2. Literature Review

Tea is an important commodity in Indian economy. It is one of the sources of revenue of India. Like many other cultivated crops tea plant is also attacked by many fungal pathogens and pests. 190 fungal pathogens and 125 pests were recorded to cause diseases of tea plants in North-East India. Losses have been estimated at 67,000,000 pounds (30,000,000 kilograms) from these pests and fungal diseases. Therefore it is an important task to control these diseases effectively. Tea plant diseases can be categorized as leaf diseases, stem diseases and root diseases. In the present study, attentions have been paid to control one of the serious root (collar rot) diseases of tea seedlings that are caused by the fungus *Rhizoctonia solani* Kukn. [Teleomorph: *Thanatephorus cucumeris* (Frank) Donk] (Eden, 1978).

Root rot diseases generally occur due to the lack of proper drainage system. The root rot causing fungus can be spread by the flow of water or by the movement of the soil. Tea seeds may be attacked by the pathogens when it remains attached to the plant or when it falls on the ground from the tree. Soil borne pathogens enter into the tea seeds after its fall from the tea plants. It is important to know about the physiology, morphology and growth patterns of a causal organism of a disease and also the host parasite interaction of the disease. Biological control methods of the plant diseases by antagonistic microorganisms and botanicals are now-a-days, increasingly popular and well accepted methods to eradicate any diseases of cultivated crop plants as they are environment friendly.

At the onset of the present study, it was considered to review the works of the previous workers in the following paragraphs. For the convenience, the observations have been divided into several sub groups, which are as follows:-

- Seed mycoflora and seed diseases of tea plants.
- Root diseases of tea plants.

- Diseases caused by *Rhizoctonia Solani.*
- Characteristics of *Rhizoctonia solani* as a pathogen.
- Studies on growth and physiology of the pathogens.
- Antigenic relationship in host and pathogen.
- Disease control by antagonistic organisms.
- Disease control by botanicals.

Seed mycoflora and seed diseases of tea plants

Rothe and Wadekar (2011) studied the seed born mycoflora of *Aegle mamelos* (L.) Corr., *Basella rubra* L., *Limonia acidissima* L., *Nyctanthus arbortristis* L. and *Tectona grandis.* They recorded total sixteen fungi using blotter technique and agar plate method. Maximum number of mycoflora was recorded in *N. arbortristic* which are *Cladosporium, Phytophthora, Brachysporium, Fusarium, Aspergillus, Trichoderma* and *Physarum.* On the other hand only two fungi (*Erysiphe* and *Ustilago*) were reported from *B. rubra.*

Javaid *et al.*, (2010) reported the presence of four fungal species of *Aspergillus* from twelve varieties of shisam (*Dalbergia sisso* Roxb.) seeds from Lahore. Pods of ten seed varieties of S-4, R-1, R-2, US-1, US-2, US-5, US-6, US-7, US-9, US-10, US-11 and US-12 were collected and seeds were incubated on a moist sterilized filter paper bed in petriplates for seven days to isolate the mycoflora. The most occurring fungal species were *A. flavus, A. fumigates, A. japonicas* in comparison to *A. teerius.* They concluded that all the twelve shisham varities were equally susceptible to the fungal pathogens and did not show any germination up to 20 days.

Mamatha *et al.*, (2000) screened the seeds of *Dandrocalamus strictus, Phyllanthus emblica, Hardwickia binata* and *Dalbergia latifolia* for presence of mycoflora and found the presence of both field and storage fungi. They tested the dominant mycoflora on quality aspects like germination and vigour and significant decreases in germination and seedling vigour was shown by the dominant fungi. From their experiment they also concluded

that Trichoderma spp was most effective both in reducing the incidence of mycoflora and in enhancing the germination and vigour.

Utobo *et al.*, (2011) studied the association of seed borne fungi with eight hybrid (H) and three local check (LC) varieties and their effects on grain germination and seedling vigour during harvesting seasons. Among nine fungal genera *Trichoconis padwickii, Helminthosporium oryzae* and *Fusarium moniliforme* were found most abundant.

Singh *et al.*, (2011) isolated sixteen fungal species namely *Alternaria alternata, Alternaria solani, Aspergillus terreus, Curvularia lunata, Fusarium roseum, Fusarium semitechtum, Penicillium citrinum, Penicilium rubrum, Rhizopus stolonifer, Trichoderma harzianum,* Dark sterile mycelium and white sterile mycelium from two cultivars of wheat (Kundan and HUW-234) after treatment with potassium nitrate and examination with agar plate and blotter method.

Afzal *et al.*, identified thirteen phytopathogenic fungi which includes *Alternaria alternata* and *A. helianthi, Aspergillus flavus, A. fumigatus A. niger, Curvularia lunata, Drechslera tetramera, Fusarium solani and F. moniliforme, Macrophomina phaseolina, Mucor mucedo, Penicillium* and *Rhizopus* spp from seeds of seven cultivars of sunflower by using agar and blotter paper methods. The isolated fungi were responsible for reduced seed germination and seedling mortality. They also concluded that Topsin and Bayleton (systemic fungicide) were found to be significantly effective in the elimination of seed-borne fungi and antifungal activity could be achieved by extract of *Azadirachta indica* (neem) and *Allium sativum* (garlic) at the concentration of 0.015%.

Bokhary *et al.*, (1986) reported thirty-seven species of seed-borne fungi from five varieties of wheat namely *Triticum cinnamon* (wheat X rye), T. vulgare (Host. Mex-Mx Paw/ Ono-III-Conch), *T. vulgare* (Host. 78/2), *T. vulgare* (Host. Var. Jori), *T. vulgare* (Host. Super X) using standard blotter and agar plate (PDA, PDA plus yeast, MEA and CZA) methods.

Fakhrunnisa *et al.*, (2006) studied seed-borne mycoflora of 19 samples of wheat, 27 samples of sorghum and 14 samples of barley using standard blotter and deep freezing method. Fungi most frequently isolated and identified by them were *Absidia* sp., *Alternaria alternata, Aspergillus* sp., *A. candidus, A. flavus, A. niger,A.sulphureus, Cephalosprium* sp., *Chaetomium globosum, Cladosporium herbarum, Curvularia lunata, Drechslera dematioidea, D. halodes, D. hawaiiensis, D. tetramera, Fusarium moniliforme, F. oxysporum, F. pallidoroseum, F. subglutinans, Nigrospora oryzae, Penicillium* spp., *Piptocephalis* sp., *Rhizoctonia solani, Rhizopus* sp., *Stemphylium* sp., *Syncephalastrum racemosum, Trichoderma hamatum, Trichothecium roseum* and *Ulocladium* sp. They also reported that deep freezing method was better for isolation of *Alternaria alternata, Cladosporium herbarum, Drechslera* spp., and *Fusarium* spp.

Nagarja *et al.*, (2009) analyzed 185 seed samples of castor from agro climatic regions of Karnataka during *kharif*. They collected seeds from fields (49), farmers (73), retail shops (16) and APMC markets (47) and isolated forty seven fungal species belonging to seven genera by using standard blotter method and samples were tested by potato dextrose agar (PDA), water agar (WA) and 2,4-Dichloro phenoxy acetic acid (2,4-D) methods. The fifteen predominant fungal species they isolated from the castor seeds were *Fusarium oxysporum* f. sp. *ricini, Alternaria ricini, A. alternata, Curvularia lunata, Macrophomina phaseolina, Sclerotinia sclerotiorum, Cladosporium herbarum, Chaetomium globosum, Botryodiplodia acerina, Stachybotrys chartarum, Aspergillus ochraceous, A. niger, A. flavus, A. versicolor* and *Rhizopus stolonifer.*

Butt *et al.*, (2011) isolated four fungal species namely *Fusarium moniliforme, Alternaria sp., Helminthosporium sp.* and *Curvularia* sp. from stored grains of five varieties of rice (*Oryza sativa* L.). They tested four chemical fungicides namely antracal, topsin, mencozeb and derosal against the isolated fungi.

Mahamune and Kakde (2011) isolated thirteen seed-borne fungi from Waghya variety of French bean and nine fungi from Varun varity by using Glucose Nitrate Agar and Rose Bengal Agar method. According to them on

both varieties Macrophomina phaseolina showed its quantitative dominance which were followed by *Aspergillus niger* and *Fusarium oxyporum* and Waghya variety showed maximum incidence of fungi as compared to Varun variety. They also studied the antagonistic activity of Trichoderma harzianum against dominant fungi.

Patil *et al.*, (2012) screened seed mycoflora from untreated and treated seeds of pigeonpea and chick pea using agar plate method. Highest percentage of fungi was found to be *Aspergillus flavus* (30%) followed by *A. niger, Penicillium notatum, Cladosporium herbarum* in untreatedseeds.

Mali *et al.*, (2008) isolated *Alternaria alternata, Aspergillus flavus, A. niger, Botrytus sp., Chaetomium globosum, Cladosporium herbarum, Curvularia lunata, Fusarium oxyporum, F. moniliforme, F. roseum, Macrophomina phaseolina, Penicillium notatum, Phytophthora sp., Rhizoctonia solani, Rhizopus stolonifer* from green gram (*Phaseolus aureus* Roxb.) and black gram (*Phaseolus mungo* L.) by using agar plate method. The untreated seeds of wild variety showed highest percent incidence of seed mycoflora than treated seeds.

Mogle and Mane (2010) reported maximum eleven genera and twelve species of fungi from untreated tomato seeds collected from local market by using potato dextrose agar (PDA), rose bengal agar (RBA) and czapek dox agar (CDA) media. They reported that the untreated seeds were found to be associated with highest percent incidence of mycoflora and minimum population was recorded in the treatment of Rhizobium and Trichoderma (RhTr) mixture followed by Azotobacter and Trichoderma (AzTr), Trichoderma (Tr) alone, Benomyl (Ben), Rhizobium and Azotobacter and control (Con). According to them seed germination percentage was highest in the treatment of AzTr followed by RhTr, RhAz and Az and minimum in the treatment of Rh, Tr, Con and Ben. From their experiment they concluded that Trichoderma and Rhizobium was found to be beneficial to reduce the pathogenic fungi and increase of germination percentage.

Rathod *et al.*, (2012) reported the presence of seed mycoflora from different cultivars of legumes using standard blotter paper, agar plate and seed washed methods. Agar plate method was reported by them as suitable

method as in less incubation there was higher percent incidence of seed mycoflora and they isolated sixteen fungi.

Mittal (1983) isolated 26 fungal species belonging to 13 genera were isolated from seeds of *Cedrus deodara* Loud by using standard moist blotters, potato–dextrose–agar plates, and moist sterilized sand. The isolated fungi were *Aspergillus flavus, A. luchuensis, Epicoccum purpurascens, Fusarium moniliforme, Pénicillium canadense,* and*Rhizopus oryzae. Aspergillus flavus, Penicillium canadense* and *Rhizopus oryzae.* According to Mittal (1983) RH-2161 and Dithane M-45 was most effective fungicides against the isolated fungi.

. Prochazkova (1990) identified 50 species of fungi identified from batches of seeds of 9 native species of broadleaved forest trees (beech [*Fagus sylvatica*], ash [*Fraxinus excelsior*], sycamore [*Acer pseudoplatanus*], lime [*Tilia* sp.], rowan [*Sorbus aucuparia*], elm [*Ulmus* sp.], hornbeam [*Carpinus betulus*], alder [*Alnus* sp.] and birch [*Betula* spp.]) in Czechoslovakia.

Pande and Gupta (2011) reported *Fusarium solani, Aspergillus niger, Aspergillus nidulens, Penicillium* sp., *Trichoderma harzianum, Alternaria solani, Alternaria alternata, Curvolaria lunata, Stachybotryis chartarum, Acremonium* sp., *Rhizoctonia solani, Chaetomium globosum, Cladosporium cladoporoides* and *Torula allii* from the seeds of *Oroxylum indicum* (L.) Vent of Kumaun region of Central Himalaya in India.

Safai and Mehrotra (1982) studied seed mycoflora of forest trees seeds of *Quercus, Cupressus, Sapium, Pyrus, Melia, Casuarina,* and *Thuja.* They studied mycoflora in fallen seeds as well as in plucked seeds. They isolated nearly forty-two species of fungi which includes *Mucor, Rhizopus, Aspergillus, Penicillum, Epicoccum, Pithomyces, Cladosporium, Paecilomyces, Fusarium, Gliocladium, Trichothecium, Trichoderma, Cephalosporium, Alternaria, Ulocladium and Curvularia.* They reported that frequency of occurrence of moulds in the fallen-off seeds was generally more in comparison to that in the plucked seeds.

Barthakur *et al.* (1998) reported that some varieties of tea TS-491 and TS-520 and S_3A_1 were damaged by the attack of the fungus *Fusarium*

solani. He also described that the fungi produced prominent black spots (about 0.75 cm × 0.50 cm in size) on the bark of the immature fruits during July-August. The hard seed coat under the cracked fruit carp turned black and the premature dropping of seed took place. The imperfect stage of the fungus i.e. the white cottony mycelial growth of Fusarium Solani and the perfect stage i.e. orange perithecial structures of Nectria were produced on fruit carps when cracks were developed on fruit carps.In the later stage of infestation by the fungus, the seeds became pinkish due to presence of powdery spores of the fungi.

Root diseases of tea plant:

Any disease symptoms of plants occur due to the attack by the pathogens, such as fungi bacteria, viruses or nematodes and also by the nutrient deficiency. Tea plants are no exception. 300 species of fungi are reported to affect different parts of the tea plant. (Chen and Chen, 1990; Agnihothrudu, 1964) Besides foliar diseases of tea, root rot is the most harmful and damaging disease to a *Camellia*. Even the healthiest plant of *Camellia* can wilt and die in just a few days when affected with root rot (Shinholster, 2009).

Chandra Mouli (1996) reported several root rot diseases of tea caused by the fungi, bacteria, and nematodes. The names of the root rot diseases and their causal organisms have been given in the following table.

Chandra Mouli (1996) reported that *Rhizoctonia solani* Kuhn causes collar rot disease of tea. Santyanarayana (1973) and Barthakur (1999) reported that *Ustulina zonata* (causal agent of charcoal stump rot), *Fomes lamaoensis* (causal agent of brown rot), *Rosellinia arcuata* and *Armillaria mella* cause serious root rot diseases of Assam (north-east India). Barthakur (1999) also reported the involvement of the pathogen *Poria hypolateritia* in causing red root rot disease of tea in Assam.

Barthakur (1999) also reported that root disease causing fungi were not host specific. They dissaminate either by direct root contact from

diseased woody remains in the soil after uprooting the diseased bushes or by the soil borne spores.

Table 2.1: Root rots of tea and their causal organisms

Name of the Disease	Causal Organism
Armillaria root rot	*Armillaria mellea* (Vahl:Fr.) Kummer.
Black root rot	*Rosellinia arcuata* petch & *Rosellinia bunodes* (Berk. & Broome) sacc.
Botryodiplodia root rot	*Lasiodiplodia theobromae* (Pat.) Griffon & Maubl.
Poria root rot	*Poria hypobrunnea* Petch
Purple root rot	*Helicobasidium compactum* (Boedijn) Boedijn
Red Root rot	*Ganoderma Philippii* (Bresad & P. Henn) Bresad *Poria hypolateritia* (Berk.) Cooke
Root rot	*Cylindrocarpon tenue* Bugnicourt *Cylindrocladiella camelliae*(Venkataramani &Venkata ram)Boesewinkel *Fomes Lamaoensis* (Murr.) Sacc. & Trott. *Ganoderma applanatum* (Pers.) Pat
Tarry root rot	*Hypoxylon asarcodes* (Theiss.) Mill
Violet root rot	*Sphaerostibe repens* Berk & Broome
White root rot	*Rigidoporus microporus* (Sw.) Overeem
Xylaria root rot	*Xylaria* sp.
Pale brown root rot	*Pseudophacolus baudonii* (Pat.) Ryu
Root-Knot nematode	*Meloidegyne* sp.
Root lesion nematode	*Pratylenchus* sp.

Armillaria root rot caused by the fungus *Armillaria heimii* and *Armillaria mellea* were also reported to cause serious tea root rot diseases in African countries like Tanzania and Kenya etc. (Onsando *et al.*, 1997; Ndunguru, 2006). Kile *et al.* (1991) reported that *Armillaria* spreads primarily through the formation of rhizomorphs or by mycelial growth directly from the diseased roots to healthy one. Tea plants of all ages are susceptible to the disease caused by *Armillaria.* Charcoal stump rot caused by the fungus *Ustulina deusta* (Fr.) Petrak was also reported to cause serious root diseases in Africa (Muraleedharan and Baby, 2007).

Four most common tea root diseases have been reported by Muraleedharan and Baby from Srilanka (Muraleedharan *et al.*, 1997; Baby *et al.*, 2004). The diseases are red root rot (caused by the fungus *Poria hypolateritia*), black root rot (caused by the fungus *Rosellinia* sp.), brown rot (caused by the fungus *Ustulina zonata*), and chorcoal stump rot (caused by the fungus *Ustulina zonata*). *Fomes applanatus, Polyporus mesotalpae, Polyporus interruptus* and *Irpsubvinosus* were also reported to attack tea roots in Srilanka (Petch, 1923).

In China, most important root rot diseases of tea, caused by the fungus were reported as red root rot (caused by the fungus *Poria hypolateritia* Berk), brown root rot (caused by the fungus *Phellinus noxius* (Corner) G. H. Cunningam (*Fomes noxius*) and charcoal stump rot (caused by the fungus *Ustulina zonata* (lev) sacc)(Premkumar *et al.*, 2006).

Damping off caused by the fungus *Hypochnus centrifugus* (Lev.) Tul was found to cause diseases in tea nurseries in South-Eastern China. Crown gall caused by *Agrobacterium tumefaciens* Smith was also found on tea cuttings in China (Chen and Chen, 1982).

In Taiwan the serious soil borne diseases of tea shrubs are white root rot caused by the fungus *Rosellinia nectatrix* (Sun *et al.*, 2007) and brown root rot caused by the fungus *Phellinus noxius* (Ann *et al.*, 2002).

Gabner *et al.* (2004) reported the white root rot disease of Sabah tea caused by the fungus *Poria hypolateritia* in Malaysia. *Poria hypolateritia* is a soil borne pathogen. In highland areas of Malaya the root of tea plants are affected by the fungus *Ganoderma Pseudoferreum* and in low land areas the root of the plants are attacked by the *Poria hypolateritia* like fungus. Both of the fungus causes the red root rot disease of the plantation. The common symptom of this disease is the – production of red rhizomorphs on the outside of the roots.

Charcoal stump rot caused by the fungus *Ustulima deusta* (Fr.) Petrak, Red root diseased caused by the fungus *Poria hypolateritia* and

Armillaria root rot caused by the fungus *Armillaria mellea* were also reported from Java of Indonesia (Muraleedharan and Baby, 2007).

The major tea root disease reported from Japan is white root rot caused by the fungus *Rosellinia necatrix* (Hartig) Berl. (Ezuka *et al.*, 1973).

Disease caused by *Rhizoctonia solani*:

Rhizoctonia solani is a soil borne Basidiomycetes fungus and it occours worldwide (Lehtonen, 2009). According to Agrios (1997) soil borne pathogenic fungi can cause disease on roots and other underground plant parts i.e. stolons, tubers and basal parts of the stems. Though *Rhizoctonia solani* is a soil borne pathogen it can attack stem and leaf of plants (Sneh *et al.*, 1991). Soil borne plant diseases increase during relatively cool and wet weather, while air borne pathogens spread better during dry conditions (Lehton, 2009). *Rhizoctonia solani* causes significant damage on crop quantity and quality of many crop species annually. (Weinhold *et al.*, 1982; Ban ville, 1989; Martin and Loper, 1999; Green and Jenson, 2000; Botton *et al.*, 2006; Wagacha and Muthomi, 2007).

Sharma and Tripathi (2001) reported that thirty three plant species would be attacked by *Rhizoctonia solani* experimentally. The plants were of families like Leguminosae (11 plants), Graminae (5 plants), Solanaceae (11 plants), Brassicaceae (5 plants), Malvaceae (3 plants), Cyperaceae (2 plants) and one each of Cucurbitaceae, Commelinaceae and Chenopodiaceae.

Host range of *Rhizoctonia solani* is very broad and several diseases have been reported by several scientists till date. A list of plants with disease symptoms along with the references has been tabulated in the following table.

Characteristics of *Rhizoctonia solani* as a pathogen

Rhizoctonia Solani is not an obligate parasite and it can stay in the soil as saprophyte for long period. Due to the lack of conidia and the scarcity of sexual spores *Rhizoctonia solani* remain as vegetative hyphae or

in sclerotial form. *Sclerotium* is an encapsulated, tightly compact hyphal clump that gives the fungus protection from environmental stress.

Table 2.2: Diseases caused by *Rhizoctonia solani* in different hosts as reported by different authors

Host name	Disease symptoms	Reference
Buck wheat	Damping off	Herr and Fulton, 1995
Barley	Root rot	Rush *et al.*, 1994
Broad bean	Reduced growth	Valkonen *et al.*, 1993
Cotton	Root rot Root canker Minor pathogen	Rothrock, 1996 Baird and Carling, 1997 Carling *et al.*, 2002 a
Corn	Leaf blight Root rot	Tomaso-Peterson and Trevathan, 2007;Mazzola *et al.*, 1996
Common bean	Leaf blight, Web blight & Root rot Reduced growth	Muyolo *et al.*, 1993 Valkonen *et al.*,1993
Cabbage	Bottom rot	Tu *et al.*, 1996
Carrot	Damping off	Grisham and Anderson, 1983
Clover	Damping off & Root rot	Wong and Sivasithamparam, 1985
Cereals	Bare patch	Mazzola *et al.*,1996
Egg plant	Brown spot	Kodama *et al.*, 1982
Flower bulbs	Root rot	Dijst and Scneider, 1996
Lettuce	Damping off	Herr, 1993
Lupin	Late emergence Minor pathogen	Valkonen *et al.*, 1993 Mac Nish *et al.*, 1995
Mycorrhiza	Mycorrhizal	Carling *et al.*, 1999
Onion	Damping off	Erper *et al.*, 2005
Oil seed rape	Damping off	Kataria *et al.*, 1991a
	Basal rot	Verma, 1996
Potato	Stem canker Stem canker & Black scurf Stem canker Minor pathogen	Chand and Logan, 1983 Bandy *et al.*, 1988 Anguiz and Martin, 1989 Carling *et al.*,1994
Pea	Stem rot & Root rot	Hwang *et al.*,2007
Rice	Sheat blight Web blight	Sayler and Yang, 2007 Hashiba and Kobayashi, 1996
Raddish	Root rot	Grisham and Anderson, 1983

Contd....

Table 2.2: (Contd.) Diseases caused by *Rhizoctonia solani* in different hosts as reported by different authors

Host name	Disease symptoms	Reference
Soybean	Bud rot Damping off Root rot Rot	Hwang *et al.*,1996 Nelson *et al.*,1996 Liu and Sinclair, 1991. Yang *et al.*,1990
Sugar beet	Root rot, Damping off & Leaf blight	Herr, 1996
Turf grass	Brown patch Large patch	Herr and Fulton, 1995 Burpee and Martin, 1996
Tree seedlings	Damping off Root rot	Hietala and Sen, 1996 Hietala and Sen, 1996
Tobacco	Target spot	Ogoshi, 1987
Tomato	Leaf blight Fruit rot	Date *et al.*,1984 Strashnov *et al.*,1985
Wheat	Root rot	Rush *et al.*, 1994

The fungus *R. solani* generally spread through selerotia, plant materials contaminated with this fungus, soil spread by wind, water or during agricultural activities such as tillage and seed transportation. Keijer(1996) reported that during initiation of infection the hyphae from the germinating selerotium starts to grow towards a suitable host by the signaling of chemical exudates, e.g. organic acids, sugars, amino acids and phenols from the plants. The unattached hypha which do not attach to the plant after the first contact, starts to grow over the plant and within a short time the hypha flattens and initiation of directional growth over the epidermal cells takes place. The T-shaped hyphal branches produce thick infection cushions which strongly attach to the host epidermis. After the

formation of infection cushions the actual active penetration of the host take place, (Keijer, 1996).

According to Lehtonen (2009) the fungus identifies the appropriate host by its surface structure. Weinhold and Sinclair (1996) reported that the fungus can take entry into the plant through a weak spot on the surface by breaking down the protecting layer.

Demirci and Doken (1998) reported that during infection the swollen hyphal tips on infection cushions produce infection pegs which penetrate the cuticle, epidermal cell wall of epidermal tissues and the outer layer of the cortex. Though the penetration occurs through the degrading enzymes such as cutinases (Baker and Bateman, 1978), Pectinases (Bertagnolli *et al.*, 1996; Jayasinghe *et al.*, 2004) and xylanases (Peltonen, 1995), it also occurs through the establishment of hydrostatic pressure. According to Demirci and Doken (1998) also reported that when the fungi grow inside the host, it spreads inter and itracellularly by degrading the tissues. As a result necrotic lesions form on the epidermal tissue of the shoots, roots and stolons. Damping off of the young seedlings was also found to take place.

Studies on growth and physiology of the Pathogen:

The genus *Rhizoctonia* is a species complex of highly heterogeneous group of filamentous fungi. These fungi have similarities in their sterile as well as anamorphic state. The fungus do not produce any sexual spores i.e. conidia. Sexual spores i.e. basidiospores occur only rarely. The asexual stages of these fungi are known as anamorphic state and the sexual stages of this fungus are known as teleomorphic state. Therefore the genus *Rhizoctonia* includes a heterogeneous group of fungi which differ in their anamorphic, teleomorphic stage and also in morphology (Vilgalys and Cubeta, 1994).

Multinucleate *Rhizoctonia solani* isolates were grouped into fourteen anastomosis group (AG). [AG-1 to AG-10 including AGBI (Sneh *et al.*,

1991), AG-II (Carling *et al.,* 1994), AG-12(Carling *et al.,*1999) and AG-13(Carling *et al.,*2002a)] based on their anastomosis behavior. Multinucleate species of *Rhizoctonia* include *R. solani, R. oryzeae* and R. zeae and R. Oryzeae isolates are classified into one AG group each, WAG-2 and WAG-O respectively. (Sneh *et al.* 1991; Carling *et al.* 1994; 1999; 2002a). Binucleate *Rhizoctonia* isolates were grouped into different AGs by various authors. Sneh *et al.* (1991) grouped binucleate *Rhizoctonia* isolates into AG-A to S on the basis of hyphal pairings. Lipps and Herr (1982) decided seven ceratobasidium anastomosis groups (CAG -1, -2, -3, -4, -5, -6, and -7). Nineteen AGs of binucleate *Rhizoctonia* have been reported by various Japanese authors (Ogoshi *et al.,* 1979), including AG -A, -B, -Ba, -Bb, -C, -D, -E, -F, -G, -H, -I, -J, -K, -L, -M, -N, -O, -P and -Q. (Carling and Summer, 1992).

Rhizoctonia solani produces thread like hyphae. Colour of the hyphae is white to brown. Immature hyphae are white in colour, as the hyphae mature it turns into brown or dark brown in colour. Dolipore septum is present within the cross wall of the hyphae. Each cell is multinucleate, though binucleate Rhizoctonia is also present. Branches produce from the main hypha at right angles. Asexual spores are not formed by the mycelium. Small, oval cells are produced in branched chains or clusters. These cells are called monilioid cells. Monilioid cells have slightly thicker walls than the mycelium. When there monilioid cells are aggregate in large amounts, they form a resting structure called sclerotia. Sclerotia are black to brown in colour and 3 to 5 mm long. Main runner hyphae are usually wider than 7 µm. Very often sexual spores i.e. basidiospores are formed after invading the host cells. Basidia are formed when the environmental condition remains moist. Sufficient growth of the fungus also occurred in moist condition. Four spores are produced on each basidium. Each basidiospore has a single nucleus. The basidiospores are not enclosed in a fleshy, fruiting body or mushroom (Uchida, 2011).

Ritchie *et al.* (2009) observed that the mycelium of *Rhizoctonia solani* isolates from potato (AGs-2-1 and AG-3) grew best between 20°C and 25°C

on all media (Potato dextrose agar, Malt yeast extract agar, water agar, soil extract agar) tested. Mycelial growth of all isolates occurred between pH4 – pH9 with an optimum pH 5.6.In AG-3 isolates sclerotia formation occurred between pH4 – pH8 and in case of AG2-1 isolates the sclerotia formed between pH5 - pH6. Sclerotia germination took place in between 20-30°C and pH 5-6 in case of AG3 on all media tested. AG 2-1 isolates grew significantly slower compared to AG-3 in soil. Mycelia grew best in soil between 20-25°C regardless of anastomosis group. Germination of sclerotia of AG3 isolates in soil took place between 10°C and 30°C. Among all the artificial media tested greatest sclerotium yields were obtained on MYA for aG3 and PDA for AG 2-1.

Gottlie (1971) observed that mycelial growth of all the fungi was not indefinite. He indicated that an age dependent, growth regulating mechanism exist in at least some fungi and is responsible for restricted growth. According to him the growth rate of *R. solani* increased to maximum at 4-5 days then decreased until growth ceased at 8-9 days. The respiration of the peripheral hyphae of mycelia that have ceased growth is greater than that of the older parts of the thallus.

According to Kliejunas and Ko (1975) *Rhizoctonia solani* grew continuously at a steady growth rate on agar media but its' growth ceased on liquid media after a certain time. Like agar media the growth of *Rhizoctonia solani* also occurred continuously in autoclaved soil media.

Elarosi (1957) stated that the optimum pH value for *Rhizoctonia solani* growth is approximately 5.9. He also observed that the growth of *R. solani* is poor in pectin media when used as a sole source of carbon. But in pectin-agar medium the growth rate of *R. solani* increased.

According to Kumar *et al.* (1999) AG-II and AG-8 of *R. solani* causes bare patch of grain crops including lupin. AG-II grew faster than AG-8 on Potato Dextrose Agar media at several temperatures (10, 15, 20, 25 or 30°C). AG-II also grew best within pH range of 4 to 7. Growth of AG-8 was

best at PH-7. At 10°C there was no difference in the linear growth of both the AGs in soil but AG-II grew at a significantly faster rate at 20°C.

Gottlieb and Etten (1966) observed that the various contents of the mycelium of *R. solani* i.e. soluble amino nitrogen, deoxyribonucleic acid (DNA), ribonucleic acid (RNA), ergosterol and protein are decreased with the age of the fungi. Total lipids and fatty acids remained constant in *R. solani.* Total carbohydrate increased with age in *R. solani. R.solani* contained myristic, Palmitic, Palmitoleic, stearic, oleic, linoleic and Pentadecanoic acids. In *R. solani* the percentage of linoleic acid per total fatty acids decreased slightly when oleic acid increased.

Harikrishnan and Yang (2001) examined the sclerotial production of three R. solani isolates (AG -1, Ag -2 -2 and AG – 4) in the presence of the three soyabean (*Glycine max*) herbicides (Glyphosphate, imazerhapyr and pendimethalin). They observed that the growth of all the 3 isolates of *R. solani* was significantly reduced in pendimethalin where as in imazerhapyr and Glyphosphate the growth reduction was not significant. AG-1 and AG-2-2 produced sclerotia both invitro and in vivo where as AG-4 isolate did not produce sclerotia in vitro. In the presence of herbicide AG-1 showed a decrease sclerotial production and AG-2 -2 showed an increased sclerotial production in vitro. AG-1 and AG-2-2 isolated showed reduction in sclerotial production in vivo compared to AG-4 isolate. AG-4 isolate showed an increase in sclerotial production in the presence of herbicide. Production of sclerotia was generally higher in vivo than in vitro.

Tiwari and Khare (2002) reported that *Rhizoctonia solani* causing diseases in different plant parts of mungbean produces both imperfect (Hyphae, sclerotia) and perfect (basidiospore) stage in soil. In their experiment hyphal stage was successfully produced at 25°C in solid Richard's medium and sclerotia was produced at 30-35°C in Czapek's liquid medium. The perfect stage i.e. basidiospores was produced by soil method at 26 to 29°C and 95-100 % relative humidity in dark.

Webb *et al.* (2011) worked with the long term preservation of *R. solani* isolates and from their experimental result they reported that cryogenic methods (storage in liquid nitrogen) are suitable for the preservation or storage of *R. solani* cultures. They also reported that efficiency may vary in different isolates.

Antigenic relationship in host and pathogen

Plant disease can be checked effectively if control measures are taken at an early stage of disease development. The early and accurate detection of plant disease plays a vital role in any disease management programme. During the last four decades much attention has been paid on the phenomenon of common antigenic relationship among the closely related organisms or among the more distantly related organisms. When both the animal and plant hosts and their parasites or pathogens come in contact with each other they establish a serological resemblance between one another involving one or more antigenic determinants. In plants, several studies have concluded 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; Kratka *et al.*, 2002; Ghosh and Purkayastha, 2003; Musetti *et al.*, 2005; Eibel *et al.*, 2005; Dasgupta *et al.*, 2005, Chakraborty and Sharma, 2007; Saha *et al.*, 2010).

Botrytis cinerea were serologically different and 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 crossed immunoelectrohoresis (CIE) techniques could be used as valuable analytical tools in resolving the spectrum of antigens present, in *Botrytis cinerea* isolates. By using CIE techniques antigenic structures of *B. cinerea, B. tulipae, B. paeoniae* and *B. allii* isolates were also compared. Antisera against antigens of these isolates gave 24, 15, 20 and 14 precipitin peaks respectively, when analyzed in homologous reactions.CIE with an intermediate gel and CIE with antibody absorption *in situ* revealed that each isolate was serologically different from the other and has species-specific antigens. *B. cinerea* has eight distinct antigens which distinguished them from the other species of *Botrytis* (Ala-El-Dein and El-Kady, 1985).

Cross-reactive antigens and lectin were the 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 (Dazzo and Hubbell, 1975).

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 by Mohan (1988). 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.*

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

The possible involvement of cross-reactive antigens in host-parasite interactions between pea and some fungal plant pathogens were analyzed by Scala *et al.* (1994). 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.*

Kitagawa *et al.* (1989) developed competitive types of two novel enzyme-linked immunosorbent assays (ELISA) for specific detection of *Fusarium oxysporum* f. sp. *cucumerinum* as well as for general detection of ten strains of common *Fusarium* species. Antiserum against a strain of *Fusarium oxysporum* f. sp. *cucumerinum* (F 504) was raised in rabbits and a highly specific, sensitive and accurate ELISA for the homologous strain was developed by using the antiserum with β-D-galactosidase-labelled anti-rabbit IgG as a secondary antibody and cell fragments of the strain attached to amino-Dylark balls as the solid-phase antigens. This assay was specific for strain F 504 and showed little cross-reactivity with nine other

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

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

Polyclonal antiserum of mycelial proteins of *Verticillium dahliae* reacted positively with 11 of 12 isolates of *V. dahliae* from potato, cotton and soil but negatively with one isolate from tomato in indirect ELISA (Sundaram *et al.*, 1991). He also found positive results in detecting, *V. dahliae* and *V. albo-atrum* from infected roots and stems of potato in a double antibody sandwich enzyme linked immunosorbent assay (DAS-ELISA).

With the help of an indirect ELISA technique, Ricker *et al.* (1991) showed that increase in cross-reactivity in late bled antiserum (anti-Bc lgG), raised against water soluble antigens from *Botrytis cinerea*

corresponded with an increase in the overall serum titers for anti-Bc IgG to antigens of *B. cinerea.*

Daniel and Nilsson (1991) raised polyclonal antiserum against mycelial extracts of the rot fungus *Phialophora mutabilis* which reacted strongly with its homologous antigen and cross-reacted strongly to moderately with six other *Philosophora* soft rot spp. in ELISA.

Lyons and White (1992) compared results of conventional isolation techniques for *Pythium violae* using polyclonal antibodies raised to *P. violae* or *P. sulcatum* in competition ELISA.

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

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

conidia following treatment with antisera of leaves (TV-28) and indirect staining with FITC (Chakraborty and Saha, 1994).

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

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

Polyclonal antibodies against prehelminthosporol, a phytotoxin produced by the plant pathogenic fungus *Bipolaris sorokiniana* were raised in rabbits immunized with a prehelminthosporol–hexon conjugate by Akesson *et al.* (1996). 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.

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

Kratka *et al.* (2002) prepared four polyclonal and two monoclonal antibodies and tested to detect *Colletotrichum acutatum,* a quarantine pathogen of strawberry. They observed that only one polyclonal antibody was sensitive enough to recognize the pathogen. The antibody was genus specific that did not cross react with several other fungal pathogens of strawberry. They also detected *C. acutatum* by Plate trap antigen enzyme linked immunosorbent assay (PTA-ELISA), dot blot and immunoprint in roots, crowns, petioles and fruits in the latent age of the disease after artificial infection of strawberry (cvs. Elsanta, Vanda and Kama).

Ghosh and Purkayastha (2003) used polyclonal antibodies and antigens of ginger and *Pythium aphanidermatum,* a causal organism of rhizome rot disease for early diagnosis of rhizome rot disease of ginger. They detected *P. aphanidermatum* in ginger rhizome after eight weeks of

inoculation by agar gel double diffusion and immuoelectrophoretic tests, but only one week after inoculation by indirect ELISA.

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

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

Indirect ELISA was used to monitor the distribution of Mycoplasma like organism (MLO) in the experimental host *Vicia faba.* Post-embedding colloidal gold indirect immunolabelling was developed to identify, without ambiguity, the various forms of MLO cells in the different infected parts of the plant by transmission electron microscopy. Silver enhancement of the gold probe gave accurate histological and cellular localization of MLOs in tissue sections, by light microscopy. Both ELISA and immunolocalization first detected MLO in roots 17 days after inoculation with infectious leafhoppers (Lherminier *et al.*, 1994).

Hema *et al.* (2001) detected *Sugarcane streak mosaic virus* (SCSMV-AP) following Double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) and direct antigen coating (DAC)-ELISA. The virus was detected up to 1/3125 and 1/625 dilutions in infected sugarcane leaf, 5 µl

and 10 µl per well in sugarcane juice, 1/3125 and 1/625 dilutions in infected sorghum leaf and 10 ng and 50 ng/ml of purified virus in DAS-ELISA and DAC-ELISA tests, respectively.

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

Several viruses were detected by Abou-Jawdah *et al.* (2001) following ELISA. Potato virus Y (PVY), potato virus A (PVA), potato virus X (PVX), potato virus M (PVM), potato virus S (PVS) and potato leaf roll virus (PVX), potato virus M (PVM) potato virus S(PVS) and potato leaf roll virus (PLRV) from main production areas of Lebanon, the Bekaa and Akkar plains.

Shahriyari *et al.* (2011) developed a rapid and efficient method for detection of witches' broom disease of lime cuased by *Canditus* Phytoplasma *aurantifolia.* They employed a sensitive seriological technique (DAS-ELISA) along with recombinant DNA technology.

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

showed that CRA was concentrated mainly in the epidermal cells and also spread throughout the cortical cells.

Immunolocalization is a powerful tool for cellular location of different proteins or antigens. Now a days immunolocalization studies are performed using immunogold labelling which is successfully used for electron microscopy (Lee *et al.*, 2000; Trillus *et al.*, 2000; Nahalkova *et al.*, 2001; Kang and Buchenauer, 2002 and Wang *et al.*, 2003). For light microscopy, silver enhancement is done after gold labelling (Santen *et al.*, 2005; Saha *et al.*, 2006, 2010).

A gold sol was found which was able to localize the ECM (Extra cellular matrix) of *C. gloeosporioides* very well. In the case of *C. gloeosporioides*, the ECM secreted out from conidium just before germination took place. The area that ECM covered was wide-spread and could reach up to several times the spore width. With gold sol, the composition and nature of the ECM could be easily identified using cytochemical and biochemical approaches (Kuo, 1999).

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

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

Two antisera against acidic β-1,3-glucanase and acidic chitinase of tobacco was raised by Kang and Buchenauer (2002). They investigated 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.

Immunogold labelling technique was used by Wang *et al.* (2003) 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.

The location of CRA in tea (*Camellia sinensis*) leaves treated with antiserum raised against *Exobasidium vexans*, causal agent of blister blight of tea have been studied by Chakraborty and Sharma (2007). 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.

Saha *et al.*, (2010) showed that the level of CRA was less in susceptible plants in comparison to the resistant eggplants. They used light microscope to observe the specially treated plant tissues. To visualize the CRA they labelled the tissues by indirect immunogold technique and the labelled tissues were subjected to silver enhancement for visualization in the light microscope. They also correlated the level of CRA with the pathogenicity of *Colletotrichum gloeosporioides* in different eggplant varieties. They also substantiated their results with indirect ELISA.

Disease control by antagonistic organisms

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. Biological control is benign to environment. 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; Evueh *et al.*, 2008; Zivkovic *et al.*, 2010; Akrami *et al.*, 2011; Parizi *et al.*, 2012; Ajith *et al.*, 2012).

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 criterions to assess its potential as biological agent (Umamaheswari *et al.* 2008)

Rhizosphere microorganisms such as *Trichoderma* spp. were found to be antagonistic against *Fusarium solani*, the causal agent of root disease of

eggplant (Hundoo and Dwivedi, 1993). 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).

Pseudomonas, an antibiotic and siderophore producing strain from virgin soils (with forest trees) which displayed *in vitro* antibiosis against many plant pathogenic fungi was isolated by Baruah and Kumar (2002). 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.*

Management of anthracnose in french bean caused by *C. Gloeosporioides* was studied by Gupta *et al.* (2005). 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. In field 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).

Effect of antagonistic microbes and extracts of botanicals on *Sclerotium rolfsii,* incitant of collar rot of brinjal was investigated by Jadon *et al.* (2005). Efficacy of isolates of *Trichoderma* spp., *Pseudomonas fluorescens,* and *Gliocladium virens* in suppressing the growth of the pathogen was tested by dual culture technique. *T. viride* isolate was found to be superior to other isolates in reducing colony diameter and sclerotial production of the pathogen.

Mycostop, 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).

Raju (1991) and Vinod *et al.* (1991) reported that *Trichoderma* resulted in dieback and disintegration of *Pythium* spp by hyphal coiling and by producing inhibitory substances. Several other works had 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 Latunde-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).

Different isolates of *Trichoderma harzianum* showed differential antagonistic potential as biocontrol agent against *Sclerotium rolfsii* (Maity and Sen, 1985; Biswas, 1999).

Filonow (1998) observed that three antagonistic yeasts competed successfully for sugars since their uptake was faster and higher than that of *Botrytis cinerea* where competitiveness plays a central role in antagonism.

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* was evaluated by Prasad and Rangeshwaran (1999). 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.

From a comparative study of chemical, biological and integrated control of wilt of pigeon pea caused by *Fusarium udum*, it was found that bavistin was 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 (Pandey and Upadhyay, 1999).

Fourteen isolates of *Trichoderma* and *Gliocladium* speices were tested *in vitro* against *Sclerotium rolfsii*, the causal organism of root/ collar rot of sunflower. 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 *et al.*, 1999).

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.

Etebarian *et al.* (2000) reported *T. harzianum* isolate T39 and *T. virens* isolate DAR 74290, as potential biological agents, controlled the rot disease in potato and tomato caused by *Phytophthora erythroseptica*.

Twenty isolates of fluorescent pseudomonads were evaluated for 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 (Ramamoorthy *et al.*, 2002).

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 produces the antifungal metabolite 2, 4-diacetylphloroglucinol. The authors suggested that producers of this metabolite may have a broader role in disease-suppressive soils worldwide.

The potential of *Trichoderma harzianum, Trichoderma aureoviride* and *Trichoderma koningii* as biocontrol agents were evaluated by Perelló *et al.* (2003). 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).

A novel indigenous *Pseudomonous aeruginousa* strain was isolated from industrial waste water by Roy *et al.* (2007) 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.

Some biocontrol agents like the isolates of *Pseudomonous fluorescence* (PfC6 and PfCIAH- 196), *Bacillus subtilis* (BSW1and BST1), Trichoderma isolates-CIAH 175 and *Trichoderma harzianum* were tested 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%) (Umamaheswai *et al.*, 2008).

Four *Bacillus* spp isolated 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 (Jadeja and Bhatt, 2008).

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 %).

Zivkovic *et al.* (2010) reported on the antagonistic activity of *Trichoderma harzianum* and *Gliocladium roseum* against the pathogen *Colletotrichum acutatum* and *Colletotrichum gloeosporioides*, the causal agents of anthracnose disease in fruit crops. They showed that *T. harzianum* and *G. roseum* was promising biocontrol agent to prevent anthracnose disease of fruits.

Akrami *et al.* (2011) evaluated the effect of three isolates of *Trichoderma* named (*T. harzianum*) T1, (*Trichoderma asperellum*) T2 and (*Trichoderma virens*) T3, against the *Fusarium* disease, caused by *Fusarium oxysporum*. They found that T1 and T2 isolates and their combination were more effective than other treatments in controlling the disease, such that it reduced disease severity from 20 to 44% and increased the dry weight from 23 to 52%.

Monteiro *et al.* (2010) reported that *Trichoderma harzianum* ALL42 were capable of overgrowing and degrading *Rhizoctonia solani* and *Macrophomina phaseolina* mycelia, coiling around the hyphae with formation of appressoria and hook-like structures. They also analyzed the extracellular proteins secreted by *Trichoderma harzianum* using two-dimensional electrophoresis and MALDI TOF mass spectrometry. Endochitinase, β-glucosidase, α-mannosidase, acid phosphatase, α-1,3-glucanase, and proteases were identified in the gel and also detected in the supernatant of culture.

Parizi *et al.* (2012) analyzed the inhibitory effect of *Trichoderma viride* in vitro against Roselle pathogens i.e. *Phoma exigua, Fusarium nygamai* and *Rhizoctonia solani* using the dual culture technique. Maximum inhibition occurred against *P. exigua*, (reduced mycelial radial growth to 71.76%). Volatile and non-volatile inhibitors of *Trichoderma* were also evaluated for this purpose and non-volatile metabolites were tested against the pathogens. Maximum inhibition occurred against *R. solani* (73.95% mycelial growth inhibition), followed by *P. exigua* (37.17% inhibition).

Ajith *et al.* (2012) reported a new fungal antagonist *Zygosporium masonii* against *Colletotrichum capsici* incitant of anthracnose on bellpeppers. Formation of clear inhibition zone in dual culture and decrease in mycelial growth of pathogen were observed when treated with volatiles and non volatile compounds from the antagonist. *Z.masonii* treated seeds showed significant increase in seed germination, shoot length, root length and dry weight of the plant. They showed that *Z .masonii* was a potential antagonist to control anthracnose and could be used as a biocontrol agent (BCA).

Evueh *et al.* (2008) evaluated the effect of phylloplane fungi i.e *Aspergillus* sp., *Trichophyton* sp., *Trichocladium* sp. and *Gliocladium* sp. against *Colletotrichum* leaf disease of rubber (*Hevea brasiliensis* Muell. Arg.). Their finding showed that *Trichocladium* sp. and *Trichophyton* sp. exhibited the highest antagonistic effects on *C. gloeosporioides*.

Diseases control by botanicals:

Several plants have shown potential antifungal activity against pathogens of crop plants. Some plant products commonly known s botanicals contain antifungal molecules that are harmless and benign to environment. In addition there are certain advantages in the deployment of botanical pesticides. These are biodegradable, safe to non-target organisms, renewable and suit to sustainability of local ecology and environment. Moreover, the need for repeated application of fungicides to attain desirable level of disease control discourages the extensive adoption of chemical control by most of the farmers (Singh and Singh, 2005).

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

During evaluating of 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* (Kirkegaard *et al.*, 1996). Singh *et al.* (1997) reported that the tissue extract of Indian mustard was equally effective and hence the role of volatile isothiocyanates is implied. Certain phytochemicals like gallic acid and abscicic acid have been shown to be antifungal. For instance, abscicic acid was shown to inhibit mycelial growth and sporidial formation and also germination of teliopores.

Garlic extracts inhibited inhibited mycelial growth of *Fusarium solani, Colletotrichum lindemuthianum, Pythium ultimum* and *Rhizoctonia*

solani i. Aueous extract of powdered oven-dried (35 °C) garlic bulbs were incorporated into the growth medium. The hyphae of *R. solani* and *C. lindemuthianum* found to collapse hyphae of *F. solani* appeared thinner than in controls (Bianchi *et al.*, 1997).

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

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 were studied by Digrak *et al.* (1999). 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.

Two hundred and four species of traditional Chinese herbal medicines belonging to 80 families from Yunnan Province in People's Republic of China were 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 (Ke *et al.*, 1999).

Three thiosulfinates with antimicrobial activity were isolated from oil-macerated garlic extract and their structures were identified by them as

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

Leaves of five *Betula* species, *B. pendula, B. browicziana, B. medwediewii, B. litwinowii* and *B. recurvata* were collected 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* (Demirci *et al.*, 2000).

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

Methanol extracts from leaves, stem bark, root bark, fruits and seed kernels of *Butyrospomum pradoxum* (*Vitellaria paradoxa*) were analyzed. It was revealed that 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) had antimicrobial activity against bacteria (*Pseudomonas aeruginosa, Escherichia coli, Salmonella typhi, Staphylococcus aureus, Ralstonia solanacearum* and *Bacillus cereus*) and fungi (*Fusarium oxysporum* and *Candida albicans*) (Ogunwande *et al.*, 2001).

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

Antifungal activities of four polymmethoxylated flavons, isolated from cold-pressed orange oil were tested 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}(Almada-Ruiz *et al.*, 2003)

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* (Curtis *et al.*, 2004).

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

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

In the search for bioactive compounds, direct bioautography of lipophilic leaf extracts of medicinal plants used by Himalayan people was used in antifungal screening by Guleria and Kumar (2006b). *Alternaria alternata* and *Curvularia lunata* were used as test organism in bioautography. The results, evaluated by the diameter of the inhibition zone of fungal growth, indicate that five plant species, among the 12

investigated, and had shown antifungal activity. They used $CHCl_3$: CH_3OH (1:9, v/v) as a solvent to develop silica gel TLC plates. Clear inhibition zones were observed for lipophilic extracts of Vitex negundo (RF value 0.85), *Zantoxylum alatum* (RF value 0.86), *Ipomea carnea* (RF value 0.86), *Thuja orientalis* (RF value 0.80) and *Cinnamomum camphora* (RF value 0.89). The best antifungal activity was shown by lipophilic leaf extract of *T. orientalis.*

Thirty plant extracts (aquous extract) were screened 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 well results (showed 72%) inhibition (Kiran *et al.*, 2006).

Two mosses viz. *Entodon plicatus* C. Muell and *Rhynchostegium vagans* jaeg showed their antimicrobial activity against *Bipolaris sorokiniana, Fusarium solani* and *Pseudomonas sclanacearum, Xanthomonas oryzae.* 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 (Mewari *et al.*, 2007).

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) reported 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%.

Malabadi and Vijoy-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.*

Yasmin *et al.* (2008) reported that the varied degrees of inhibitory effects of fifty five aqueous extracts of angiospermic plants on vegetative growth of *Fusarium moniliforme* Sheldon *in vitro.* They reported the antifungal properties of *Andrographis paniculata* leaves and *Lagerstroemia speciosa* against bakanae disease or the first time .The leaf extract of *Lawsonia inermis* showed maximum inhibition (60.65 %) followed by roots of *Asparagus racemosus* (50.59 %) and also suggested that the possibility to control bakanae disease of rice using these plant extracts in seed treatment.

Bhosale *et al.*(2008) reported that the mancozeb (0.3%) controled 51 per cent leaf blight disease of onion followed by *Trichoderma viride* (38.84%). They also reported that highest onion bulbs were produced (10.57 t/ha and 10.31 t/ha) following application of the mancozeb and *Trichoderma viride* respectively. Two botanicals (produced from *Lantana camara* and *Psorelea pinnata*) were effective and controlled disease (27.08 % and 22.44 % respectively).

Parekh and Chanda (2008) evaluated the methanol extract of 9 Indian medicinal plants of different families, for in vitro antifungal activity against some yeasts including *Candida albicans* (1) ATCC2091, *C. albicans* (2) ATCC18804, *Candida glabrata* NCIM3448, *Candida tropicalis* ATCC4563, *Cryptococcus luteolus* ATCC32044, *Cryptococcus neoformans* ATCC34664, *Trichosporon beigelli* NCIM3404, and some moulds such as *Aspergillus candidus* NCIM883, *Aspergillus flavus* NCIM538, *Aspergillus niger* ATCC6275 and *Mucor heimalis* NCIM873. The *in vitro* antifungal activity was evaluated at three different concentrations by agar disc diffusion method and the activity obtained was not concentration dependent. A. flavus was the most susceptible fungal strain while *C. glabrata* was the most resistant one.

Aslam *et al.* (2010) reported the antifungal activity of plant diffusates from 5 indigenous medicinal plant species (*Adhatoda zeylanica, Azadirachta indica, Capparis decidua, Dodonaea viscosa* and *Salvadora oleoides*) of Potohar region. They tested the antifungal activity against 3 pathogens (*Alternaria solani, Rhizoctonia solani* and *Macrophomina phaseolina*) attacking commercial crops. All selected medicinal plants exhibited considerable reduction in radial growth of mycelia of the pathogens tested. They also observed that radial growth of selected pathogens reduced with the increase of concentration of plant diffusates. Among 5 concentrations of plant diffusates, the highest inhibition in radial growth of all 3 pathogens were observed at 200g/l. Minimum concentration found to control mycelial growth was 10g/l.

Al-Askar *et al.* (2010) investigated antifungal activity of ethanol-water extracts of four medicinal plants, cinnamon (*Cinnamomum verum* Presl.), anise (*Pimpinella anisum* L.), black seed (*Nigella sativa* L.) and clove (*Syzygium aromaticum* L. Merr. & Perry.) against pea (*Pisum sativum* L.) root-rot fungus *Rhizoctonia solani* and the efficacy of clove extract (concentration 4%) on disease incidence of *Rhizoctonia* root-rot of pea in the pot experiment in greenhouse. The highest antifungal activity (zero percent disease) was recorded in case of 1% clove extract application.

Singh and Kumar (2011) isolated seven *Trichoderma harzianum* coded as T1, T2, T3, T4, T5, T6 and T7 for their biocontrol potential against highly virulent *Fusarium oxysporum* f. sp *chrysanthemi* (Foc) isolate FO-10 and also evaluated Eight botanicals namely *Mentha arvensis* (MA), *Tagetus patula* (TP), *Eucalyptus sp* (ES), *Datura stramonium* (DS), *Calotropis procera* (CP), *Lantana sp* (LS), *Ricinus communis* (RC) and *Catharanthus roseus* (CR) for their biocontrol potential against Foc using food poison technique. Based on the performance of *Trichoderma harzianum* isolates T3, T4, T5 and botanical MA, TP and DS were selected for the biological control trials in pot conditions. Maximum disease control was recorded by the treatment of soil with botanicals [MA (70.0%), TP (61.0%) and DS (50.0%)].

Nisha *et al.* (2011) investigated the antifungal activity of aqueous extract of *Cannabis sativa*, *Parthenium hysterophorus*, *Urtica dioeca*, *Polystichum squarrosum* and *Adiantum venustum* against *Alternaria solani*, *Alternaria zinniae*, *Curvularia lunata*, *Rhizoctonia solani* and *Fusarium oxysporum* at different concentrations (5, 10, 15 and 20%). The extract of *C. Sativa* at 20% concentration showed maximum antifungal activity 100% and 59.68% against *C. lunata* and *A. zinniae* respectively. Leaf extract of *P. hysterophorus* controlled growth of *A. solani* upto 50%.

Abu-Taleb *et al.* (2011) reported that the extracts of *Rumex vesicarius* L. and *Ziziphus spina-christi* (L.) Willd. were antifungal compounds against two root rot pathogens (*Drechslera biseptata* and *Fusarium solani*) *in vitro*. Eight flavonoid subfractions (rutin, quercetin, myricetin, apigenin, quercetin-3-O-galactoside, luteolin, kaempherol and kaempherol-3-O-robinoside) and six flavonoid subfractions (apigenin-7-O-glucoide, quercitrin, quercetin, isovitexin, rutin and quercetin-3-O lucoside-7-O-rhamnoside) were isolated from the remaining aqueous layer fraction of *R. vesicarius* and *Z. spina-christi*, respectively. *F. solani* failed completely to produce spores when treated with ethanolic extract of *Z. spina-christi* at the concentration of 20% but plant extracts were more effective against to *D. biseptata*.

Seema *et al.* (2011) evaluated the antifungal effect of 10 plant extracts viz., *Thevetia peruviana*, *Ocimum basilicum*, *Piper betel*, *Murraya*

koenigii, *Chrysanthemum coronarium*, *Polyalthia longifolia*, *Catharanthus roseus*, *Pelargonium graveolens*, *Moringa officinalis* and *Lawsonia inermis* by poisoned food technique against *Rhizoctonia solani* Kuhn, the causal organism of sore shin disease of tobacco. Among them only four plants (*Lawsonia inermis*, *Piper betel*, *Polyalthia longifolia* and *Pelargonium graveolens*) showed significant antifungal activity against *Rhizoctonia solani*. Organic solvents viz., n-hexane, ethyl acetate and methanolic extracts of four plants of *Lawsonia inermis*, *Piper betel*, *Polyalthia longifolia* and *Pelargonium graveolens* showed 100% inhibition of mycelial growth and sclerotia formation of *Rhizoctonia solani* at 1000 ppm concentration.

Sasode *et al.* (2012) evaluated the effectiveness of some botanicals (viz., Neem, Eucalypts, *Datura*, Pudina, Tulsi and Lantanas). They tested crude as well as 10% extract against *Alternaria brassicae* under *in vitro* condition by poisoned food technique. Neem and *Eucalyptus* were also evaluated in the oil forms and boiled extract of Neem showed the minimum radial growth of the pathogen. The oil extract (Neem and *Eucalyptus*) were found less effective as compared to crude and boiled extracts.

Adejumo *et al.* (2012) reported that the antimicrobial activity of Partially (Pp) and completely purified (Cp) methanolic extracts of leaves of *Cassia alata*, *Cymbopogon citratus* (lemongrass), *Mangifera indica* (mango), *Carica papaya* (pawpaw), *Citrus limon* (lemon), fruits of *Xylopia aethiopica*, seeds of *Aframomum melegueta* (alligator pepper), *Citrus aurantifolia* (lime), *Garcinia kola* (bitter kola), *Piper guineense* (brown pepper), and rhizome of *Zingiber officinale* (ginger) using the poisoned medium and disc diffusion assay techniques on maize mycotoxigenic fungus: *Fusarium verticillioides*. *P.* guineense, *G. kola* and *A. melegueta* showed consistent higher growth inhibitions while *C. papaya* and *M. indica* had the least activities. They reported that *P. guineense*, *G. kola* and *A. melegueta* could be successfully used as environmentally-friendly, cheap, available, effective and sustainable alternative biopesticides.