2. Literature review

Plants have evolved sophisticated defense mechanisms including preformed barriers, constitutively expressed antimicrobials and inducible defense mechanisms against potentially pathogenic fungi, bacteria and viruses. Pathogenic microorganisms, non-pathogenic microorganisms and synthetic chemicals can induce resistance when it come in contact with the host plant and provide protection against a broad spectrum of pathogens. It has been shown that plants can recognize general structures associated with microorganisms. The pathogen associated molecular patterns (PAMPs) including some proteins from bacteria and chemicals from fungi (viz. chitin, ergosterol, several cell wall glucans, and proteins) after binding to specific receptor of plant elicits or trigger a signalling cascade which finally resulting to biochemical defense mechanisms such as production of phytoalexins and proteins with antimicrobial activities and mechanical strengthening of the cell walls (Thuerig *et al.* 2006). Like many other plants economically important plants are also attacked by several pathogens and cause substantial yield loss. To control diseases of plants effectively, it is necessary to understand different aspects of host parasite interactions. In case of plant disease management, biological control and botanicals also play important role as they are environment-friendly.

At the onset of the present study, it was considered to review the works of the previous workers. The observations of the previous workers in concord with the present line of investigation are being presented, in a selective manner, in the following paragraphs. For convenience, the observations have been grouped into some aspects. The different aspects of this review are:

- Diseases of niger.
- Diseases caused by *Alternaria alternata.*
- Studies on growth and physiology of the pathogens.
- Antigenic relationship in host and pathogen.
- Induction of systemic resistance (SAR and ISR).
- Disease control by antagonistic organisms.
- Disease control by botanicals.

Diseases of niger

Diseases have been reported on niger plants. Getinet and Sharma, 1996, reported approximately 16 diseases of niger caused by fungi and bacteria. They reported that some *Alternaria* sp. cause stem and leaf blight of niger. They also mentioned that niger blight (*Alternaria* sp.) and leaf spot are most serious diseases among the diseases listed by them (Table 2.1).

Table 2.1 : Diseases of niger

Pathogen	Disease	distribution	References
Alternaria dauci	On seeds and leaf	Ethiopia	Stewart and Yirgu (1967)
Alternaria porri sp. *dauci*	Leaf spot	Ethiopia	Yirgu (1964)
Alternaria sp.	Stem and leaf blight	Ethiopia	Yitbarek (1992)
Aspergillus sp.	-	Ethiopia, India	Kolte (1985)
Bremia lactucae	Downy mildew	Ethiopia	Stewart and Yirgu (1967)
Cercospora guizoticola	Leaf spot	Ethiopia, India	Yirgu (1964)
Cladosporium sp.	-	Ethiopia, India	Yirgu (1964)
Emericella sp.	-	Ethiopia India	Kolte (1985)
Fusarium sp.	-	Ethiopia, India	Kolte (1985)
Ozonium taxanum var. *parasiticum*	Ozonium wilt	India	Kolte (1985)
Macrophomina phaseolina	-	Ethiopia, India	Chaven (1961)
Phoma sp.	Stem lesion, wilting	Ethiopia	Yitbarek (1992)
Phyllosticta spp.	Tar spot	Ethiopia, India	Yirgu (1964)
Plasmopara halstedii	Downy mildew	Ethiopia	Yitbarek (1992)
Puccinia guizotiae	Rust	Ethiopia	Yirgu (1964)
Rhizoctonia solani	Root rot	Ethiopia	Yirgu (1964)
Rhizoctonia bataticola	Seed rot	India	Yitbarek (1992)
Sclerotium rolfsii	Seed rot	India	Kolte (1985)
Sphaerotheca sp.	Powdery mildew	India	Yirgu (1964)
Xanthomonas campestris pv. *guizotiae*	Leaf spot	Ethiopia	Yirgu (1964)
Anguina amsinckia	Leaf gall	Ethiopia	Stewart & Yirgu (1967)
Epicoccum nigrum	-	Ethiopia	Yirgu (1964)
Erysiphe cichoraceurum	-	Ethiopia	Yirgu (1964)
Coniothyrium sp.	-	Ethiopia, India	Kolte (1985)
Penicillium spp.	-	Ethiopia, India	Yirgu (1964)
Xanthomonas campestris pv. guizota var. *indicus*	-	India	Kolte (1985)
Septoria sp.	-	Ethiopia	Stewart & Yirgu (1967)

Disease caused by *Alternaria alternata*

Genus *Alternaria* is one of the most common fungal pathogen of plants. Different species of the fungi attack a large number of vegetables, ornamental plants, orchard plants and cause substantial yield losses (Stranberg, 1992; Farrar *et al.*, 2004). They are polyphagus in nature and have ability to produce mycotoxins and other toxic metabolites that are potentially dangerous food spoilage agents (Repeckiene *et al.*, 2005; Solfrizzo *et al.*, 2005). Alternaria disease decreases nutritive value of vegetables, their storability and resistance to rot (Azevedo *et al.*, 2000; Sidlauskiene & Surviliene, 2002).

Maiti *et al.* (2007) reported *Alternaria alternata* causing leaf spot and leaf blight diseases of some cultivated medicinal plants. They reported leaf spot of *Aloe vera* L.Burm, leaf blight of *Rauvolfia serpentina* L. Benth.ex Kurz., leaf blight of *Mentha arvensis* L., leaf blight of *Ocimum gratissium* L., leaf blight of *Plantago ovata* Forsk., leaf blight of *Catharanthus roseus* L.G. Don., leaf spot of *Cassia angustifolia* Vahl. and leaf blight of *Datura metel* L. of lower gangetic plain of West Bengal.

Chakraborty *et al.* (2006) reported *Alternaria alternata* as a new foliar fungal pathogen of tea in North Bengal, India. They described that disease symptoms first appeared as grayish brown patches around tips and margins of young tea leaves. These lesions extended towards the midrib, resulting in leaf curl, death and defoliation. Microscopic observations showed brown and septate hyphae and conidiophores (17-28 X 3-6 µm). Muriform conidia (23-34 X 7-10 µm) were usually solitary but occasionally in short chains.

Akbari and Parakhia (2007) reported the huge yield loss of sesame, an important oil seed crop due to *Alternaria* blight caused by *Alternaria alternata* (Fr.) Keissler in Saurashtra region of Gujrat, India.

Verma *et al.* (2007) reported leaf spot of Safed Musli (*Chlorophytum borivilianum*), an important medicinal plant, caused by *Alternaria alternata*. The fungus attack only leaves of the plant. The lesions are minute with light brown punctuation in the centre. In later stages the central portions commonly become serious and in a few cases this portion fall-off giving a sort hole like appearance.

Alternaria alternata has been reported in Pakistan as a saprophytic pathogen of tomato causing post harvest losses in high frequency (Akhtar *et al.*, 1994). Among 35 *A. alternata* isolates collected from rotted fruits from fields and markets but only

one isolate from the field was able to produce leaf blight symptoms. Thus they could separate one pathotype of *A. alternata* causing leaf blight. Akhtar *et al.* (2004) also reported that *A. alternata* causing leaf blight in tomato plants in Pakistan. Symptoms on affected plants started with yellowing and browning of the lower leaves, progressing upwards under high humidity conditions. Symptoms often developed from the leaf tips and along the margins of the leaf petiole. Under severe infection, lesions enlarged and coalesced causing blighting of the leaves. Concentric circles with dark layers of spores were observed under moist conditions on blighted leaf portions. Infection under favorable conditions was found to cause severe defoliation, with considerable yield losses when it occurred prior to flowering. In microscopic study showed that conidia formed in long chains and were obclavate and muriform, often with a short conical or cylindrical, pale beak, less than one third of the length of the conidium. Conidia had 3-7 transverse septa and usually several longitudinal or oblique septa.

Maiti *et al.* (2006) first reported that *Alternaria alternata* causing leaf spot on *Stevia rebaudiana.* Symptoms initially appeared as small circular spots, light brown in colour. Later, many became irregular and dark brown to grey, while others remained circular with concentric rings or zones. On severely infected leaves several spots coalesced to form large necrotic areas. On older leaves concentric spots were more common at the tips. Leaf spots varied from 2-18 mm in diameter. Conidial dimensions varied from 10-40 × 6-12 µm, mid to dark brown or olive-brown in colour, short beaked, borne in long chains, oval and bean shaped with 3-5 transverse septa.

Bashan *et al.* (1991) reported that wind dispersal of *Alternaria alternata* spore is the cause of leaf blight of cotton plants. The number of air-borne spores of *A. alternata* was significantly increased by the presence of diseased cotton plants, being highest close to the diseased plants. *Alternaria* blight epidemics occurring in the fields twice a year. The two peaks recorded for the number of spores present in the air above cotton crops correlate with the annual two outbreaks of *Alternaria* blight epidemics. In addition, both wind and plant row direction affect disease development in the fields.

A severe leaf spot disease of cucumber caused by a pathtype of *Alternaria alternata* (Fr.) Keissler was reported by Vakalounakis and Malathrakis (1988), in plastic houses in Crete, Greece. Lesions ranged in size of a pin point to over 5 cm in

diameter, with necrotic tissue on most of their area and a surrounding yellow zone. The pathogen grew satisfactorily on PDA at temperatures between 5°C–40°C and spore germination occurred in the range less than 10°C to over 37°C. Optimum temperature in both cases was near 26°C.

Roy (1976) reported leaf blight spot of *Adhatoda* vasica caused by *Alternaria alternata* at several location of Rajasthan. Singh *et al.* (2006) reported *Altenaria* blight of *Adhatoda vasica* Nees caused *Alternaria alternata* (Fr.) Keissler. They confirmed pathogenicity of the fungus both on leaves and inflorescence of *A. vasica.*

Sreekantiah *et al.* (1973) reported *Alternaria* leaf and fruit spot of Chilli (*Capsicum annuum* L.) caused by a virulent strain of *Alternaria alternata.* Pandey and Vishwakarma (1999) reported leaf blight of brinjal caused by *A. alternata* from Uttar Pradesh. Mangala *et al.* (2006) reported that *Alternaria alternata* is one of the important pathogen causing chilli leaf blight. They investigated the pathogenicity of *Alternaria alternata* on chilli cultivars. They also showed host range of one *Alternaria alternata* pathotype *causing* leaf blight disease in different plants. The host range has been presented in the Table 2.2.

2.2: Table Host range of *Alternaria alternata*

Host plant	Symptoms
Tomato (*Lycopersicon esculentum* Mill.)	Necrotic spots
Redgram(*Cajanus cajan* Millsp)	Necrotic spots
Blackgram(*Vigna mungo* L. Hepper	Necrotic spots
Greengram (*Vigna radiata* L. Hepper)	Necrotic spots
Groundnut (*Arachis hypogea* L.)	Necrotic spots
Cabbage (*Brassica oleracea capitata* L.	Necrotic spots
Mustard (*Brassica nigra* L.)	Necrotic spots
Brinjal (*Solanum melongena* L.)	blighted Symptoms
Tobacco (*Nicotiana tabacum* L.)	blighted Symptoms
Soybean (*Glycine max* L.)	blighted Symptoms
Cluster bean (*Cymposis tetragonaloba* L. Tank)	blighted Symptoms
Potato (*Solanum tuberosum* L.)	blighted Symptoms
Cauliflower (*Brassica oleracea campestris* L.)	blighted Symptoms

Studies on growth and physiology of the pathogens

Knowledge about fungal growth and physiology has immense importance for control of fungal diseases. Use of resistant varieties is one of the important alternatives to overcome the disease problems. A variety which exhibits resistance in one area may show susceptibility in another area due to variation in weather (Dubey, 2005) and variation in pathogen (Paulkar and Raut, 2004). Infection rate and disease development were significantely related with the temperature, wind velocity, relative humidity, soil pH and other physiological factors of nature. Therefore, studies of growth and sporulation of fungi in different conditions is very much helpful in determining various control measures.

Alternaria species are well-adapted to natural conditions with daily fluctuations in temperature and light, but there is considerable variability in the requirements for sporulation in culture (Rotem, 1994). Photosporogenesis in many *Alternaria* spp. consists of two distinct phases: the inductive phase, leading to the formulation of conidiophores; and the terminal phase, leading to the formation of conidia (Aragaki *et al.*, 1973).

It has been shown that nutrition, temperature, light conditions and moisture affected conidiation of *A. alternata* on various agar media (Shabana *et al.*, 1996; Sidky *et al.*, 1999). Maximum production of virulent *A. alternata* conidia was obtained on PDA at 20°C under constant NUV (near ultra violate light), incubated for 4 weeks. Conidiophore induction occurred on nutrient rich media and was stimulated by NUV. Formation of conidia proceeds best in darkness when nutrients are depleted under warm/dry conditions or cool moist conditions (Masangkay *et al.*, 2000).

Babu *et al.*, (2004) reported that conidia production and virulence of *A. alternata* were affected by temperature, light and incubation period. The highest number of conidia were produced on rice seed (120.6×10^5 g^{-1} substrate) followed by wheat (66.2×10^5 g^{-1} substrate), sorghum (60.3×10^5 g^{-1} substrate), maize seeds and cornmeal at 20°C when exposed to near-ultraviolet than on the other substrates, while least conidia (23.2×10^5 g^{-1} to 12.5×10^5 g^{-1} substrate) were observed on these substrates under light conditions. At 20°C, large numbers of virulent conidia (26.8×10^5 g^{-1} substrate) were produced on rice seeds after 4 weeks of incubation under constant dark conditions.

Shahin and Shepard (1979) reported that CaCO3 was required for sporulation to occur, but 10 to 50 g l^{-1} of CaCO3 gave comparable conidial production of *A. solani*. Similarly, when CaCO3 was added to standard PDA medium, *A. solani* mycelium growth was significantly inhibited and sporulation enhanced (Moretto and Barreto, 1995).

Response to light conditions using the S-medium (water agar amended with calcium carbonate [$CaCO_3$] and sucrose) was similar to that on standard agar media. Dark conditions were required to produce large numbers of conidia. Incubation in the light completely inhibited conidial production and exposure to 12 h of alternating light and dark periods dramatically reduced conidial production on all the primary agar media. The effect of temperature on conidiation on S-medium was the reverse of that on standard agar media. Conidiation was inhibited by high temperatures (24 and 28°C) and stimulated by a lower temperature (18°C). Once conidiophores were formed, removal of blue light and lowering of temperature below 20° C was required for conidia production to continue (Aragaki *et al.*, 1973). The wetting of the conidiophores at warmer temperatures stimulated vegetative growth of conidiophores to sterile hyphae (Vakalounakis, 1986).

Consistency in production of inoculum, germination capacity and spore concentration, age of plant and environmental conditions are responsible for disease development. Selection of suitable medium is necessary for the production of inoculums with a high degree of sporulation, minimal aerial mycelia growth and prossessing ability of inoculum to cause a high level of infection (Kong *et al.* 1995).

Prasad *et al.* (2008) reported that growth and sporulation of *Alternaria helianthi*, a pathogen causes leaf blight disease in sunflower were maximum in sunflower leaf extract followed by carrot agar medium whereas *A. helianthi* shows less growth and sporulation on potato dextrose agar (Allen *et al.* 1983; Mukewar *et al.* 1974). Subculturing of *A.helianthi* continuously up to 60 days after isolation reduced the germination capacity of conidia and for successful infection inoculam concentration suggested tobe 1×10^6 spores/ ml and 20-30 days old plants were ideal. Older plants (60 days old) failed to show disease symptom.

Normally, *Alternaria* blight was high under high humidity of 80-90%. Temperature of 25°C could stimulate the disease in glass house conditions. The optimum condition determined for lesion development in case of *Alternaria* blight of

Paulownia trees were 25-30°C temperature and RH of 98-100% (Pleysier *et al.* 2006).

The effect of temperature and pH on the growth and sporangial sporulation of isolates from each of the four known races of *Phytophthora clandestine* Taylor, Pascoe & Greenhalgh were investigated by Harden *et al.* (2002). Mycelial growth occurred at temperatures from 10°C to 30°C and pH 3.5 to 9.0 with highest growth rates of all isolates being at 25°C with a pH of 6.0 to 6.5. Sporangial production was greatest between 20°C to 25°C and pH 5.0 to 7.0 with all races.

Umamaheswari *et al.* (2008) reported growth and sporulation of six isolates of two *Alternaria* species (*A.alternata* and *A.cucumerina*) isolated from leaf blight disease of curcarbits. They used four different media e.g. Potato dextrose agar (PDA), Oats agar, watermelon agar and sucrose-calcium carbonate agar to determine the growth and sporulation. Growth of all the six isolates of *Alternaria* spp was slow on PDA and it influenced the sporulation of only one isolate of *A.alternata.*The oats agar hastened the growth of all the *Alternaria* spp. but it influenced sporulation of only two isolates. The fungus took 27-31 days to attain 90 mm growth in special medium amended with calcium carbonate. Watermelon leaf extract was better to induce sporulation of different isolates of *A. alternata* and *A. cucumerina. Pleospora infectoria* (perfect stage of *A.alternata*) with ascoscarps and ascospores were observed in *A.alternata* isolates from pumpkin (*Cucumis pepo*) on oats agar.

Ho and Ko (1997) reported that *B. theobromae* produce conidia when they were grown on 10% V-agar (10% V-8 juice, 0.02% $CaCO^3$ and 2% Bacto agar) at 24° C for 2 weeks under light. Fatty acid and their solvent (ethanol) had no adverse effect upon spore germination.

Rani and Kumar (2007) tested five culture media for variability in culture and morphological characters of six geographical isolates of *Pythium aphanidermatum* (Edson) Fitz. causing damping off of tomato. The isolates varied in mycelia growth, shape, position of sporangia and oospore in thickness. Among different solid media tested, potato dextrose agar supported maximum growth, while the growth was poor in carrot agar. Variations in mycelia, sporangial and oospore characters among six isolates of *P. aphanidermatum* revealed that three isolates produced fluffy aerial

mycelium with inflated sporangia, while two others produced moderately abundant surface mycelium with lobulate sporangia. Another isolate produced sparse aerial mycelium with lobulate sporangia.

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.

Nirmalkar and Lakpale (2007) reported the optimum conditions of some physical factors (*i.e.* temperature, duration of incubation, colours of light and relative humidity) for uredospore germination and subsequent germ tube elongation. The results obtained revealed that uredospore germination and germ tube length were maximum at 20°C followed by 25 and 15°C. Germinatin of uredospore started 8h after incubation, increased up to 20 h and then remained constant up to 24 h. Diffused light, complete dark condition and 75% relative humidity were found ideal for maximum spore germination and germ tube elongation of uredospores of *Uromyces achori.*

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 11.2×10^5 spores ml^{-1}, 32 °C and pH 6.75 respectively. Temperature pretreatment of 50 °C for 20 minutes significantly reduced spore germination, whereas pretreatment at 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.

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.

Antigenic relationship in host and pathogen

The early and accurate diagnosis of plant disease is a crucial component of any crop managmant system. Plant disease can be managed effectively if control measures can be started at an early stage of disease development. 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 or 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, Chakraborty and Sharma, 2007). Recent advances in molecular biology and biotechenology are being applied to the development of rapid specific and sensitive tools for the detection of plant pathogen. The novel evolutionary steps in plant pathological research is the development of antibody based immunodiagnosis.

Dazzo and Hubbell (1975) reported cross-reactive antigens and lectin was determinants of symbiotic specificity in the Rhizobium-clover association. Cross-

reactive antigens of clover roots and *Rhizobium trifolii* were detected on their cell surfaces by tube-agglutination, immunofluorescent, and radioimmunoassay techniques. Anti-clover root antiserum had a higher agglutinating titer with infective strains of *R. trifolii* than with noninfective strains. The root antiserum previously adsorbed with noninfective *R. trifolii* cells remained reactive only with infective cells, including infective revertants. Radioimmunoassay indicated twice as much antigenic cross-reactivity of clover roots and *R. trifolii* 403 (infective) than *R. trifolii* Bart A (noninfective). Immunofluorescence with anti-R. trifolii (infective) antiserum was detected on the exposed surface of the root epidermal cells and diminished at the root meristem.

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.

Evaluation of antisera raised against pooled mycelial suspensions from five isolates (Pf-1, Pf-2, Pf-3, Pf-10 and Pf-11) representing five physiologic races of *Phytophthora fragariae* for detecting the red core disease of strawberries by enzyme-linked immunosorbent assay (ELISA) were performed. Cross-reactivity of antiserum raised against *P. fragariae* with other *Phytophthora* as a genus detecting antiserum has been reported. 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* (Mohan, 1988).

Agar gel double diffusion between cotton seed globulins and the antisera specific to each of the tested *Fusarium oxysporum* f. sp. *vasinfectum* isolates were determined by Abd-El-Rehim *et al.* 1988. The antiserum of *F. moniliforme* revealed that all the tested antisera of *F. oxysporum* f. sp. *vasinfectum* reacted with seed globulins except one cultivar (Menoufi cultivar) globulins. No precipitin lines were detected in the reaction between the antigen of the cotton cultivar Acala SJ2 versus the antiserum of P10 isolate. Five cultivars reacted differently with each fungal antiserum to the extent that they could be distinguished accordingly. When the seed globulins of the susceptible cultivars (Giza 74, and Bahtim 110) reacted with antiserum of the tested *F. oxysporum* f. sp. *vasinfectum* isolates, more precipitin lines were found than the resistant cultivars.

Antigenic relationships between soybean and *Colletotrichum dematium* var. *truncata* using immunodiffusion, immunoelectrophoresis and indirect ELISA technique was studied by Purkayastha and Banerjee (1990). 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 (UPSM-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.

Pathogenicity test of *Fusarium oxysporum* on ten cultivars of soybean revealed Soymax and Punjab-1 to be most resistant while JS-2 and UPSM-19 were most susceptible. Antigens were prepared from the roots of all the ten varieties of soybean and the mycelium of *F. oxysporum*. Polyclonal antisera were raised against the mycelial suspension of *F. oxysporum* and the root antigen of the susceptible cultivar UPSM-19. Cross reactive antigens shared by the host and the pathogen

were detected first by immunodiffusion. The immunoglobulin fraction of the antiserum was purified by ammonium sulfate precipitation and DEAE-Sephadex column chromatography. The immunoglobulin fractions were used for detection of cross-reactive antigens by enzyme-linked immunosorbent assay. In enzyme-linked immunosorbent assay, antigens of susceptible cultivars showed higher absorbance values when tested against the purified anti-*F. oxysporum* antiserum. Antiserum produced against UPSM-19 showed cross-reactivity with the antigens of other cultivars. Indirect staining of antibodies using fluorescein isothiocyanate indicated that in cross-sections of roots of susceptible cultivar (UPSM-19) cross-reactive antigens were concentrated around xylem elements, endodermis and epidermal cells, while in the resistant variety, fluorescence was concentrated mainly around epidermal cells and distributed in the cortical tissues. CRAs were also present in microconidia, macroconidia and chlamydospores of the fungus (Chakraborty *et al.* 1997).

Scala *et al.* (1994) analyzed the possible involvement of cross-reactive antigens in host-parasite interactions between pea and some fungal plant pathogens. Antiserum to pea was used to analyse cross-reactive antigens (CRA) between pea and some fungal plant pathogens with different levels of specificity towards this host by using both double diffusion and immunoblotting techniques. Non pathogens of pea were also included in the study. The three *f. sp. of Nectria haematococca* MPVI (Viz. *dianthi, lycopersici* and *pisi*) of *Fusarium oxysporum* and *Ascochyta pisi* produced strong reactions in both techniques. No CRA was observed in the non-specific pathogens *Rhizoctonia solani, Sclerotium rolfsii* and *Sclerotinia sclerotiorum*, as well as in the non-pathogen *Phytophthora capsici.* The immunoblotting patterns of the most reactive fungi showed common bands with molecular weights of 84, 75 and 62 kDa. Some bands were present only in the specific pathogens *N. haematococca* MPVI and *F. oxysporum* f.sp. *pisi.*

Chakraborty *et al.* 1995, discussed the detection of grey blight of tea caused by *Pestalotiopsis theae* through cross reactive antigen between *P. theae* antigen of tea leaves. Among the 12 varieties of tea tested against three isolates of *Pestalotiopsis theae*, causal agent of grey blight disease, Teen Ali-17/1/54 and TV-23 were found to be highly susceptible while CP-1 and TV-26 were resistant under

identical conditions. Leaf antigens were prepared from all the tea varieties, three isolates of P. theae and a non-pathogen of tea (*Bipolaris tetramera*). Polyclonal antisera were raised against mycelial suspensions of *P. theae* (isolate Pt-2) and leaf antigens of Teen Ali-17/1/54 and CP-1. These were compared in immunodiffusion test and enzyme-linked immunosorbent assay to detect cross reactive antigens (CRA) shared btween the host and the parasite. CRA were found among the susceptible varieties and isolates of P. theae (Pt-1, 2 and 3). Such antigens were not detected between isolates of *P. theae* and resistant varieties, B. tetramera and tea varieties or isolates of *P. theae*. Indirect staining of antibodies using fluorescein isothiocyanate (FITC) indicated that in cross sections of tea leaves, the CRA was concentrated in the epidermal cells and mesophyll tissues. CRA was present in the young hyphal tips of the mycelia and on the setulae and appendages of the conidia of *P. theae*.

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. Priestley and Deway (1993) developed a double antibody sandwich ELISA test 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. 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.

Polyclonal antibodies against pre helminthosporol, 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 performed, indirect immunoflourescens appeared to be the most rapid and amenable assay for the detection of resting spores of *P. brassicae* in soil.

Polyclonal antibodies were raised against mycelium from the logarithmic growth phase of a shake culturen of *Ustilago nuda,* and a double antibody sandwich enzyme-linked immunosorbent assay (ELISA) with biotinylated detection antibodies was developed. The detection limit of the assay was 15 ng total protein ml^{-1} for the homologous antigen and 50 ng ml^{-1} for a spore extract, 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 ELISA has potential for field application including 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 characterisation of resistance mechanisms (Eibel *et al.*, 2005).

Besides fungus 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).

Abou-Jawdah *et al.* (2001) in a survey detected some potato viruses by ELISA from potato fields in the two main production areas of Lebanon, the Bekaa and Akkar plains. Hema *et al.* (2001) evaluated double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) and direct antigen coating (DAC)-ELISA 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.

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 (Wang *et al.* 2006)

Several other workers also used ELISA for detecting virus (Petrunak *et al.*, 1991; Abou-Jawdah *et al.*, 2001; Hema *et al.*, 2001; Chen *et al.*, 2005) and bacterial (Mazarei and Kerr, 1990) pathogens of plants.

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.

Cellular location of different proteins or antigens can be done by immunolocalization. Location of cross reactive antigens (CRA) was successfully done by several workers. 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.

In a similar fashion, Chakraborty and Saha (1994) labelled polyclonal antiserum with FITC and found CRA between tea leaves and the pathogen *Bipolaris carbonum*. CRA 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. Location of CRA was also studied in tea leaves that were treated with antiserum raised against two pathogens of tea. Indirect labelling of the antibodies with FITC showed that CRA was concentrated mainly in the epidermal cells and also spread throughout the cortical cells.

Immunogold labelling followed by electron microscopy have been successfully performed by previous workers(Lee *et al.*, 2000; Trillus *et al.*, 2000; Nahalkova *et al.*, 2001; Kang and Buchenauer, 2002; Wang *et al.*, 2003). They used the technique for immunolocalization studies. For light microscopy, silver enhancement is done after gold labelling (Santen *et al.*, 2005; 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 case of *C. gloeosporioide*s, 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) performed immunogold labelling and 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) and 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 invasion.

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.

Chakraborty and Sharma (2007) studied the location of CRA in tea (*Camellia sinensis*) leaves treated with antiserum raised against *Exobasidium vexans*, causal agent of blister blight of tea. Indirect staining of antibodies using FITC indicated cross reactive antigens (CRA) were concentrated mainly around epidermal and mesophyll cells in susceptible tea variety (T-78). This finding was substantiated by ultrastructural studies using gold labelled antibodies through transmission electron microscopy (TEM) which shows specific localisation in the chloroplast and host cytoplasm.

Induction of systemic resistance:

Systemic acquired resistance (SAR) & induced systemic resistance (ISR):

Plant pathogen intractions are governed by specific interactions between avr (avirulence) gene loci and alleles of the corresponding plant disease resistance (R) locus. When corresponding R and avr genes are present in both host and pathogen,

the result is disease resistance. Another type of resistance is horizontal or quantitative resistance that depends upon multiple genes in the host. According to Agrios (1988) resistance is the ability of an organism to exclude or overcome, completely or in some degree, the effect of a pathogen or other damaging factor. Disease resistance in plants is manifested by limited symptoms, reflecting the inability of the pathogen to grow or multiply and spread, and often takes the form of a hypersensitive reaction (HR), in which the pathogen remains confined to necrotic lesions near the site of infection. Induced resistance is the phenomenon that a plant, once appropriately stimulated, exhibits an enhanced resistance upon 'challenge' inoculation with a pathogen.

The plant immune response is much more like the innate immune response of animals than the adaptive. Plant does not develop specific resistance to the challenging pathogen but developed a broad spectrum resistance to several (Sticher *et al.* 1997). Plant cannot move to escape enviormantal challenges so they have evolved sophisticated mechanisms to perceive such attacks and to translate that perception into an adaptive response through signal molecules either synthesized by the invading organisms or released from plant cell walls. These signal molecules are collectively termed as 'elicitors', due to the presence of elicitors plant only receive general information that the plant is under attack (Wojtaszek 1997). Plants have several challenged-inducible resistance mechanisms, broadly divisible into local and systemic defences. Local defences include structural changes, such as the formation of papillae, tyloses and abscission zones, necrotic changes etc. Accumulation of phytoalexins, the synthesis of phenolic compounds and their subsequent oxidation of quinines by polyphenol oxidase and peroxidase are also important in plant defence (Agrios, 1997). Systemic defences involve the accumulation of antimicrobial compounds in parts of the plants distinct from the site of infection. Four main classes of compounds can accumulate: hydrolases, particularly the pathogen related proteins (PR), defensins (Broekaert *et al.,* 1995), proteinase inhibitors (Schaller and Ryan, 1996), cell wall componants, particularly hydroxyprolin rich glycoproteins (HRGP) and lignin and its precursor (Sticher *et al.* 1997).

Mainly five types of systemic induced resistance are known: (a) Local acquired resistance (LAR), express in immediate vicinity of the hypersensitive zone caused by attempted pathogen invation. (b) Sytemic acquired resistance (SAR),

expressed in the plant as a whole in response to pathogen attack. (c) Systemic gene silencing (SGS), a putative explanation for the recovery phenomenon by which systemic parts of the plants can exhibit resistance to the virus in infected parts. (d) Induced systemic resistance (ISR), which is induced by plant growth promoting rhizobacteria (PGPR) and expressed systemically. (e) Systemic wounding response (SWR), caused by the wounds inflicted on the plant by chewing insects and leading to the induction of proteinase inhibitors in systemic parts of the plant.

SAR development is mediated by a mobile signal that originates at the primary infection or treatment site and thought to be translocated systemically in the phloem (Rasmussen *et al.,* 1991; Smith-Becker *et al.,* 1998; Kaur and Kolte, 2001; Sharma *et al.,* 2001; Paul and Sharma, 2002). Systemic acquired resistance (SAR) can be induced in plants following a localised infection with a necrotizing pathogen or treatment with chemical elicitors (Mauch-Mani and Metraux, 1998; Sticher *et al.*, 1997). SAR was induced in mature plant part after localized treatment with 0.2 mM salicylic acid (SA) or previous inoculation with the same pathogen. SAR was expressed in adjacent untreated leaves as a reduction in lesion diameter (Reglinski *et al.*, 2001).

Willits and Ryals (1998) reported that Probenazole was the first commercialized disease resistance inducer and was widely used for the control of rice blast in Japan. Besides probenazole, some other chemical inducers of disease resistance in different plants have been described whice include salicylic acid (SA), 2,6-dichloro isonicotinic acid (INA) and 3-aminobutyric acid (BABA) (Kessmann *et al.*, 1994; Cohen, 1996; Sticher *et al.*, 1997). One of the benzothiadiazole compounds (BTHs), acibenzolar-S-methyl (CGA245704: benzo[1,2,3]thiadiazole-7-carbothioic acid S-methyl ester) was developed by Novartis Crop Protection AG and was introduced in 1996 as a 'plant activator' for the control of wheat powdery mildew in Germany and Switzerland (Ruess *et al.*, 1996 ;Buonaurio *et al.* 2002).

In the last two decades many biotic and abiotic inducers have been used for establishment of SAR in different plants. Meena *et al.* (2001) used salicylic acid in groundnut, Higa *et al.* (2001) used active oxygen radicals in rice, O'Donnell *et al.* (1996) used ethylene in tomato, Smith-Becker *et al.* (1998) used SA and 4–hydroxybenzoic acid in cucumber, Cohen *et al.* (1993) used jasmonic acid and

methyl jasmonate in potato and tomato, Siegrist *et al.* (2000) used β - aminobutyric acid in tobacco, Kaur and Kolte (2001) and Stadnik and Buchenauer (2000) used benzothiadiazole in mustard and wheat plant respectively, Brederode *et al.* (1991) used UV-light in tobacco, Ernst *et al.* (1992) used ozone in tobacco, Klessig *et al.* (2000) used nitric oxide and Kaku *et al.* (1997) applied N-acetylchitooligosaccharide in barley.

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). It has been described that SA coordinately induces the full spectrum of SAR genes, encompassing all well-characterized PRs (Ward *et al.*, 1991). Salicylic acid (SA) is an endogenous signal for the development of SAR and it is transported by phloem from the sites of its origin. Leaves inoculated with pathogen exhibits high level of endogenous SA (Malamy *et al.,* 1990). Foliar application of SAsignificantly increased the activity of Phenylalanine ammonia-lyase (PAL), Chitinase, β-1,3-glucanase, Peroxidase, Polyphenol Oxidase and Phenolic content in groundnut (Meena *et al.*, 2001). Jasmonic acid (JA) plays an important role in plant defense response. Its level is increased under wounding and treatment with pathogen-elicitors that induce genes encoding enzyme involved in flavonoid biosynthesis, chalcone synthase (Creelman *et al.*, 1992) and Phenylalanine ammonia-lyase (Gundlach *et al.*, 1992).

Schweizer *et al.* (1999) showed the induction of resistance in rice seedlings by *Pseudomonas syringae*, a biological inducer of resistance, and the chemical inducers benzothiadiazole (BTH) and 2, 6-dichloroisonicotinic acid (INA). Both INA and BTH induced similar patterns of genes, suggesting that these compounds were functional analogues. In contrast, the patterns induced by the chemical inducers and by *P. syringae* were clearly dissimilar.

Dann *et al.* (1998) assessed for severity of white mould disease caused by *Sclerotinia sclerotiorum* following induction of resistance by 2,6 dichloroisonicotinic acid or benzothiadiazole in field or greenhouse grown soybeans. They hypothesized that the decrease in disease severity following treatment with INA or BTH is a result of resistance induction.

Ishii *et al.* (1999) suggested that acibenzolar-S-methyl (CGA 245704) induced resistance to some but not all diseases on cucumber and Japanese pear. Induction of disease resistance in cucumber was rapidly triggered after treatment with acibenzolar-S-methyl (CGA245704: benzo [1,2,3] thiadiazole-7- carbothioic acid S-methyl ester) which showed no antifungal activity *in vitro*.

Cohen (1996) reported a new class of resistance-inducing compound belongs to aminobutyric acids. Sticher *et al.* (1997) reported local treatments with DL-3-aminobutyric acid (BABA) can protect tomato, potato, and tobacco, systemically, against *Phytophthora infestans* and *Peronospora tabacina*, respectively.

Acibenzolar-S-methyl (Novartis) induces defense-related compounds in apple seedlings. The protection was associated with the activation of two families of defense-related enzymes, peroxidases and β-1,3-glucanases. Accumulation of both enzymes were induced locally in treated leaves and systemically for β-1,3-glucanases in upper untreated leaves and was sustained for at least 17 days. A pre-flowering foliar spray of the plant activator acibenzolar-S-methyl combined with a fruit dip in guazatine at harvest substantially decreased disease in stored melons caused by *Fusarium* spp., *Alternaria* spp., *Rhizopus* spp. and *Trichothecium* sp. (Huang *et al.*, 2000).

An obligate fungus, *Albugo candida* infects all aerial parts of the mustard (*Brassica juncea*) plants. Plants treated with benzothiadiazole (BTH) exhibited high level of the enzyme PAL, peroxidase and cell wall-bound phenolic compounds (Coumarics acid and ferulic acid) than in untreated control. It was been shown that transcripts of six typical defense response genes, POX (peroxidase), PR-1. PR-2 (β-1,3-glucanase), PR-3 (chitinase), PR-4 and PR-5 (thaumatin-like protrein) were induced in spray-inoculated heads of the susceptible cv. of wheat . There was activation of SAR by 2-6-dichloro isonicotinic acid (INA) or benzo (1,2,3) thiodiazol-7- carbothioic acid S- methyl ester (BTH) (Görlach *et al.*, 1996). Similar activation was reported in barley (Kogel *et al.*, 1994) and maize (Morris *et al.*, 1998). The chemical BTH had no antifungal activity *in vitro* against the pathogen *A. candida*. Under the field conditions, plants treated with BTH at concentrations of 100mgL^{-1}, 250mg L^{-1}and 500mgL^{-1} showed protection from staghead development by 59.2%,

61.4% and 82.6% respectively when challenge inoculated with *A. candida* (Kaur and Kolte, 2001).

Buonaurio *et al.* (2002) used acibenzolar-S-methyl to induce resistance in pepper plants against *Xanthomonas campestris* pv. *vesicatoria* in both growth chamber and open field conditions. In growth chamber experiments of acibenzolar-S-methyl treatment in pepper plants showed resistance expression systemically and locally that lead to the reduction in the number and diameter of bacterial spots and bacterial growth. Systemic protection was also noticed by the acibenzolar-S-methyl acid derivative, CGA 210007. Under open field conditions both leaves and fruits were protected from the disease perhaps due to SAR activation.

Emmanuel *et al.* (2001) applied 'Phytogard' and BABA to induce systemic resistance in lettuce against downy mildew. Phytogard and BABA completely protected the disease. Pathogenesis related (PR) protein analysis showed that BABA induced weak accumulation of PR-2, but not PR-1, PR-5 and PR-9. Phytogard induced none of these proteins.

Cohen *et al.* (1999) reported the non-protein amino acid BABA (DL-3-aminobutyric acid) to induce local and five other isomers of aminobutyric acid, [Viz. L-2 aminobutyric acid, 2-amino isobutyric acid, DL-2-aminobutyric acid (AABA), DL-3-amino isobutyric acid, and 4-aminobutyric acid (GABA)] gave no protection against the downy mildew fungus.

Park *et al.* (2002) observed that the wall glucan elicitor (WGE), mycolaminarin, jasmonic acid (JA), methyl jasmonate and ethelene precursor, 1-amino-cyclopropane carboxylic caid (ACC) were effective in protecting the cells distal to the point of treatment from the site of infection of *Phytophthora sojae* in soybean.

Penninckx *et al.* (1996) purified a 5-kD plant defensin from *Arabidopsis* leaves after challenged inoculation with the fungus *Alternaria brassicicola* and shown to possess antifungal properties. The corresponding plant defensin gene was induced after treatment of leaves with methyl jasmonate or ethylene but not with salicylic acid *or* 2,6-dichloroisonicotinic acid. When challenged with *A. brassicicola*, the levels of the plant defensin protein and mRNA rose both in inoculated leaves and in nontreated leaves of inoculated plants (systemic leaves). The results indicated

that systemic pathogen-induced expression of the plant defensin gene in *Arabidopsis* was independent of salicylic acid but requires ethylene and jasmonic acid to response.

Ton *et al.* (2002) observed that three signals salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) played an important role in inducing defense resistance in plants. Salicylic acid is a key regulator of pathogen-induced systemic acquired resistance (SAR), whereas jasmonic acid and ethylene are required for rhizobacteria-mediated induced systemic resistance (ISR). Both types of induced resistance were effective against a broad spectrum of pathogens (oomycete, fungal, bacterial, and viral pathogen). In non-induced *Arabidopsis* plants, these pathogens were primarily resisted through SA-dependent basal resistance (against *Peronospora parasitica* and Turnip crinkle virus [TCV]), JA/ET-dependent basal resistance responses (against *Alternaria brassicicola*), or a combination of SA-, JA-, and ET-dependent defenses (against *Xanthomonas campestris* pv. *armoraciae*). They suggested that SAR and ISR constitute a reinforcement of extant SA- or JA/ET-dependent basal defense responses, respectively.

Meera *et al.* (1994) reported that plant growth promoting rhizobacteria (PGPR) can induce systemic resistance. Some fungal isolates collected from the rhizospheres of zoysiagrass enhanced the growth of a variety of crop plants and thus these isolates were designated as plant growth-promoting fungi (PGPF). The PGPF belonged to the genera *Fusarium*, *Penicillium*, *Phoma*, *Trichoderma* and sterile fungi. It was found that systemic resistance was induced in cucumber using the *Phoma* sp. and the sterile fungus against anthracnose caused by *C. orbiculare*. Cucumber roots treated with culture filtrates (CFs) of PGPF isolates also induced resistance against anthracnose. CF-treated plants expressed resistance to pathogen infection by an alteration of various metabolisms, such as high increases in activities of chitinase, β-1,3-glucanase, peroxidase, polyphenol oxidase and phenylalanine ammonia lyase, indicating that an elicitor substance(s) existed in the CFs.

Plant growth promoting rhizobacteria (PGPR) can suppress the disease caused by foliar pathogen by triggering plant-mediated resistance mechanism called induced systemic resistance, so called ISR (Dube, 2001). Van Loon *et al.* (1998) reported that the rhizosphere bacteria were present in large numbers on root

surface; few of them stimulated plant growth. The strains that were isolated from naturally disease-suppressive soil were mainly fluorescent *Pseudomonas* sp. It was demonstrated to reduce plant disease by suppressing soil-borne pathogens. Some of those biological strains had the ability to reduce disease caused by foliar pathogens by triggering a plant-mediated resistance mechanism called induced systemic resistance.

Ongena *et al.* (2002) demonstrated the ability of *Pseudomonas putida* BTP1 to induce resistance in bean to *Botrytis cinerea. In vivo* assays with samples from successive fractionation steps of the BTP1 supernatant suggested that salicylic acid, pyochelin and pyoverdin, previously identified as *Pseudomonas* determinants for induced systemic resistance (ISR), were not involved in systemic resistance triggered by BTP1 but one main metabolite (not characterized) retained most of the resistance-inducing activity in bean.

Conrath *et al.* (2001) reported that pre-treatment of cultured parsley cells with inducers of systemic resistance, salicylic acid or a benzothiadiazole, leads to the direct activation of a set of defence-related genes and also primes the cells for stronger elicitation of another set of defence genes including those encoding phenylalanine ammonia-lyase. In *Arabidopsis*, pre-treated plants with benzothiadiazole was found to augment the subsequent activation of phenylalanine ammonia-lyase genes by *Pseudomonas* infection, wounding and osmotic stress and also to enhance wound/osmotic stress-induced callose production. From these results, it was concluded that the resistance inducers have at least a dual role in plant defence-gene activation. Chowdhury *et al.* (2003) evaluated the effect of biotic and abiotic elicitors for the managnent of sheath blight of rice caused by *Rhizoctonia solani*. Thre fungal bioagents e.g, *Trichoderma viride*, *T. harzianum* , *T.virens* and nine antagonistic wheat rhizopheric bacteria (WRb) were evaluated *in vitro* by dual culture method for their antagonistic activities against *Rhizoctonia solani*. A maximum inhibition (62.96%) in growth of the pathogen was observed by wheat rhizosphere fluorescent pseudomonas bacteria (WRPf) followed by WRb8 (56.67%) and *T. virens*. Out of 4 plant extracts (*Polyalthia longifolia*, *Polygonum* sp. *Allium sativum* and *Gingiber officinale*), extracts of *Allium sativum* (at 10% concentration) inhibited maximum mycelial growth (100%) and sclerotial production *in vitro*. Among

abiotic elicitors seeds and seedlings treated with K_2HPO_4 (20mM) followed by $FeCl_3$ (10mM), reduced maximum disease incidence.

Abd-El-Kreem, (2007) reported that root rot caused by *Fusarium solani* or *Rhizoctonia solani* and *Alternaria* leaf spot of bean plants (*Phaseolus vulgaris* L.) were controlled with integrated treatment of *Trichoderma harzianum* and humic acid. It was observed that intregated treatments with seed coating with *Trichoderma harzianum* followed by seedling spray with humic acid at the concentration of 6 or 8 ml/L reduced the root rot incidence maximum (more than 80.5%) as compared with plant treated singly with *T.harzianum* or humic acid at concentration of 6 or 8 ml/L (more than 65.7%). *Alternaria* leaf spots were also appeared less when the plants weresubjected to the same treatment. In addition, more increase in chitinase activity was observed.

Role of certain elicitors like jasmonic acid (JA), salicylic acid (SA) and PGPR (*Pseudomonas*) were evaluated on the chemical induction of resistanse in tomato (*Lycopersicum esculantum* Mill.) against the leaf caterpillar *Spodoptera litura* Fab. by Malvin and Muthukumaran (2008). Elicitor was used to manipulate the activities of four putative defense related proteins like protein inhibitors, polyphenol oxidase, peroxidase and lypoxigenase in the leaves of tomato plants. When activities of protinase inhibitors and polyphenol oxidase in leaf tissue were high, growth rates of *S.litura* were low and *vice versa*. In contrast, high activities of peroxidase and lypoxigenase have no effect of growth and development of *S. litura*.

Plants face many biotic and abiotic challenges in the enviorment including drought and pathogen attack and combination of such stresses. Local and systemic induced defense responses were investigated by Fossdal *et al.* (2007) in the Norway spruce-*Rhizoctonia* sp. pathosystem and compared with drought alone or in combination of the two stresses using real-time reverse transcriptase (RT- PCR). They showed that compatible pathogen stress resulted in a transient systemic induction of selected defense related gene transcripts in the shoot. They also showed a persistant local induction in the roots. Drought lead to similar but delayed host response, while the combine stress gave, large and earlier changes in the transcripts than the two stresses separately.

Cellodextrins (CD), water soluable derivatives of cellulose composed of β-1,4 glucosidas residues, have been shown to induce a variety of defense responses in grapevine (*Vitis vinifera* L.) when challenged with *Botrytis cinerea.* The treatment has also resulted in significant reduction of the disease. The large oligomers of CD rapidly induced transient generation of H_2O_2, followed by a differential expression of genes encoding key enzymes of the phenyl propanoid pathway and pathogenesis related proteins. It also stimulated chitinase and β-1,3 glucanase activities(Aziz *et al.* 2007).

Lui *et al.* (2008) investigated the antagonism between acibenzolar-S-methyl (ASM) induced systemic acquired resistance and Jasmonic acid (JA) induced syatemic acquired susceptibility (SAS) to *Colletotrichum orbiculare* infection in cucumber plants. ASM treatment of cucumber plants resulted in much higher accumulation of class III chitinase (*CHI2*) gene, and lesion suppression, than in plants treated with distilled water (DW). In contrast, JA treatment suppressed expression of the *CHI2* gene and caused plant to be more susceptible to *C. orbiculare* infection. In the ASM +JA treatment, the number of lesions and the hybrid signal intensity fell midway between the ASM- and JA-only treatments. There was clear intimation about the antagonism or negative crosstalk between ASM-induced SAR and JA induced SAS.

Role of ethylene in plant development has been well estrablished but role of ethylene in plant defence is contradictory. Ortuno *et al.*, (2008) proposed ethylene as a possible marker of susceptibility of citrus fruits against *A. alternata* pv. *Citri.* They established a positive correlation between susceptibility to *A. alternata* pv. *Citri. and* the different citrus fruits with that of ethylene levels(produced *in vitro*).

Dutsadee and Nunta (2008) purified a novel 75 kDa protein-elicitor from the culture filtrate of *Phytophthora pulmivora*, a pathogen of *Hevea braziliensis.* The protein-elicitor was compared with another renound elicitor 'elicitin'. The new protein elicitor (75 kDa protein) activated defence at a concentration lower than those required for elicitin. The 75 kDa protein induced peroxidase enzyme, scopoletin, phenolic compounds and local resistance of rubber plants against *Phytopthora palmivora*, at about a 2-fold lower concentration than 'elicitin'.

Induction of resistance to downy mildew in sunflower caused by *Plasmopara halstedii* was studied by Nandeeshkumar *et al.* (2008) after treatment with chitosan. Treatment of sunflower with 5% chitosan resulted in decreased (46-52%) disease in field conditions. There was enhanced activation of defense related enzymes like catalase (CAT), phenylealanine ammonia lyase (PAL), peroxidase (POX), polyphenol oxydase (PPO) and chitinase (CHI) in chitosan-pretreated and *P. halstedii* inoculated plants. Northern hybridization analysis revealed increased levels of transcripts for five known defense response genes viz.,pr- 1a, β-1,3 glucanase, chitinase, peroxidase and chalcone synthase.This enhanced and early activation of defense-related responses (due to pretreatment with chitosan) in the susceptible cultivars were comparable to that of resistant cultivars.

Disease control by antagonistic organisms

Plant diseases occur regularly in plants and cause severe economic loss due to low harvest or production of oilseeds/foodgrains from diseased plants. Due to variation in cultivars and climate the resistance towards pathogens also vary. Application of chemical fungicides leads to destroy beneficial microbes on the crop milieu and thus alters the crop scenario and also causes toxicity to human and natural biota (Patro *et al.* 2008). Biological control of plant diseases involves the use of one nonpathogenic organism to control or eliminate a pathogenic organism. Hence, biological control has attracted a great interest in plant pathology (Goto, 1990) and it becomes importent to develop cheaper management practices to control disease and obtain higher yield. To develop biological control strategies for controlling any disease, a thorough knowledge of life cycle of the pathogen(s), their mode of survival, the plant-pathogen interaction processes, the physical relationship of the pathogen to its host during pathogenesis, the time of infection, 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).

Plant growth promoting rhizobacteria (PGPR) can suppress pathogen and reduce disease incidence by several ways like competition for nutrient and space, production of antibiotics, production of HCN, production of siderophores, increase in

salicylic acids, excreation of lytic enzymes, enhancement of plant defense through Induced systemic resistance (ISR), plant growth promotion by production of auxins and gibberalins etc. In *Trichoderma*, the production of secondary volatile and non-volatile metabolites is one of the criterion to assess its potential as biological agent (Umamaheswari et al. 2008)

Fungal population in the rhizosphere of eggplant was studied by Hundoo and Dwivedi (1993) showing that rhizosphere microorganisms 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 microorganisms 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.

Trichoderma harzianum has antagonistic effect against four fungal pathogens (viz. *Phytophthora parasitica, Colletotrichum capsici, Sclerotium rolfsii* and *Rhizoctonia solani*) of betel vine (D'souza *et al.* 2001). 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. viride* 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.* (2005) carried out experiment with antagonistic microbes and extracts of botanicals on *Sclerotium rolfsii,* incitant of collar rot of brinjal. They 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.

Some other 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). Mycostop when used 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 other works 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; 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* (10^7conidia/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.

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).

Roy *et al.* (2007) isolated a novel indigenous *Pseudomonous aeruginousa* strain from industrial waste water following dilution plate technique in nutrient agar (pH 7) medium. They used the *Pseudononas* strain as biocontrol agent against several species of *Phytophthora* (viz. *P. nicotianea*, *P. capsici*, *P. colocasiea* and *P. melonis*) and effectively controlled their growth.

Patro *et al.* (2008) reported that *Pseudomonous fluorescence* (*Pf* -1@ 0.6%) can be effectively used as a seed treatment and foliar spray for the management of blat in finger millet in addition to the edifenphos (0.1 %).

Jadeja and Bhatt (2008) isolated four *Bacillus* spp from mango fruit surface and tested against *Lasiodiplodia theobromae*, causing stem end rot disease of mango fruit. The application of any of the four Bacillus species on fruits resulted in reductions by more than 50% of the natural incidence of stem end rot.

Umamaheswai *et al.* (2008) evaluated some biocontrol agents like the isolates of *Pseudomonous fluorescence* (PfC6 and PfCIAH- 196), *Bacillus subtilis* (BSW1and BST1), Trichoderma isolates-CIAH 175 and *Trichoderma harzianum* against *Alternaria alternata* in watermelon. Their result showed that the antibiotics produced by B. subtilis caused swelling of the germ tube while *P. fluorescence* modified hypha into a chain of knotted cells. Volatile metabolites of Trichoderma isolate (CIAH-175) caused a maximum reduction in growth of *A. alternata* (92.2%).

Diseases control by botanicals

Plants naturally synthesize several carbon compounds, basically for their physiological functions or for use as chemical weapons against pathogens, insects and predators (Fatope, 1995). It has been estimated that 70-80% of total world population largely depends on traditional herbal medicine to meet their primary

health care need (Hamayun *et al.* 2006). 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, repeated application of fungicides to attain desirable level of disease control has been discouraged by some of the farmers (Singh and Sinha, 2005).

Terras *et al.* (1993) noticed synergistic enhancement of antifungal activity of wheat thionins by 2- to 72- folds when combined with 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 was implied. Certain phytochemicals like gallic acid and absisic acid have been shown to be antifungal. For instance, absisic 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. Ethanol extract of *Melia azadirachta* fruit showed fungistatic (MIC 50-300 mg/ml) and 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 [AllS(O)SPn-(Z,E)], 2-propene sulfinothioic acid S-methyl ester [AllS (O)SMe] and methane sulfinothioic acid S-(Z,E)-1-propenyl ester [MeS(O)SPn-(Z,E)]. Antimicrobial activities of AllS (O) SPn-(Z, E) and AllS (O) SMe against gram positive and gram negative bacteria and yeasts were compared with 2-propene-1-sulfinothioicacids-2-propenylester [AllS(O)SAll, allicin]. Antimicrobial activity of AllS(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-phytotoxic toon sugarcane germination and growth.

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 of either compound at concentrations of 0.33% controlled bunch rot and necrotic leaf lesion colonization by *B. cinerea.* Spray applications of Thyme R oil (0.33%) at 8-10 day intervals from flowering to harvest controlled *B. cinerea* bunch rot but also made senesce to floral tissues.

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 μg 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) 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). The isolated compound showed inhibitory activity on growth, sporulation, and germination of the fungus in "agar dilution" bioassay *in vitro.*

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 officianale*) exhibited remarkable antisporulant effect even after 10-fold dilution of the crude extracts. But in the case of remaining 15 plants the crude extracts lost 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).

Guleria and Kumar (2006) searched for bioactive compounds from lipophilic leaf extracts of medicinal plants used by Himalayan people. They screened antifungal properties by direct bioautography. *Alternaria alternata* and *Curvularia lunata* were used as test organism in bioautography. The results were evaluated by the diameter of the of fungal growth. They showed five effective plant species with antifungal activity among the 12 investigated. 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*.

Kiran, *et al.* (2006) screened thirty plant extracts (aquous extract) against the pathogen *Sclerotium rolfsii in vitro* to examin the inhibitory effect on mycelial growth and sclerotial production. Maximum inhibition (74%) of mycelial growth was recorded at 10% concentration of plant extract (*Prosopis juliflora)*. Other two antifungal plant extracts were from *Agave Americana* (showed 68% overall inhibition) and *Nerium indicum* (showed 54% overall inhibition). The inhibition(94%) of sclerotial production was exhibited by *Agave americana* and almost similar inhibition was shown by *Clerodendron inerme leaf extracts*. Leaf extract of *Riccinus communis* and fruit extract of *Riccinus communis* also gave good results (showed 72%) inhibition.

Reddy, *et al.* (2007) reported the antifungal component of cloves. They isolated, characterized and tested the efficacy of cloves against *Aspergillus* spp. The major component, eugenol was identified on TLC plate as dark coloured spot with R_f 0.5 along with standard. In TLC plate bioautography test, TLC plates were spray inoculated with four species of *Aspergillus* (*A. flavus*, *A. paraciticus*, *A.niger*, *A. ochraceus*) and eugenol on TLC plates inhibited mycelia growth of all four species of *Aspergillus*.

Meena et al.(2007) evaluated antibacterial activity of seven semi purified plant extracts made from flowers, leaves, fruits, stems, pods and seeds of some

plants and four antimicrobial chamicals. The bacterial plant pathogens were *Pseudomonas solanacearum*, *Xanthomonas campestris* pv. *Campestris*, *Xaxonopodis* and *Xanthomonas* pv. *Citri. Evaluation were done* by disc diffusion technique. Product componantes from mahua flowers and Satyanashi leaves were found effective against *Pseudomonas solanacearum* at 1000 ppm.

Mewari *et al.* (2007) screened two mosses viz. *Entodon plicatus* C. Muell and *Rhynchostegium vagans* jaeg for their antimicrobial activity against *Bipolaris sorokiniana* (Sacc. and Sorok), *Fusarium solani* (Mart.) Sacc.(fungi) and *Pseudomonas sclanacearum*, *Xanthomonas oryzae* pv.*oryzae* (bacteria). Aquous extracts of the two mosses were found to be ineffective. Ethanolic extracts of E. plicatus showed maximum inhibition (42%) of *B.Sporokiniana* and petroleum ether extract of *R.vagans* exhibited max. inhibition (45%) of *B.Sporokiniana.* Extract of *R. vagans* were found to be more effective inhibitors of *F. solani* than those of *E. plicatus.* Ethanolic extract of *R.vagans* showed maximum inhibition (44%) of *F. solani* whereas alcoholic extracts of both the mosses showed more effective antimicrobial activity.

Phyton-T, an extract of seaweed (*Sargassum wightii*) reduced disease incidence, induces defense enzymes against late blight of potato caused by *Phytophthora infestants* and enhances quality of potato. Siddagangaiah *et al.* (2008) reorted that tuber soaking and foliar spray in combination with Phyton-T (0.4%) and mancozeb (0.3%) for thrice at 15 days interval reduced the disease incidence up to 80%.

Phytochemical compound and antimicrobial properties of methanolic extracts of *Aspilia mossambicensis* (Compositae) were evaluated by Musyimi *et al.* (2008) against clinical strain of *Streptococcus pyogenes* (gram positive) and *Salmonella typhi* (gram negative) bacteria and one strain of fungi *Aspergillus niger.* Methanolic plant extract of leaves was found to be more active against the three microorganisms than the root extract.

Malabadi and vijay kumar (2007) evaluated the antifungal activities of aceton, hexane, dichloromethen and methanol extracts of leaves of four plant species (*Acacia pennata*, *Anaphylis wightiana*, *Capparis pepiaria* and *Catunaregum spinosa*) against pathogen viz. *Candida albicans*, *Kluyeromyces polysporus*, *Aspergillus niger*,

Aspergillus fumigantus. High antifungal activity was observed with methanolic extract of *Anaphylis wightiana* against all the test pathogens with the MIC values ranging from 0.02 to 0.06. Methanolic extract of *Acacia pennata*, *Anaphylis wightiana*, *Capparis pepiaria* have very stroung antifungal activity against tested pathogens particularly *C. albicans* and *K. polysporus*.