initial incubation period of infection of *Passiflora alata* and *P. edulis* f. *Flavicarpa* by two isolates of *C. gloeosporioides*. The disease symptoms were reproduced only when the pathogen was inoculated on the leaves after wounding. Of the five leaves studied, the third and the fourth, counted from the apex were the most susceptible. Disease severity was higher when incubation of the inoculated leaves or plants was carried out for 48 h in dark and high relative humidity.

Infection of mango fruit by C. *gloeosporioides* was studied by Dinh *et al.* (2003) using artificial inoculation. They observed that under optimal condition (95-100% relative humidity, 25 °C), germination and appressoria formation started at 12 h and 14 h respectively, after deposition of conidia on the peel After 48 h, 60% of the fungal propagules present were appressoria.

The infection process of *C. gloeosporioides* was examined on papaya fruit by Casarrubias-carrillo *et al.* (2002) and showed that conidial germination occurred between 48 and 72 hour after inoculation. Kumar *et al.* (2002) compared the morphology of conidia and growth rate of isolates of the fungus causing raised, anthracnose and papery lesions of *Colletotrichum* leaf disease on rubber. Growth rate of isolates from raised lesions was significantly lower than that from anthracnose and papery lesions.

Studies on Growth and physiology of the pathogen

Knowledge of fungi and fungal processes has immense importance for control of fungal pathogen. Hence understanding of the physiological processes of a pathogen is also important while understanding the plant-pathogen relationship and the reasons of pathogenicity. Studies on the growth characteristics and sporulation are therefore, required before formulation of any type of control measure.

Thakare and Patil (1995) described *C. gloeosporioides* as a causative agent for leaf blight disease of *Chrysanthemum*. Mycelia of the fungi were septate, hyaline branched, vacuolated and produced cylindrical, single celled hyaline conidia and dark brown acervuli with septate setae. Bean meal agar, potato dextrose agar, and Richard's agar culture media, mannitol, dextrose, D-glucose carbon sources and DL-threonium, L-leucine and ammonium phosphate nitrogenous compounds supported good growth. The optimum temperature and pH for growth were 25-30 ^{0}C and 4.1-6.8 respectively.

Manandhar *et al.* (1995b) observed conidial germination and appressorial formation of *C. gloeosporioides* on pepper fruits and in association with some organic and inorganic compounds. They noticed that conidial gernination was significantly higher for both the fungi as concentration of either sucrose or KCl increased. Appressorial formation of *C. capsici* was highest when sucrose was 10 mM and for *C. gloeosporioides* at 0.01 mM. Appressorial formation was reduced and mycelia formed for both the fungi at higher sucrose concentration. Among six compounds tested, spore germination and appressoria formation was significantly increased when tested with $CaCl_2$ and sucrose while KCl is an intermediate inducer. Appressorial formation of *C. capsici* was completely inhibited by tricyclazole and stimulated by fthalide and isoprothiolane while appressorial formation of *C. gloeosporioides* was completely inhibited and stimulated by isoprothiolone and tricyclazole respectively. They suggested that inorganic and organic compounds that affect conidial germination and appressorial formation may play a role in preinfection process of *Colletotrichum* spp. on pepper fruits.

Conidial germination and appressoria formation of *Glomerella cingulata* causing the brown blight disease of tea were studied *in vitro*. Spore germination and appressoria formation were optimum at a temperature of 25 ^{0}C, pH 5.0, a 7 hours light/day regime and a 24 h incubation period. At a concentration of conidia of 1200/ 10 days old culture, *G. cingulata* exhibited a maximum germination and appressoria formation. Maximum production of lesions was also evident on detached tea leaves at this spore concentration and in diffuse light. Diffusates of phenolic nature collected from tea varieties susceptible and resistant to *G. cingulata* inhibited spore germination and appressoria formation. Diffusates from resistant varieties were more fungitoxic than from susceptible varieties (Chakraborty *et al.*, 1995).

Kuo (1999) studied the germination and appressorium formation in *C. gloeosporioides* and observed that the size of the conidia of the fungus ranged between 10.7-24.1 µm x 4.0-6.7 µm (15.4 µm x 4.8 µm). They studied the conidial germination and appressorium development by a two step method during a nine hour period. At first mango decoction was added as supplemental nutrients into the spore suspension in order to trigger germination and this was followed by depletion of the mango decoction to induce the formation of appressorium. It was noticed that in mango decoction, the germlings formed long germ tubes and abundant hyphal branches without forming appressorium during 9 hour period. Appressorium formed mostly at the end of the long germlings or at the end of the hyphal branches if the incubation time was extended. When the spore suspension was first incubated in sterilized mango decoction for two hours and the decoction then removed and replaced with ddH_2O, the percentage of appressorium formation was enhanced dramatically.

Physiological processes of several other pathogens have also been studied by several authors. Saha and Chakraborty (1990) reported the effect of some environmental factors on spore germination of *Bipolaris carbonum* Nelson, a pathogen of tea. Under identical humid condition, the optimal concentration of spores, temperature, and pH for spore germination were recorded to be 11.2 x 10^5 spores ml^{-1}, 32 ^{0}C and pH 6.75 respectively. Temperature pretreatment of 50 ^{0}C for 20 minutes significantly reduced spore germination, whereas pretreatment at 0 ^{0}C for even 12 hours had no effect on spore germination and germ tube elongation. Light condition and age of the conidia did not affect the spore germination.

Achar (2000) showed that the mycelial growth of three isolates of *Stenocarpella maydis* from maize seeds increased progressively from 15 ^{0}C to a maximum of 30 ^{0}C. The maximum number of conidia was produced by all three isolates after 8 days of incubation at temperatures ranging from 22 ^{0}C to 30 ^{0}C. Harden *et al.* (2002) reported the effects of temperature and pH on the growth and sporangial production of isolates from each of the four known races of *Phytophthora clandestine* Taylor, Pascoe & Greenhalgh. Mycelial growth occurred at temperature from 10-30 ^{0}C and pH 3.5-9.0 with highest growth rates of all isolates being at 25 ^{0}C with a pH of 6.0 – 6.5. Sporangial production was greatest between 20 ^{0}C-25 ^{0}C and pH 5.0 – 7.0 with all races. However, sporulation occurred over a temperature range from 10-30 ^{0}C and from pH 4.0-9.0 with all isolates. There were no consistent

differences between the four pathogenic races of *P. clandestine* in their relative growth rate or extent of sporangial production over a range of temperatures and pH values.

Jash *et al.* (2003) observed the effect of different culture media, pH and carbon sources on growth and sporulation of *Alternaria zinniae* Pape causing leaf and flower blight of marigold. They reported that among the different culture media, maximum growth and sporulation of the fungus was obtained in both solid and liquid form on leaf extract dextrose followed by potato dextrose medium. The optimum pH for growth of the pathogenic fungus was found in the range of pH 6.0-6.5. Maximum growth and sporulation of this fungus were obtained with sucrose as carbon source followed by starch and maltose.

Detached strawberry leaves were inoculated with the powdery mildew pathogen, then held in controlled environments of constant temperature (4-36 °C) and relative humidity (RH, 32-100%) representing the range of these variables observed under California commercial production conditions. Percent germination and lesion expansion rate were determined by destructive sub sampling over time. Conidia germinated at all temperatures by 6 hours and reached a maximum by 48 h, with the optimum near 20 °C. Lesions were marked with the aid of a microscope and measured by computer-assisted image-analysis to determine expansion rate. Maximal rates occurred at 25 °C. Several growth models were fit to the expansion rate data with high significance. Predicted optima from these models ranged from 22 °C-27 °C and / or 17-27 mm Hg VP (water @ 100% RH). Neither RH, partial vapor pressure of water (VP (water)), nor vapor pressure deficit (VPD) correlated with lesion expansion rate, adding to studies minimizing the importance of RH and VPD as determinants of asexual phase powdery mildew growth other than specifically at spore germination (Miller *et al.*, 2003).

Amborabé *et al.* (2005) studied on environmental factors and the nutritional requirements on *Eutypa lata* causing *Eutypa* dieback (dying arm disease, eutypiosis), a devastating disease in many grape-producing areas. This work shows that the isolated strain of *E. lata* was able to grow in a large temperature range (2-30 °C). However, a higher temperature (35 °C) presented inhibitory effects on mycelial growth. *E. lata* was able to use various osidic molecules (C5, C6, C12, C18, C24, and starch), showing thus a large adaptation to the carbon source supplied. As nitrogen source, it used salts and numerous natural amino acids. A significant result was obtained with cysteine presenting obvious antifungal properties. This effect can further be used with the aim of setting up a curative treatment of the disease.

Antigenic relationship in host and pathogen

The discovery of the precipitation reaction arose from the demonstration of precipitates formed when the cell free filtrates by typhoid cultures were mixed with corresponding antiserum. Precipitation reactions in which antigens and antibodies diffuse through and react in semisolid matrices (i.e. agar gel) have become essential tools in biochemical analysis (Clausen, 1969). A phenomenon of common antigenic relationship has received attention during the last three decades. The presence of common antigens among closely related organisms or even among more distantly related organisms is surprising. Studies on both animal and plant hosts and their parasites and pathogens suggest that whenever an intimate continuing association of cells of host and pathogen occurs, partners of this association have a unique serological resemblance to one another involving one or more antigenic determinants. In plants, several studies have shown that the possibility of susceptibility is greater when antigenic similarity is greater. Thus the concept of common antigens between a plant and a pathogen is a notable feature in determining resistance or susceptibility. It is believed that the degree of compatibility and susceptibility of a plant cultivar to a pathogen is correlated to levels of common antigens present in both host and pathogen (Alba *et al.,* 1983; Purkayastha and Banerjee, 1990; Chakraborty and Saha, 1994; Ghosh and Purkayastha, 2003; Kratka *et al.*, 2002; Musetti *et al.*, 2005; Eibel *et al.*, 2005; Dasgupta *et al.*, 2005).

Crossed immunoelectrohoresis (CIE) techniques were used by Ala-El-Dein and El-Kady (1985) to show that the tested isolates of *Botrytis cinerea* were serologically different; some antigens were specific for each isolate. Isolate no.1 of *Botrytis cinerea* had four specific antigens; although these antigens were absent in other isolates. At least sixteen antigens were common in the isolates tested. Some isolates were serologically similar when tested by double gel diffusion test while they were distinguishable when CIE techniques were used. Numbers of precipitin peaks obtained with CIE techniques were more than double the number of precipitin lines detected with double gel diffusion test. Results revealed that CIE techniques could be used as valuable analytical tools in resolving the spectrum of antigens present, in *Botrytis cinerea* isolates. By using CIE techniques antigenic structures of *B. cinerea, B. tulipae, B. paeoniae* and *B. allii* isolates were also compared. Antisera against antigens of these isolates gave 24, 15, 20 and 14 precipitin peaks respectively, when analyzed in homologous reactions.CIE with an intermediate gel and CIE with

antibody absorption *in situ* revealed that each isolate was serologically different from the other and has species-specific antigens. *B. cinerea* has eight distinct antigens which distinguished them from the other species of *Botrytis*.

Amouzon-Alladaye *et al.* (1988) reported that antiserum obtained against the mycelial proteins of a strain of *Phytophthora fragariae* could detect 11 different strains of *P. fragariae* in pure culture and pathogen in naturally infected or inoculated roots. The antiserum failed to react with 18 fungal species isolated from underground parts of strawberry but reacted with some strains of *P. cactorum*, which parasitized only rhizomes but not roots. In inoculated strawberry roots, *P. fragariae* was detected reliably by ELISA several days before oospores were found and before symptoms developed.

Mohan (1988) evaluated antisera raised against pooled mycelial suspensions from five isolates (Pf-1, Pf-2, Pf-3, Pf-10 and Pf-11) representing five physiologic races of *Phytophthora fragariae* for detecting the red core disease of strawberries by enzyme-linked immunosorbent assay (ELISA). Cross-reactivity of antiserum raised against *P. fragariae* with other *Phytophthora* as a genus detecting antiserum has also been reported by Mohan (1989). Antiserum of *P. fragariae* isolates (Anti-PfM) reacted strongly with antigens from several *Phytophthora* species. Some cross-reaction with antigens from *Phythium* species was decreased by fractionating on an affinity column of sepharose 4 B bound to extracts of *Fragaria vesca* roots infected with *P. fragariae*. The affinity purified anti PfM retained its high cross-reactivity with the various *Phytophthora* species. Anti-PfM could not be made specific for *P. fragariae* because it was raised against components shown to be antigenically similar in all *Phytophthora* species tested. However, immunoblotting with the affinity purified anti-PfM produced distinct patterns for *P. fragariae*, *P. erythroseptica* and *P. cactorum*.

Competitive types of two novel enzyme-linked immunosorbent assays (ELISA) for specific detection of *Fusarium oxysporum* f. sp. *cucumerinum* as well as for general detection of ten strains of common *Fusarium* species has developed by Kitagawa *et al.* (1989) that show specific pathogenicities to different plants. Antiserum against a strain of *Fusarium oxysporum* f. sp. *cucumerinum* (F 504) was elicited in rabbits and a highly specific, sensitive and accurate ELISA for the homologous strain was developed by using the antiserum with β-D-galactosidase-labelled anti-rabbit IgG as a secondary antibody and cell fragments of the strain attached to amino-

Dylark balls as the solid-phase antigens. This assay was specific for strain F 504 and showed little cross-reactivity with nine other strains of *Fusarium* species including strain F 501 of *F. oxysporum* f. sp. *cucumerinum* (FO). F 501 possesses pathogenicity against cucumber similar to that of strain F 504, although slight differences have been observed between these two strains regarding their spore formation and pigment production. Cell fragments of strain F 501 absorbed on amino-Dylark balls possessed sufficient immune activity against anti-FO antibody to use in a heterologous ELISA for general detection of ten *Fusarium* species with high sensitivity.

Purkayastha and Banerjee (1990) studied common antigenic relationships between soybean and *Colletotrichum dematium* var. *truncata* using immunodiffusion, immunoelectrophoresis and indirect ELISA technique. Cross-reactive antigens were detected between susceptible soybean cultivars and the virulent strain of *C. dematium* but no cross-reactive antigen was detected between soybean cultivars and avirulent pathogen (*C. dematium*) or non-pathogen *C. corchori.* Results of immunodiffusion and immunoelectrophoresis showed absence of common antigen between resistant cultivars (UPS M-19) and the pathogen, while the results of indirect ELISA indicated the presence of common antigen between the two at a very low level. They compared antigenic patterns of untreated and cloxacillin treated soybean leaves which induced resistance of soybean against anthracnose disease. The disappearance of one antigen from cloxacillin treated leaves of susceptible soybean cv. "Soymax" was correlated with alteration of disease reaction.

Daniel and Nilsson (1991) raised polyclonal antiserum against mycelial extracts of the rot fungus *Phialophora mutabilis* which reacted strongly with its homologous antigen and cross-reacted strongly to moderately with six other *Philosophora* soft rot spp. in ELISA. With the help of an indirect ELISA technique, Ricker *et al.* (1991) showed that increase in cross-reactivity in late bled antiserum (anti-Bc IgG), raised against water soluble antigens from *Botrytis cinerea* corresponded with an increase in the overall serum titers for anti-Bc IgG to antigens of *B. cinerea.* Polyclonal antiserum of mycelial proteins of *Verticillium dahliae* reacted positively with 11 of 12 isolates of *V. dahliae* from potato, cotton and soil but negatively with one isolate from tomato in indirect ELISA (Sundaram *et al.*; 1991). He also found positive results in detecting, *V. dahliae* and *V. albo-atrum* from infected roots and stems of potato in a double antibody sandwich enzyme linked immunosorbent assay (DAS-ELISA).

Lyons and White (1992) compared results of conventional isolation techniques for *Pythium violae* using polyclonal antibodies raised to *P. violae* or *P. sulcatum* in competition ELISA. A double antibody sandwich ELISA test was developed for the detection of *Pseudocercosporella herpotrichoides* using a highly specific monoclonal antibody pH 10 as the capture antibody and genus specific polyclonal rabbit antisera as test antibody by Priestley and Deway (1993). The assay recognized extracts from plants both artificially and naturally infected with *P. herpotrichoides*, at least three-fold higher absorbance values with extracts of *P. herpotrichoides* infected tissue than with extracts from healthy tissues. The high molecular weight fraction of immunogen (mycelial extracts) was shown to contain cross-reactive antigens; it induced antiserum in mice that cross-reacted with the other stem base fungi even at high dilution.

Chakraborty and Saha (1994) compared antigens obtained from tea varieties, isolates of *Bipolaris carbonum* and non-pathogens of tea (*Bipolaris tetramera* and *Bipolaris setariae*) by immunodiffusion, immunoelectrophoresis and enzyme linked immunosorbent assay to detect cross reactive antigens (CRA) shared by the host and the parasite. CRA were found among the susceptible varieties (TV 9, 17 and 18) and isolates of *B. carbonum* (BC-1, 2, 3 and 4). Such antigens were not found between isolates of *B. carbonum* and resistant varieties (TV 16, 25 and 26), non-pathogens and tea varieties, as well as non-pathogen and *B. carbonum*. CRA were also found concentrated mainly around the epidermal cells of leaves of TV-18 in cross section following indirect staining of antibodies using fluorescein isothiocyanate (FITC). They indicated the presence of CRA in the young growing hyphal tips and conidia following treatment with antisera of leaves (TV-28) and indirect staining with FITC.

Brill *et al.* (1994) prepared polyclonal antibodies (PAbs) produced against culture filtrates and mycelial extracts immunogen from the soybean (*Glycine max*) and fungal pathogen *Phomopsis longicolla*. Polyclonal antibodies were purified to the immunoglobulin fraction and tested in indirect ELISA and in direct DAS-ELISA the PAbs raised to culture filtrate were more specific but less active in binding to members of Diaparthe-*Phomopsis* complex than were those to mycelial extract immunogen preparation. DAS-ELISA was more specific and 100-fold more sensitive in detecting members of the complex than was indirect ELISA. Variability in specificity between different PAbs was lower in DAS-ELISA compared to indirect ELISA.

White *et al.* (1994) found an extensive cross reaction, when two monoclonal and three polyclonal antisera, raised against the cell wall/membrane fractions of *Pythium violae* and *P. sulcatum* screened with a collection of 40 isolates of the genus *Pythium* including 20 species and the H-S group. However, when the binding of the antibodies was assessed in an enzyme-linked immunosorbent assay (ELISA) using cytoplasmic fraction antigens, the combined recognition patterns produced profiles unique to each species.

Polyclonal antibodies against prehelminthosporol, a phytotoxin produced by the plant pathogenic fungus *Bipolaris sorokiniana* were raised in rabbits immunized with a prehelminthosporol–hexon conjugate. The IgG was isolated from the serum and the specificity of the purified antibodies was investigated with indirect ELISA. The antibodies bound both to free prehelminthosporol and to a prehelminthosporol-bovine serum albumin conjugate bound to micro titer wells. The antibodies showed less affinity to structurally related compounds from the fungus. No cross-reactivity was shown for proteins extracted from mycelium of *B. sorokiniana*. Low-temperature preparation techniques for electron microscopy were used in combination with immunogold labeling for localization of prehelminthosporol in hyphae and germinated conidia of *B. sorokiniana*. A low level of labeling was obtained throughout the cytoplasm, and the main labeling was seen in membrane-bound organelles identified as Woronin bodies (Akesson *et al.*, 1996).

Polyclonal antisera against whole (coded: 16/2) and sonicated (coded: 15/2) resting spores of *Plasmodiophora brassicae were* raised as well as soluble components prepared by filtration and ultracentrifugation (coded:SF/2), cross-reactivity of all three antisera with a range of soil fungi, including *Spongospora subterranean* was low (Wakeham and White, 1996). Test formats including western blotting, dipstick, dot blot, indirect ELISA and indirect immunofluorescence were assessed for their potential to detect resting spores of *P. brassicae* in soil. Dot blot was least sensitive, with a limit of detection level of $1x10^7$ resting spores/ g in soil. With western blotting, the lower limit of detection with antiserum 15/2 was $1x10^5$. This antiserum showed the greatest sensitivity in a dipstick assay, indirect ELISA and indirect immunofluorescence, for all of which there was a limit of detection of $1x10^2$. Of the assays tested, indirect immunoflourescens appears to be the most rapid and amenable assay for the detection in soil low levels of resting spores of *P. brassicae* in soil.

Four polyclonal and two monoclonal antibodies were prepared and tested to detect *Colletotrichum acutatum*, a quarantine pathogen of strawberry by Kratka *et al.* (2002). They observed that only one polyclonal antibody was sensitive enough to recognize the pathogen. The antibody was genus specific that did not cross react with several other fungal pathogens of strawberry. They also detected *C. acutatum* by Plate trap antigen enzyme linked immunosorbent assay (PTA-ELISA), dot blot and immunoprint in roots, crowns, petioles and fruits in the latent age of the disease after artificial infection of strawberry (cvs. Elsanta, Vanda and Kama). Ghosh and Purkayastha (2003) used polyclonal antibodies and antigens of ginger and *Pythium aphanidermatum*, a causal organism of rhizome rot disease for early diagnosis of rhizome rot disease of ginger. They detected *P. aphanidermatum* in ginger rhizome after eight weeks of inoculation by agar gel double diffusion and immuoelectrophoretic tests, but only one week after inoculation by indirect ELISA.

Eibel *et al.* (2005) raised polyclonal antibodies against mycelium from the logarithmic growth phase of a shake culture of *Ustilago nuda*, and developed a double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) with biotinylated detection antibodies. Other species of *Ustilago* reacted with the antibodies. Cross-reactivity was highest with *U. tritici*. No signal was obtained with the tested isolates of *Tilletia, Rhizoctonia, Pythium* and *Fusarium*. With naturally infected barley seeds, the results of the ELISAs were always in good agreement with those obtained with the routinely used seed embryo test. They suggested that potential fields of application of the ELISA include the early prediction of the efficacy of protection agents, e.g. in screenings for seed treatments, the elucidation of the biology of the fungus and characterization of resistance mechanisms.

Besides fungus, virus (Petrunak *et al.*, 1991; Abou-Jawdah *et al.*, 2001; Hema *et al.*, 2001; Devaraja *et al.*, 2005; Chen *et al.*, 2005) and bacterial (Mazarei and Kerr, 1990) pathogens of plants could be successfully detected by various ELISA formats.

Indirect ELISA was used to monitor the distribution of Mycoplasma like organism (MLO) in the experimental host *Vicia faba*. Post-embedding colloidal gold indirect immunolabelling was developed to identify, without ambiguity, the various forms of MLO cells in the different infected parts of the plant by transmission electron microscopy. Silver enhancement of the gold probe gave accurate histological and cellular localization of MLOs in tissue sections, by light microscopy. Both ELISA and

immunolocalization first detected MLO in roots 17 days after inoculation with infectious leafhoppers (Lherminier *et al.*, 1994).

Double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) and direct antigen coating (DAC)-ELISA tests were evaluated for detection of sugarcane streak mosaic virus (SCSMV-AP). The virus was detected up to 1/3125 and 1/625 dilutions in infected sugarcane leaf, 5 µl and 10 µl/well in sugarcane juice, 1/3125 and 1/625 dilutions in infected sorghum leaf and 10 ng and 50 ng/ml of purified virus in DAS-ELISA and DAC-ELISA tests, respectively (Hema *et al.*, 2001). Abou-Jawdah *et al.* (2001) in a survey detected potato virus Y (PVY), potato virus A (PVA), potato virus X (PVX), potato virus M (PVM), potato virus S (PVS) and potato leaf roll virus (PVX), potato virus M (PVM) potato virus S(PVS) and potato leaf roll virus (PLRV) by ELISA from potato fields in the two main production areas of Lebanon, the Bekaa and Akkar plains.

Wang *et al.* (2006) observed that an indirect enzyme-linked immunosorbent assay (ID-ELISA) protocol is capable of detecting *Rice black-streaked dwarf virus* (RBSDV) in very dilute wheat leaf extracts. Based on the results, they concluded that efficient and economic detection of RBSDV can be performed routinely using polyclonal antiserum against outer capsid protein (P10) expressed in prokaryotic cells.

Immunolocalization is a powerful tool for cellular location of different proteins or antigens. This method has been utilized for location of CRA in tissues of the host and also in pathogen. In a study, DeVay *et al.* (1981) inoculated young cotton (Acala 2) roots with antiserum to *Fusarium oxysporum* f. sp. *vasinfectum* and stained with FITC conjugated, antirabbit globulin-specific goat antiserum. Strong fluorescence was observed at the epidermal and cortical cells, and the endodermis and xylem tissues that indicated a general distribution of the CRA determinants in roots. Chakraborty and Saha (1994) labelled polyclonal antiserum with FITC and found that CRA between tea leaves and the pathogen *Bipolaris carbonum* was present mainly around the epidermal cells and mesophyll tissues of leaves of the host and in hyphal tips and in patch like areas on conidia and mycelium of the pathogen. Dasgupta *et al.* (2005) also studied the location of CRA in tea leaves that were treated with antiserum raised against the pathogen *C. gloeosporioides*. Indirect labelling of the antibodies with FITC showed that CRA was concentrated mainly in the epidermal cells and also spread throughout the cortical cells.

Present day immunolocalization studies are performed using immunogold labelling which is successfully used for electron microscopy (Lee *et al.*, 2000; Trillus *et al.*, 2000; Nahalkova *et al.*, 2001; Kang and Buchenauer, 2002 and Wang *et al.*, 2003). For light microscopy, silver enhancement is done after gold labelling (Santen *et al.*, 2005 and Saha *et al.*, 2006). However immunogold labeling has not yet been utilized for location of CRA in compatible host and pathogens. Kuo (1999) used a gold sol which was found to be able to localize the ECM (Extra cellular matrix) of *C. gloeosporioides* very well. In the case of *C. gloeosporioides*, the ECM secreted out from conidium just before germination took place. The area that ECM covered was wide-spread and could reach up to several times the spore width. With gold sol, the composition and nature of the ECM could be easily identified using cytochemical and biochemical approaches.

Lee *et al.* (2000) reported that immunogold labelling showed specific labelling of chitinase in the interaction of pepper stems with *Phytophthora capsici.* Chitinase was found on the cell wall of the oomycete in both compatible and incompatible interactions at 24 h after inoculation. In particular, numerous gold particles were deposited on the cell wall of *P. capsici* with a predominant accumulation over areas showing signs of degradation in the incompatible interaction. Chitinase labelling was also detected in the intercellular space and the host cytoplasm. However, healthy pepper stem tissue was merely free of labelling.

Nahalkova *et al.* (2001) performed immunolocalization experiments for locating *Pinus nigra* ARN lectin (PNL). They observed that the protein was mainly located on the cytoplasmic membranes and on the primary cell walls. In infected seedlings (infected by *Heterobasidium annosum* and *Fusarium avenaceum*), a strong labelling of hyphal materials with PNL antisera was recorded only at the early stages of infection but not at the later stages of hyphal invation.

Kang and Buchenauer (2002) raised two antisera against acidic β-1,3-glucanase and acidic chitinase from tobacco and used to investigate the subcellular localization of the two enzymes in *Fusarium culmorum*-infected wheat spike by means of the immunogold labelling technique. These studies demonstrated that the accumulation of the enzymes in the infected wheat spikes differed distinctly between resistant and susceptible wheat cultivars. Wang *et al.* (2003) used immunogold labelling technique for localization of PB90 which is a novel protein elicitor secreted by *Phytophthora boehmeriae*. The anti-90 kDa protein antiserum

was used for immunocytolocalization studies of PB90 elicitor, on the mycelium and encysting zoospores of *P. boehmeriae* grown *in vitro* in liquid culture and also in solid medium. In liquid culture, immunogold labelling was located mainly in the cell wall. In solid medium gold particles were observed not only in the cell wall, but also in the solid medium near the hypha.

Plant disease alternation by chemical treatment

Resistance to plant disease is often specific and metabolites and receptors controlling to this specificity may have specific structures. However, simple, structurally unrelated compounds induce systemic resistance in unrelated plants to diverse pathogens including fungi bacteria and viruses. Both resistance and induced systemic resistance (ISR) are associated with the rapid accumulation of the same structurally unrelated putative defense compounds that have diverse functions (Kuc, 2001). Several physical and chemical agents (X-rays, UV-rays and biological agents) have been used for alteration of disease reaction. Systemic acquired resistance (SAR) of plants against pathogens is a widespread phenomenon with respect to the underlined signaling pathways as well as to its potential use in plant protection. Plants respond with a salicylic acid dependent signaling cascade which leads to the systemic expression of a broad spectrum and long-lasting disease resistance (Heil and Bostock, 2002). Salicylic acid is a natural phenolic compound present in many plants that play an important role in the signal transduction pathway and involved in local and systemic resistance to pathogens (Delaney *et al.*, 1995). During the last decade extensive research work has been performed for the establishment of SAR by the application of a variety of biotic and abiotic inducers (Meena *et al.*, 2001; Ryals *et al.*, 1996).

Salicylic acid, as already stated, acts as an endogenous signaling molecule that mediates SAR in several host-pathogen systems. Jasmonic acid or methyl jasmonate also has an important role in signal transduction following host-pathogen interaction. *Artemisia tridentate* (sagebrush), a plant shown to possess methyl jasmonate in leaf surface structures when incubated at chambers with tomatoplants, proteinase inhibitor accumulation is induced in tomato leaves, demonstrating that inter plant communication, through airborne methyl jasmonate can occur from leaves of one species of plants to leaves of another species and simultaneously the defensive genes are also expressed (Farmer and Ryan, 1992).

Schneider-Muller*et al.* (1994) reported Ca^{2+} ions playing an important role in the induced production of salicylic acid and chitinase, one of the pathogenesis-related proteins.

Pieterse *et al.* (1998) reported that induced systemic resistance is mediated by a jasmonate/ethylene sensitive pathway and does not involve expression of PR proteins. Pretreatment of tomato fruit with low concentration (0.01 mM) of methyl jasmonate (MeJA) or methyl salicylate (MeSA) induces the synthesis of some stress proteins, such a PR proteins which leads to increased tolerance to chilling temperature and resistance to pathogens, thereby decreasing the incidence of decay (Ding *et al.*, 1999). Genetic analysis in tomato indicates that systemin and its precursor protein prosystemin are upstream components of a wound-induced inter-cellular signaling pathway that involves both the biosynthesis and action of jasmonic acid. Activation of jasmonate biosynthetic pathway in response to wounding or prosystemin in required for the production of a long distance signal, whose recognition in distal leaves depends on jasmonate signaling, which may act as a transmissible wound signal (Lil *et al.*, 1999).

Besides salicylic acid and jasmonic acid, numerous chemicals have been tested for induction of SAR in various plants. Leroux (1996) reported that SAR can be accomplished by the exogenous application of aspirin or its derivatives. This biorational approach has been the basis of the development of a new fungicide BTH (CGA 245704) by Ciba Geigy. BTH has been shown to offer prophylactic protection when applied @ 20-30 g.ha at early tillering stage against powdery mildew in wheat, rice blast in rice and blue mould in tobacco.

Reuveni *et al.* (1997) sprayed solutions of 0.005 M H_2BO_3, 0.0025 M $CuSO_4$ and 0.0025 M $MnCl_2$, on the upper surface of the leaves of cucumber plants 2 h before inoculation with a conidial suspension of *Sphaerotheca fuliginea,* and noticed induced systemic protection against powdery mildew in leaves. A similar level of systemic protection was observed when plants were induced by a variety of micronutrients or microelements with various concentrations 2, 24 and 72 h before challenge with *S. fuliginea*. They also observed that a single spray of micronutrient solutions on the upper surface of the leaves of cucumber plants significantly inhibited powdery mildew development.

Meena *et al.* (2001) reported that salicylic acid induces resistance in groundnut against the late leaf spot caused by *Cercosporidium personatum*. They noticed the

changes in the activities of phenyl alanin ammonia-lyase, chitinase, β-1-3-glucanase, peroxidase, polyphenol oxidase and phenolic contents of groundnut after application of SA and inoculation with *C. personatum*. Increase in phenolic contents was observed one day after inoculation with *C. personatum*.

Siegrist and Buchenauer (2002) reported that K_2HPO_4 was effective in inducing a high level of systemic protection in cucumber plants against anthracnose caused by *Colletotrichum lagenarium*. Resistance induction by K_2HPO_4 was associated with localized cell death in cucumber leaves treated with the phosphate salt. The cell death observed, subsequently resulted in the appearance of macroscopically visible, necrotic spots. Appearing lesions resembled those provoked by tobacco necrosis virus (TNV) during a hypersensitive response (HR) that leads to pathogen-induced activation of SAR. Phosphate-mediated cell death was preceded by a rapid generation of superoxide and hydrogen peroxide. As a further consequence of phosphate application, a local and systemic increase in free and conjugated salicylic acid (SA) levels was detected. They also suggested that BTH-mediated SAR was different from that of K_2HPO_4-induced SAR and TNV-induced SAR.

Paul and Sharma (2002) observed that the leaves of barley treated with aqueous leaf extract of neem (*Azadirachta indica* Juss.) exhibited significantly high activity of enzymes PAL and tyrosine ammonia lyase (TAL) along with rapid and distinct accumulation of fungitoxic phenolic compounds. The population of most of the phylloplane mycoflora species remained unaltered.

Ghosh and Purkayastha (2003) studied on induced systemic protection against rhizome rot of ginger caused by *Pythium aphanidermatum*. They noticed that systemic protection against *P. aphanidermatum* was induced in ginger (Cv. Suprabha) by soaking rhizome seeds separately in selected synthetic chemicals or specific herbal extracts for 1 h prior to sowing. Among 12 plant defense activators tested, jasmonic acid (JA, 5 mM) and 10% leaf extract of *Acalypha indica* (ALE) reduced disease significantly, with concomitant increase of defense-related proteins (DRPs). Growth response of pathogen to both JA and ALE was evaluated *in vitro*. ALE stimulated growth, while JA inhibited growth at high concentration (0.5 mM) and slightly stimulated growth at low dose (0.005 mM). Results suggest their host-mediated role in induced systemic protection against disease.

Veronese *et al.* (2003) reported that an incompatible interaction results in an increase in endogenous levels of reactive oxygen intermediates, nitric oxide, salicylic

acid (SA), jasmonic acid (JA) and ethylene that trigger the defense responses through different signalling pathways. Signalling pathways activate a series of defense responses that curb or eliminate the pathogen. These responses include the hypersensitive response (HR), up-regulation of phenylalanine ammonia lyase (PAL), a key enzyme in plant defense, deposition of cell wall reinforcing materials, and synthesis of a wide range of antimicrobial compounds including pathogenesis related (PR)-proteins and phytoalexins.

Baysal *et al.* (2005) inoculated leaves of pepper (*Capsicum anuum* L.) with *Phytophthora capsici* 3 d after treatment with acibenzolar-S-methylbenzo [1,2,3]thiadiazole-7-carbothioic acid-S-methyl ester (ASM) and observed resistance to *Phytophthora* blight disease. They noticed that *P. capsici* was significantly inhibited by ASM treatment by up to 45 % *in planta*. The pepper plants responded to ASM treatments by rapid and transient induction of L-phenylalanine ammonia-lyase (PAL), increase in total phenol content and activities of chitinase and α-1,3-glucanase. No significant increase in enzyme activities were observed in water-treated control plants compared with the ASM-treated plants. Therefore, it may be suggested that ASM induces defense-related enzymes, PAL activity, PR proteins and phenol accumulation in ASM-treated plants and contribute to enhance resistance against *P. capsici*.

Conceicao *et al.* (2006) observed changes in phenolic metabolism after elicitation with *Colletotrichum gloeosporioides* (CG) in *Hypericum perforatum* L. (HP) cell suspension cultures. Soluble phenolics were analysed by HPLC–DAD and HPLC–DAD–MS/MS. HP cultures elicited with the CG elicitor showed a significant increase in xanthone accumulation. Xanthone accumulation increased twelve fold when the cells were primed with methyl-jasmonate (MeJ) or salicylic acid (SA), before elicitation. HP cultures exposed only to MeJ produced a set of flavonoids, the flavones which represent a substantial part (approx. 40%) of the total flavonoids accumulated in these cells. The results indicated that xanthones are important component of defense mechanism of HP against biotic stress.

Guleria and Kumar (2006a) reported that induced resistance is an important component of disease-resistance response of plants, and is accompanied by increased capability for activating defense responses upon pathogen ingress or elicitor treatment. They observed that aqueous leaf extract of neem (*Azadirachta indica* Juss.) provided the control of *Alternaria leaf* spot pathogen (*Alternaria sesami*) of sesame (*Sesamum indicum* L). Treatment with this extract led to the changes in plant

metabolism as leaves of the treated plants exhibited significantly high level of enzymes phenylalanine ammonia-lyase (PAL), peroxidase (PO) and content of phenolic compounds. Furthermore, germination of *A. sesami* spores was not significantly inhibited by neem extract. It is therefore, suggested that, protection of sesame plants against *A. sesami* by neem extract might be due to stimulation of plants natural defense response.

Besides chemicals several virulent and avirulent pathogens, nonpathogens, herbivore attack and wounding might induce resistance. Hammerschmidt (1999) reported the local or systemic induction of disease resistance in the treated plant to subsequent pathogen attack. Signal transduction pathways that operate both at the site of wounding and undamaged distal leaves regulate plant defense responses to wounding and herbivore attack.

Similarly, some biotic inducers have also been used to enhance plant defense reaction. Some of them are *Reynoutria sachaliensis* in cucumber (Daayf *et al.*, 1995), plant growth promoting rhizobacteria (PGPR) in cucumber (Chen *et al.*, 2000; Liu *et al.*, 1995; Wei *et al.*, 1991), *Pseudomonas fluorescens* strain CHAO in tobacco (Maurhofer *et al.*, 1994), *Pseudomonas syringae* in cucumber (Rasmussen *et al.*, 1991), *Pyricularia oryzae* and *Bipolaris sorokiniane* in rice (Manandhar *et al.*, 1999).

Disease control by fungicides

The introduction of benzimidazole fungicides such as benomyl, carbendazim and thiophanates in the early 1960s revolutionized disease control (Russell, 1995). Since then, chemical control plays an important role in the integrated control of the diseases (Muraleedharan and Chen, 1997). Several workers have suggested chemical control of crop diseases. Some of the previous works are being included in this review.

Kaushal and Sugha (1995) conducted an experiment to control damping-off of seedlings in eggplant caused by *Phomopsis vexans* through seed dressing by fungicides. The pathogen caused 29.9-31.8% pre- and 25.6-30.9% post emergence damping-off of seedlings. Seed treatment with captan, carbendazim, carbendazim + thiram, carboxin, metsulfovax, thiram and triadimenol improved the seedling stand significantly over the control. Seed treatment with carbendazim alone or in combination with thiram provided better control of pre- and post-emergence damping-off than the other fungicides.

Ide and Tashiro (2004) evaluated azoxystrobin, dithianon, fluazinam, kresoxymmethyl, captan and benomyl for residue, rainfastness and efficacy of disease inhibition in Japanese pear by spraying on leaves after inoculation with *Colletotrichum gloeosporioides*. They found azoxystrobin and dithianon as more efficient than other three fungicides and produced 80% disease suppression over control until 14 days after spraying in the field. They also noticed that among the five fungicides, azoxystrobin was the most efficacious when sprayed 2 days after inoculation with the pathogen. Gupta *et al.* (2005) used the fungicide carbendazim and carboxin integrated with biocontrol agents for controlling anthracnose of french beans caused by *C. gloeosporioides*.

Several chemicals have also been used against pathogens of different plants. *In vitro* activity of azoxystrobin, dimethomorph and fluazinam on growth, sporulation and zoospore cyst germination of *Phytophthora capsici, P. citrophthora* and *P. parasitica* to that of fosetyl-Al and metalaxyl were compared. The activity of azoxystrobin, dimethomorph and fluazinam on one or more stages of the life cycle of *P. capsici, P. citrophthora* and *P. parasitica* suggested that these compounds potentially could provide *Phytophthora* spp. disease control comparable to that of the established fungicides fosetyl-Al and metalaxyl (Matheron and Porchas, 1999).

Cromey *et al.* (2001) investigated the control of *Fusarium* head blight (FHB) of wheat using fungicides in two field trials. The first trial examined the effects of tebuconazole applied at a range of crop growth stages around flowering, whereas the second trial compared nil fungicide, tebuconazole, carbendazim and azoxystrobin, applied at full ear emergence or mid anthesis. Levels of both *Fusarium* and resulting mycotoxins were substantially reduced following treatment with tebuconazole or carbendazim but were not affected by treatment with azoxystrobin.

O' Neill *et al.* (2002) in a series of experiments, examined fungicides with different modes of action to the commonly used phenylamide-based products against downy mildew of rose (*Rosa* spp.) and blackberry (*Rubus fructicosus*), caused by *Peronospora sparsa*. Cymoxanil + mancozeb + oxadixyl and fluazinam gave good downy mildew control on both rose and blackberry. On outdoor container-grown rose, high volume sprays of fosetyl-aluminium were also effective, but on young micropropagated blackberry plants, application as a drench treatment was better than as a spray. Good control was also achieved on blackberry with chlorothalonil and with metalaxyl in formulation with either thiram or mancozeb.

Disease control by antagonistic organisms

Biological control of plant diseases involves the use of one nonpathogenic organism to control or eliminate a pathogenic organism. Frequent use of chemical pesticides and fungicides is increasingly causing concern in modern society in term of human toxicity and hazardous effect on natural environment. Hence, biological control has attracted a great interest in plant pathology (Goto, 1990). To develop biological control strategies of any disease, a thorough knowledge of life cycle of the pathogens(s), their mode of survival, the plant pathogen interaction processes starting from, the physical relationship of the pathogen to its host during pathogenesis, and the time and factors leading to infection and disease development are needed. Several authors have reported antagonistic activity of microorganisms in different crops (Droby *et al.*, 1992; Prasad *et al.*, 1999; Meena *et al.*, 2000; Dwivedi and Johri, 2003; Jadeja, 2003; Kohli and Diwan, 2003; Vestberg *et al.*, 2004; Brewer and Larkin, 2005; Sudha *et al.*, 2005; Singh and Sinha, 2005).

Fungal population in the rhizosphere of eggplant was studied by Hundoo and Dwivedi (1993) showing that rhizosphere microorganism such as *Trichoderma* spp. was found to be antagonistic against *Fusarium solani*, the causal agent of root disease of eggplant. Bucki *et al.* (1998) observed the presence of some biocontrol microorganism viz., isolates of Actinomycetes, fluorescent *Pseudomonads* and *Trichoderma* sp. in the soil which prevent the damping off of egg plant caused by *Fusarium* sp., *Pythium* sp. and *Rhizoctonia* sp.

D' Souza *et al.* (2001) reported that *Trichoderma harzianum* has antagonistic effect against four fungal pathogen of betel vine viz. *Phytophthora parasitica*, *Colletotrichum capsici, Sclerotium rolfsii* and *Rhizoctonia solani.* Ramamoorthy and Samiyappan (2001) suggested that *Pseudomonas flourescens* isolates were effective bacterial antagonist for the management of fruit rot of chilli caused by *Colletotrichum capsici.* Jadeja (2003) observed that fungal antagonists like *Trichoderma* spp. were highly effective for inhibiting mycelial growth and retarding pycnidial formation of *Phomopsis vexans* causing disease in brinjal. *T. koningii* exhibited the maximum antagonistic activity. Bacterial antagonists, e.g. *Bacillus* spp. and *Pseudomonas fluorescens* were also highly effective against the pathogen (Meena *et al.*, 2000).

Baruah and Kumar (2002) isolated an antibiotic and siderophore producing *Pseudomonas* strain from virgin soils (with forest trees) which displayed *in vitro* antibiosis against many plant pathogenic fungi. They noticed that seed bacterization

improved germination, shoot height, root length, fresh and dry mass, enhanced yield and chlorophyll content of leaves in the five test crop plants under field conditions. Seed bacterization also reduced the number of infected brinjal plants grown in soil infested with *Rhizoctonia solani.*

Gupta *et al.* (2005) studied on management of anthracnose in French bean caused by *C. gloeosporioides.* On the basis of *in vitro* studies they found *Trichoderma viride* isolate (Tv2), neem extract, carboxin and carbendazim as best treatments in inhibiting the growth of the pathogen. They were then tested in field at different combinations. The most effective combinations comprised of seed treatement with carboxin and *T. virive* followed by foliar spray of neem extract and carbendazim. This combination treatment resulted in the least disease incidence (1.45%) and severity (0.50%) and maximized yield (126 q/ha).

Jadon *et al.* (2005b) carried out experiment with antagonistic microbes and extracts of botanicals on *Sclerotium rolfsii,* incitant of collar rot of brinjal. The tested the efficacy of isolates of *Trichoderma* spp., *Pseudomonas fluorescens,* and *Gliocladium virens* in suppressing the growth of the pathogen by dual culture technique. They observed that *T. viride* isolate was superior than other isolates in reducing colony diameter and sclerotial production of the pathogen.

Several pathogens of other crops were also controlled by several workers using biological control strategies. For instance, mycostop was a biofungicide that has been effectively used to control a number of soil and seed-borne pathogens like *Botrytis cinerea, Rhizoctonia solani* etc. and seed borne foot rot disease of wheat and barley (Tahvonen and Lahdenpera, 1988; Tahvonen and Avikainen, 1990). The active component of mycostop was the spores and mycelium of *Streptomyces griseoviridis.* The product has been used successfully in seed treatment, soil drench, drip irrigation and as a transplant dip to control various disease causing fungi (Lahdenpera, 1987; Lahdenpera *et al.,* 1990 and Mohammadi, 1992). Use of mycostop at the rate of 0.35 g/l or greater reduced spore germination, plasmolysed germlings and reduced sporulation of *C. radicicola.* In essence, it reduced the inoculum potential of *C. radicicola* (Suleman *et al.*, 2002).

The antagonistic effect of *Trichoderma viride* was well established as reported by several workers. The hyphal coiling and production of inhibitory substances by different species of *Trichoderma,* resulting in dieback and disintegration of *Pythium* spp. were reported by Raju (1991) and Vinod *et al.* (1991). Several authors have shown considerable potential of *Trichoderma* and *Gliocladium* in controlling disease

caused by *Sclerotium rolfsii* in snap bean, sugar beet, tomato, chickpea and cotton in greenhouse and field studies.(Elad *et al.*, 1983; Upadhyay and Mukhopadhyay, 1983; Punja, 1985; Papavizus and Lewis, 1989; Wokocha, 1990; Ciccarese *et al.*, 1992 and Latunda Dada, 1993). Efficient control of chickpea wilt complex was found when seeds were treated with *Gliocladium virens* (10^7 conidia/ml) and carboxin 0.1% (Mukhopadhyay *et al.*, 1992).

Maity and Sen (1985) and Biswas (1999) reported that different isolates of *Trichoderma harzianum* showed differential antagonistic potential as biocontrol agent against *Sclerotium rolfsii.* Filonow (1998) observed that three antagonistic yeasts competed successfully for sugars since their uptake was faster and higher than that of *Botrytis cinerea.* He concluded from this that high competitiveness plays a central role in antagonism.

A comparative study of chemical, biological and integrated control of wilt of pigeon pea caused by *Fusarium udum* was done by Pandey and Upadhyay (1999). In chemical control, bavistin was found highly effective, while *Trichoderma viride* and *T. harzianum*-C isolates were found best among biocontrol agents. Integration of biocontrol agents with bavistin was not beneficial. However, integration of the bioagents with thiram reduced wilt incidence significantly. Thus, seed coating with bioagents proved better and safe for the management of wilt of pigeon pea.

Fourteen isolates of *Trichoderma* and *Gliocladium* speices were tested *in vitro* against *Sclerotium rolfsii,* the causal organism of root/ collar rot of sunflower by Prasad *et al.* (1999). Two isolates of *T. viride,* four isolates of *T. harzianum,* one each of *T. hamatum, T. koningii, T. polysporum, G. virens, G. deliquescens* and *G. roseum* inhibited mycelial growth of the pathogen significantly. Complete inhibition of sclerotial germination was obtained with the culture filtrates of *T. harzianum* (PDBCTH 2, 7 and 8), *T. pseudokoningii* and *G. deliquescens.* The three *T harzianum* isolates and the *T. viride* isolate (PDBCTV4) were superior under greenhouse conditions with PDBCTH 8 showing maximum disease control (66.8%) followed by PDBCTH 7 (66.0%) PDBCTV 4(65.4%), PDBCTH 2 (61.6%) and were even superior to fungicide captan. *G. deliquescens* gave maximum (55.7%) disease control among *Gliocladium* spp.

Prasad and Rangeshwaran (1999) evaluated a modified granular formulation containing powdered wheat bran, kaolin, acacia powder and biomass of isolates of *Trichoderma harzianum* (PDBCTH 10 and PDBCTH 8), *T. virens* (PDBCTV$_s$ 3 and ITCC 4177) and *Gliocladium deliquescens* (ITCC 3450) for their effect on the

reduction of chickpea damping off caused by *Rhizoctonia solani*. Granules with all isolates of bioagents significantly reduced damping off. The above two *T. harzianum* isolates were more effective in reducing saprophytic growth of the pathogen compared to other bioagents.

Ahmed *et al.* (2000) studied the effect of pepper seed and root treatments with *Trichoderma harzianum* spores on necrosis caused in stems by *Phytophthora capsici* inoculation and on the course of capsidiol accumulation in the inoculated sites. They suggested that the treatments significantly reduced stem necrosis, which fell by nearly a half compared with the values observed in plants grown from non-treated seeds. Necrosis was also reduced in plants whose roots were drenched with various doses of *T. harzianum* spores. As potential biological agents *T. harzianum* isolate T39 and *T. virens* isolate DAR 74290 controlled the rot disease in potato and tomato caused by *Phytophthora erythroseptica* (Etebarian *et al.*, 2000).

Ramamoorthy *et al.* (2002) characterized twenty isolates of fluorescent pseudomonads and evaluated their ability to control damping-off in tomato (*Lycopersicon esculentum*) and hot pepper (*Capsicum annuum*). Among these isolates, *P. fluorescens* isolate Pf1 showed the maximum inhibition of mycelial growth of *Pythium aphanidermatum* and increased plant growth promotion in tomato and hot pepper. *P. fluorescens* isolate Pf1 was effective in reducing the damping-off incidence in tomato and hot pepper in greenhouse and field conditions. Moreover, the isolate Pf1 induced the production of defense related enzymes and chemicals in plants.

Weller *et al.* (2002) reported the microbial basis of specific suppression to four diseases, *Fusarium* wilts, potato scab, apple replant diseases and take-all disease. One of the best-described examples occurs in take-all decline soils. In Washington State, take-all decline results from the buildup of fluorescent *Pseudomonas* spp. that produce the antifungal metabolite 2,4-diacetylphloroglucinol. The authors suggested that producers of this metabolite may have a broader role in disease-suppressive soils worldwide.

Perelló *et al.* (2003) evaluated the potential of *Trichoderma harzianum*, *Trichoderma aureoviride* and *Trichoderma koningii* as biocontrol agents of *D. tritici-repentis* under *in vitro* and greenhouse conditions. Dual cultures in petridishes containing potato dextrose agar showed that the isolates of *Trichoderma* spp. tested inhibited significantly the mycelial growth of *D. tritici-repentis* between 50% and 74%.

The results of the greenhouse tests indicated that seven strains of *Trichoderma* spp. significantly reduced the disease severity on wheat plants compared with untreated plants. In general, there was a significant decrease in *Trichoderma* spp. population on the wheat phylloplane with time. Additional greenhouse studies using other isolates and under a wide range of temperature conditions are needed to fully assess the potential and limitations of *Trichoderma* spp. as biocontrol agents of *D. tritici-repentis*.

Perello *et al.* (2006) also evaluated six isolates of *Trichoderma harzianum* and one isolate of *T. koningii* on the incidence and severity of tan spot (*Pyrenophora tritici-repentis*) and leaf blotch of wheat (*Mycosphaerella graminicola*) under field conditions and noticed significant differences between wheat cultivars, inoculum types and growth stages. Three of the isolates tested showed the best performance in controlling leaf blotch and tan spot when coated onto seed or sprayed onto wheat leaves at different growth stages, with significant severity reduction up to 56%. In some experiments, the biocontrol preparation (T2 and T5) gave a level of disease control similar to that obtained with Tebuconazole (70 and 48%, respectively).

Diseases control by botanicals

Plants have been proved as useful source of several antifungal molecules that are harmless and benign to the environment. There are certain advantages in the deployment of botanical pesticides. These are biodegradable, safe to non-target organisms, renewable and suit to sustainability of local ecology and environment. Moreover, the need for repeated application of fungicides to attain desirable level of disease control discourages the extensive adoption of chemical control by most of the farmers (Singh and Singh, 2005).

Terras *et al.* (1993) noticed synergistic enhancement of antifungal activity of wheat thionins by 2- to 72- folds when combined with 2 S albumins of radish or rape and being effective against filamentous fungi and some gram-positive bacteria. Permeabilization of the hyphal plasmalemma of thionins has been shown to be the mode of action. Soil amendments with crop residues lead to build up of allelochemicals and plant nutrients. In a comparative study, it was shown that incorporation of straw was found more effective than burning of straw in containing the symptoms of eye spot disease (*Pseudocercosporella herpotrichiodes*) and sharp eye spot disease (*Rhizoctonia cerealis*) of wheat (Prew *et al.*, 1995).

Kirkegaard *et al.* (1996) while evaluating rape and Indian mustard as companion crop showed that the latter was more effective in minimizing the incidence not only of take-all disease of wheat but also *Rhizoctonia solani, Pythium* and *Cochliobolus sorokiniana.* The tissue extract of Indian mustard was equally effective and hence the role of volatile isothiocyanates is implied. Certain phytochemicals like gallic acid and abscicic acid have been shown to be antifungal. For instance, abscicic acid was shown to inhibit mycelial growth and sporidial formation and also germination of teliopores (Singh *et al.*, 1997)

Bianchi *et al.* (1997) tested *Fusarium solani, Colletotrichum lindemuthianum, Pythium ultimum* and *Rhizoctonia solani* and found that garlic extracts inhibited mycelial development *in vitro.* They also used aqueous extract of powdered oven-dried (35 °C) garlic bulbs incorporated into the growth medium and reported that the hyphae of *R. solani* and *C. lindemuthianum* showed collapse and for *F. solani* hyphae appeared thinner than in controls.

Ali *et al.* (1999) screened hexane and methanol extracts of sixteen plants of the family Caesalpiniaceae, collected around Karachi, Pakistan and were tested for their antibacterial and antimicrobial activity. As compared to hexane extracts, the methanol extracts of all the examined plants showed stronger growth inhibition against bacteria and fungi, *Cassia* species being the biologically more active plant. Ethanolic extract of *Melia azadirachta* rip fruit showed fungistatic (MIC 50-300 mg/ml) a fungicidal (MFC60-500 mg/ml) activity against *Aspergillus flavus, Fusarium moniliforme, Microsporum canis* and *Candida albicans* (Carpinella *et al.,* 1999).

Digrak *et al.* (1999) studied the antimicrobial activities of *Valex* (the extract of Valonia), the extracts of mimosa bark, gallnut powders, *Salvia ancheri* Benthum. Var. *ancheri* and *Phlomis bourgei* Boiss. The results of the study indicated that mimosa bark extracts had the greatest antibacterial activity, followed by the *Valex,* gullnut powders, *Salvia ancheri* var. *ancheri* and *Phlomis bourgeie* extracts, respectively. Furthermore, it was found that gullnut powders and the extracts of mimosa bark contained high amounts of tannins and showed antifungal activity.

Ke *et al.* (1999) collected two hundred and four species of traditional Chinese herbal medicines belonging to 80 families from Yunnan Province in People's Republic of China and tested for antifungal activities using a *Pyricularia oryzae* bioassay. Twenty-six herbal medicines form 23 families were active against *P. oryzae* and the ethanol extract of *Dioscorea camposita* (dioscoreaceae) exhibited the most bioactivity among the entire tested sample.

Yoshida *et al.* (1999a) isolated three thiosulfinates with antimicrobial activity from oil-macerated garlic extract and their structures were identified by them as 2-propene-1-sulfinothioic acid S-(Z,E)-1-propenyl ester [AIIS(O)SPn-(Z,E)], 2-propenesulfinothioic acid S-methyl ester [AIIS (O)SMe] and methane sulfinothioic acid S-(Z,E)-1-propenyl ester [MeS(O)SPn-(Z,E)]. Antimicrobial activities of AIIS (O) SPn-(Z, E) and AIIS (O) SMe against gram positive and gram negative bacteria and yeasts were compared with 2-propene-1-sulfinothioicacids- 2-propenylester [AIIS(O)SAII, allicin]. Antimicrobial activity of AIIS(O) S Me and All S(O)S Pn-(Z,E) were comparable and inferior to that of allicin, respectively. In another study, Yoshida *et al.* (1999b) isolated and identified an organosulfur compound from oil-macerated garlic extract by silica gel column chromatography and preparative TLC. The antimicrobial activity of isoE-10-DA was inferior to those of similar oil-macerated garlic extract compounds such as E-ajoene, Z-ajoene and Z-10-DA.

Demirci *et al.* (2000) collected the leaves of five *Betula* species, *B. pendula, B. browicziana, B. medwediewii, B. litwinowii* and *B. recurvata* from different parts of Turkey. The leaves were hydro distilled to yield the consequent essential oils. The essential oils showed antifungal activity against various phytopathogenic fungi like *Cephalosporium aphidicola, Drechslera sorokiniana, Fusarium solani* and *Rhizoctonia cereals.*

Limonene is the major constituent of essential oil of exocarpic part of *Citrus sinensis* which possessed strong and broad-spectrum antifungal activity against important fungal pathogens of sugarcane (Rao *et al.*, 2000). The mycelial growth of *Ceratocystis paradoxa* at 2000 ppm and that of *Fusarium moliniforme* and *Curvularia lunata* at 3000 ppm concentration of limonene were completely inhibited. It proved fungistatic at minimum inhibitory concentration and exhibited non-phytotoxicity on germination and growth of sugarcane.

Ogunwande *et al.* (2001) analysed methanol extracts from leaves, stem bark, root bark, fruits and seed kernels of *Butyrospomum pradoxum* (*Vitellaria paradoxa*) and revealed the presence of alkaloids (in leaves and stem barks), flavones (in stem and root bark), saponins (in root bark), steroids (in stem bark, fruits and seed kernels) and tannins (in leaves and root bark) which have antimicrobial activity against bacteria (*Pseudomonas aeruginosa, Escherichia coli, Salmonella typhi, Staphylococcus aureus, Ralstonia solanacearum and Bacillus cereus*) and fungi (*Fusarium oxysporum* and *Candida albicans*).

Jaspers *et al.* (2002) studied the control of *Botrytis cinerea* Pers. leaf colonization and bunch rot in grapes with oils in laboratory and field tests. In detached lateral experiments, the essential oils from thyme (*Thymus vulgaris* L.) and clove (*Syzygium aromaticum* L.), as well as massoialactone (derived from the bark of the tree *Cryptocarya massoia* R.Br.) were not phytotoxic on leaves at concentrations of 0.33% or less. *B. cinerea* sporulation on artificially induced necrotic leaf lesions was significantly reduced by thyme (Thyme R) and masoialactone oils at 0.33%. A single application at veraison (1997/98) of either compound at concentrations of 0.33% controlled bunch rot and necrotic leaf lesion colonization by *B. cinerea* compared with *Botrytis* control treatments. Spray applications of Thyme R oil (0.33%) at 8-10 day intervals (1998/99) from flowering to harvest controlled *B. cinerea* bunch rot but also caused floral tissues to senesce.

Bautista-Banos *et al.* (2003) also evaluated the *in vitro* fungicidal effect of chitosan and aqueous extracts of custard apple leaves, papaya leaves and papaya seeds, and the combination of chitosan and plant extracts on the development of *Colletitrichum gloeosporioides,* causative agent of anthracnose on papaya. They found that chitosan had a fungicidal effect on *C. gloeosporioides.* Extracts alone did not show any fungicidal effect while the combination of 2.5% chitosan with all the tested extracts had a fungistatic rather than fungicidal effect. Changes in the conidial morphology of *C. gloeosporioides* were observed with 1.5% chitosan concentration after 7 h incubation. For in situ studies, control of anthracnose disease was obtained with 1.5% chitosan applied before *C. gloeosporioides* inoculation

Almada-Ruiz *et al.* (2003) evaluated antifungal activities of four polymmethoxylated flavons, isolated from cold-pressed orange oil against *Colletotrichum gloeosporioides,* a major plant pathogen of fruits that causes significant damage to crops in tropical, sub-tropical and temperate regions. They noticed that methoxylated flavones were effective in inhibiting mycelial growth of the fungus. Complete inhibition of the growth of the pathogenic fungus *C. gloeosporioides* was observed at a concentration of 100 $\mu g\ ml^{-1}$.

Curtis *et al.* (2004) reported that garlic extract showed activity against the plant pathogenic bacteria *Agrobacterium tumefaciens, Erwinia carotovora, Pseudomonas syringae* pv. *maculicola*, *P.s.* pv. *phaseolicola*, *P.s.* pv. tomato, *Xanthomonas campestris* pv. *campestris*, the fungi *Alternaria brassisicola, Botrytis cinerea, Plectosphaerella cucumerina*, *Magnaporthe grisea*, and the oomycete *Phytophthora infestans.*

Peraza-Sánchez *et al.* (2005) also screened seven Yucatecan plant extracts to look for fungicidal activity for the control of *C. gloeosporioides*. Bioassay-directed purification of the root extract of one of the most active plants, *Acacia pennatula*, resulted in the isolation of the new compound 15,16-dihydroxypimar-8(14)-en-3-one (1), which in the *in vitro* bioassay "agar dilution" was shown to have growth, sporulation, and germination inhibition activity.

Deepak *et al.* (2005) used methanolic extracts of forty plant species commonly growing across India and screened for antisporulant activity against *Sclerospora graminicola* , the causative organism of pearl millet downy mildew. The methanolic extracts of nine species did not show any effect, whereas the activity of the extracts of *Clematis gouriana, Evolvulus alsinoides, Mimusops elengi, Allium sativum* and *Piper nigrum* were commensurable to that of the marketed botanical fungicides. The extracts of 11 species (*Agave americana, Artemisia pallens, Citrus sinensis, Dalbergia latifolia, Helianthus annus, Murraya koenigii, Ocimum basilicum, Parthenium hysterophorus, Tagetes erecta, Thuja occidentalis* and *Zingiber offinale*) exhibited remarkable antisporulant effect even after 10-fold dilution of the crude extracts while in the case of remaining 15 plants the crude extracts loosed activity after 10-fold dilution. The antisporulant activity of commercialised *Azadirachta* preparation (Nutri-Neem) was more pronounced than that of Reynutria based on (Milsana) and Sabadilla (veratrin), however, these botanical preparations held off the extracts of *C. gouriana* and *E. alsinoides* and synthetic fungicides.

In the search for bioactive compounds, direct bioautography of lipophilic leaf extracts of medicinal plants used by Himalayan people was used in antifungal screening by Guleria and Kumar (2006b). *Alternaria alternata* and *Curvularia lunata* were used as test organism in bioautography. The results, evaluated by the diameter of the inhibition zone of fungal growth, indicate that five plant species, among the 12 investigated, had shown antifungal activity. They used $CHCl_3$: CH_3OH (1:9, v/v) as a solvent to develop silica gel TLC plates. Clear inhibition zones were observed for lipophilic extracts of *Vitex negundo* (RF value 0.85), *Zantoxylum alatum* (RF value 0.86), *Ipomea carnea* (RF value 0.86), *Thuja orientalis* (RF value 0.80) and *Cinnamomum camphora* (RF value 0.89). The best antifungal activity was shown by lipophilic leaf extract of *T. orientalis*.