LITERATURE REVIEW

Immunoassays are being used routinely for the detection of plant pathogens in vegetatively propagated plant material and seeds in conjunction with quarantine seed-testing, seed-certification, and pathogen-indexing programmes. While the majority of these programmes are directed toward the detection of viruses and bacteria, immunoassays have also been developed that detect fungi in seed and vegetative tissue. Tests such as Enzyme linked immunosorbent assay (ELISA), Indirect immunofluorescence assay (IFA), Dot blot and Western Blotting have demonstrated the sensitivity and specificity required to replace such time-consuming and expensive bioassays as indicator-plant inoculation, growing on tests, and dilution plating. These assays will help growers, crop-consultants and plant health care professionals from having to rely excessively on symptomatology and/ or time-consuming diagnostic procedures, and permit early detection of pathogens. This should be viewed as management tools. One critical application of immunoassay technology is the monitoring of pathogen spread in a crop system. Immunoassays have the potential to detect and quantify pathogen propagules in soil and other complex matrices. Direct detection and quantification of pathogens in soil may be more difficult to achieve as a result of low propagule counts, soil complexity, and other factors. However, highly sensitive tests using immunoassay and immunofluorescence combined with appropriate soil preparation measures will likely overcome some of these obstacles to provide information that can be used in making crop-management decisions.

A short and comprehensive review on (a) Immunodetection of plant pathogenic fungi and (b) Potentiality of biocontrol fungi for crop management have been presented below.

(A) Immunodetection of Plant Pathogenic fungi

Each and every living plant has its own immune system functionally similar to that of animals. Conclusive evidences are now available to confirm the existence of phytoimmunity but untike humoral immunity or specific target of antibodies that commonly operates in animals. The serological cross reactivity between host and pathogen has been a subject of considerable interest to a number of workers and a number of reviews pertaining to this area have been published previously (Damian, 1964, Devey *et. al.,* 1967; Devay *et. al.,* 1972; Purkayastha, 1973; Devay and Adler, 1976; Damian 1979; Clark, 1981; Chakraborty 1988; Purkayastha, 1989; Purkayastha *et. al.,* 1991, Purkayastha, 1994). Detection of plant pathogenic fungi within host tissues by serological means is a relatively recent development in the field of plant pathology. The merit of this method of detection lies in its ability to detect very small amounts of pathogen in tissues which is generally not detected by conventional techniques. Recent reviews have been published by Hansen and Wick (1993), Werres and Steffens (1994) and Chakraborty and Chakraborty (2002).

Protein extracts of resistant and susceptible cabbage seedlings inoculated with *Fusarium oxysporum* f. sp. *conglutinans* were subjected to electrophoretic and immuno-chemical analysis. By electrophoresis in starch gel, 4 components were separated but no significant differences were observed in uninoculated or inoculated resistant seedling extracts. In contrast, immunochemical analysis with rabbit antisera revealed upto 7 components in extracts of infected susceptible cabbage, compared with 4 infected susceptible cabbage, compared with 4 components in healthy susceptible and healthy and inoculated resistant plants. The additional components detected in infected susceptible cabbage were not original fungus protein. They may have been formed either by the fungus after infection or more likely by the infected plant cell. On the basis of different immunological experiments it was suggested that these components were not merely breakdown products but antigenic substances (Probably proteins) which differed from the

normally present substances in the healthy plants (Heitefuss *et. al.*, 1960). The relationship between antigenic substances produced by sweet potato in response to black rot infection caused by *Ceratocystis fimbriata* and the magnitude of disease resistance was pointed out by Uritani and Stahmann (1961). Tissue extracts of healthy sliced and black rotted sweet potato roots of several Japanese varieties showed precipitation lines with antisera towards corresponding extracts from an American variety (Sunny-side). Antigenic components designated as A and C were distributed in tissue extracts of all varieties. However, B and D were produced in response to the infection. The amount produced in several Japanese varieties was correlated with the degree of resistance. In healthy root tissue B and D seemed to be present in very small amounts and increased in response to simple injury or slicing but to a much lesser extent than that after infection.

The immunological responses of *Verticillium alboatrum* an *V. nigrescens* pathogenic to cotton were compared by Wyllie and DeVay (1970). On the basis of antigenic pattern *Verticillium* species were distinctly differentiated from one another. Defoliating strain of *V. albo-atrum* (T9) was shown to differ antigenically from the non-defoliating strain (SS4). It appeared to be more closely related serologically to the mildly virulent *V. nigrescens* isolates than was the defoliating T9 isolates.

Serodiagnostic methods for the differentiation between resistant and susceptible varieties of cotton infected with *Fusariam oxysporum* and *Citrus* sp. with *Phytophthora citrophthora* have been described by Abd-El, Rehim and Hashan (1970) and Abd-El-Rehim *et al.* (1971a). Serological and immunoelectrophoretical investigation on watermelon varieties, resistant and susceptible to *Fusarium semitectum* also revealed that the cultivars could be differentiated by the titre or the time after which reaction occurred between antisera specific to the pathogen and seed globulins. It was noted that a_2b globulin fraction was present only in resistant varieties (Abd-El-Rehim *et al.*, 1971b).

Wimalajeewa and DeVay (1971) detected common antigenic relationship between *Zea mays* and *Ustilago maydis*. A pair of compatible haploid lines and two dipoloid solopathogenic lines of *U. maydis* were used in serological studies. *Avena sativa* var. "Victory" and *Hordium vulgare* var. "California Mariout" were taken as resistant hosts. Certain antigens were found common between corn and *U. maydis*. A strong antigenic relationship existed between the solopathogenic lines 132 and 3-day-old Oat seedlings. Barley did not have any antigen in common with any of the *U. maydis* lines tested, Antigenic comparison of the four lines of *U. maydis* did not indicate any qualitatively significant serological difference among them.

Common antigens among four varieties of cotton (*Gossypium hirsutum*) and isolates of *Fusarium* and *Verticillium* species were compared by Charudattan and DeVay (1972). One antigenic substance was common among the varieties of cotton and isolates of *F. oxysporum* f. sp. *vasinfectum*, F. *solani* f. sp. *phaseoli*, *V. albo-alerum* and *V. nigresens*. Cotton varieties which were resistant or susceptible to *Fusarium* wilt shared the common antigen with both pathogenic and nonpathogenic isolates of *F. oxysporum* f. sp. *vasinfectum*. However, the common antigen was not shared between cotton and non-pathogenic isolates of *F. moniliforme*. In immunodiffusion tests five to eight precipitin bands were observed in homologous reactions; of these only one or two bands were common in heterologous reactions between the fungal and the cotton preparations. The common antigenic determinant shared by cotton and fungal isolates does not appear to be related to the severity of wilt symptoms, but it may affect host pathogen compatibility during the process of root infection.

Antigenic affinity among the saline soluble proteins of *Triticum aestivum* and *Avena sativa* and soil borne fungus *Ophiobalus graminis* were determined by Abbott (1973). Single precipitin band in immuodiffusion test was formed when antisera of the wheat and Oat roots were allowed to diffuse with the antigens of *O. graminis*. Common antigen was also shared by both avirulent and virulent

isolates of *F. oxysporhm* f. sp. *vasinfectum* with disease resistant and susceptible line of cotton. In all cases, the fungal isolates invaded and parasitized cortical tissues of cotton roots, but only those fungal isolates that caused disease become established in the vascular system (Kalyanasundaram *et al.*, 1978).

Rabbit antisera were raised against soluble extracts of *Phytophthora infestans* (race 4) and tubers of "Arran Banner" and "Golden wonder" potato cultivars showing field susceptibility and resistance respectively to late blight. Their antisera were then used to test for the presence of common antigens between extracts of the fungus and various host and non-host plants (Palmerley and Callow, 1978). Cross reactive antigen was detected between *P. infestans* (race 4) and potato tubers of both the field susceptible and field resistant cultivars and also between the fungus and leaves of tomato and tobacco. Common antigens were not detected between *P. infestans* (race 4) and leaves of non hosts (mung-bean, pea, radish, cucumber and maize), nor between potatoes and the alternative pathogen, (*Fusarium solani*) and two non-pathogens (Ustilago *maydis* and *Phytophthora cinnamoni*)

Charudattan and DeVay (1981) isolated, purified to homogeneity and partially characterised a major cross reactive antigenic substance (CRA) from conidial culture of *Fusarium oxysporum* f. sp. *vasinfectum* common to roots of cotton (*Gossypium hirsutum*). The tissue and cellular location of the CRA and their possible role in host parasite compatibility has been subsequently described by Devay *et al.*, (1981). Indirect staining of antibodies using fluorescein isothiocyanate (FITC) indicated that in cross reactions of cotton roots cut near or just below the root hair zone, the CRA was concentrated mainly around xylem elements, the endodermis and epidermal cells and was present throughout the cortex tissue. Protoplast prepared from cross sections of young cotton roots also contained the CRA which was concentrated in the region of plasmalemma. Treatment of conidia and mycelia of *F. oxysporum* f. sp. *vasinfectim* with

asntiserum to cotton and indirect staining with FITC indicated that the CRA was mainly present in hyphal tips and in patch like areas on conidia.

Chakraborty and Purkayastha (1983) detected cross reactive antigen shared between soybean cultivars and *Macrophomina phaseolina* causing charcoal rot disease. Rabbit antisera were raised against root antigens of soybean cultivars (soymax and UPSM-19) and *M. phaseolina* isolate (M.P1) and tested against homologous and heterologous antigens following immunodiffusion test. When antiserum of *M. phaseolina* was reacted against its own antigens and antigens of susceptible soybean cultivars (Soymax, R-184), strong precipitation reactions were observed. In case of resistant cultivars, (UPSM-19 and DS-73-16) no such reactions were observed. Reciprocal cross reactions between antiserum of the resistant cultivar and antigens of three isolates of *M. phaseolina* also failed to develop even weak precipitation bands. Four antigenic substances were found to be common between the susceptible soybean cultivars and isolates of *M. phaseolina* in immunoelectrophoretic tests, but no common antigens were detected between resistant cultivars and the fungus. Common antigens were also detected in extracts of urediniospores of *Hemileia vastatrix* and in leaf and root extracts of coffee plant. An antigenic disparity was observed between coffee plants of physiologic group D and E. Common antigens shared between coffee plants and urediniospores of *H. vastatrix* and their possible involvement in such interaction were discussed by Alba *et. al.* (1983). Serological relationship between *Colletotrichum corchori* and jute cultivars (JRC-212) was detected by Bhattacharyya and Purkayastha (1985).

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

Immunodiffusion, immunoelectrophoretic and cross immunoelectrophoretic analysis of rice antigens and their serological relationship between *Acrocylindrium oryzae* was determined by Purkayastha and Ghosal (1985). One precipitation band was observed when the antigen of *A. oryzae* was cross reacted with its own antiserum or against the antisera of four susceptible rice cultivars (Jaya, Ratna, IR-8, CR-126, 42-1). No precipitin band was detected between the antiserum of the resistant cv. Mahasuri and antigen preparation from three isolates of *A. oryzae* or between the antigens of resistant cvs. Mahasuri and Rusail and the antiserum of *A. oryzae.* Cross reactive antigens were detected in crude preparations and in purified preparations from mycelia of *Phytophthora infestans* race-4, and race -1, 2, 3, 4, 7 with antisera for potatoes cv. king Edward and cv. Pentland Dell by using an indirect enzyme linked immunosorbent assay (Alba and Devay 1985). They suggested that the fungal mycelia do not easily release cross reactive antigens (CRA) into synthetic media where they grow and most *P. infestans* CRA are thermolabile and can be concentrated by precipitation in the presence of 40% saturated amonium sulphate (SAS). An antigenic disparity was noticed when 40% SAS from *P. infestans* Race-4 mycelia preparation was assayed with asntisera for cvs. king Edward and Pentland Dell. The occurrence of CRA in *P. infestans* mycelium and their involvement in such interactions were discussed.

The common antigenic relationship between soybean cultivars and *Colletotrichun dematium* var. *treeneata* was ascertained following immunodiffusion, immunoelectrophoretic and crossed immunoelectrophoretic tests (Purkayastha and Banerjee 1986). At least one antigen was found to be common between host cultivar and the pathogen. No antigenic relationship was observed either between soybean cultivars and the non-pathogen (*C. corchori*) or avirulent pathogen (*C. dematium*).

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

Cross reactivity of antiserum raised against *Phytophthora fragaria* with other *Phytophthora* species and its evaluation as a genus detecting antiserum has also been discussed by Mohan (1989). Antiserum of *P. fragariae* isolates (Anti-PfM) reacted strongly with antigens from several *Phytophthora* species. Some cross reactions with antigens from *Pythium* species are decreased by fractionating on an affinity column of sepharose 4B bound to extracts of *Fragaria vesca* roots infected with *P. fragariae.* The affinity purified anti-PfM retained its high cross reactivity with the various *Phytopthora* species Anti-PfM could not be made specific for *P. fragariae* because it was raised against components shown to be antigenically similar in all *Phytophthora* species tested. However, immunoblotting with the affinity purified anti-PfM produced distinct patterns for *P. fragariae, P. erythroseptica* and *P. cactorum.*

Kitagawa *et al.,* (1989) has also developed 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 that show specific pathogenicities to different plants. Antiserum against a strain of *F. oxysporum* f.sp. *cucumerinum* (F504) was elicited in rabbits, and a highly specific, sensitive and accurate ELISA for the homologous strains was developed by using the antiserum with B-D-galactosidase-labelled antirabbit IgG as the secondary antibody and cell fragments of the strain attached to amino-Dylark balls as the solid-phase antigens. This assay was specific for strain F504 and showed little cross reactivity with nine other strains of *Fusarium* species including strain 501 of *F. oxysporum* f. sp. *cucumerinum.* Strain F501 possess pathogenicity against cucumber similar to that of strain F504, although slight differences were observed between these two strains regarding their spore formation and pigment production.

A polyclonal antibody was prepared by immunizing rabbit with mycelial extract of *Phytophthora infestans* reacted in an enzyme linked immunosorbent assay with mycelial extracts of two *Phytophthora* species but not with those of ten

were compared by immunodiffusion, immunoelectrophoretic and cross immunoelectrophoretic techniques for the presence of cross reactive antigens. Common antigens were found among the susceptible cultivars of groundnut and two isolates of *M. phaseolina,* but not between nonpathogens and groundnut cultivars. No antigenic similarity was found between non-pathogen and *M. phaseolina* isolates. Cross immunoelectrophoretic tests confirmed that at test one antigen was common between cvs. J-11 and TMV-2; Kadiri-71-1 and TMV-2 and kadiri-71-1 and isolates of *M. phaseolina* (Purkayastha and Ghosal 1987).

Changes in antigenic patterns were detected after chemical induction of resistance in susceptible soybean cultivar (soymax) to *Macrophomina phaseolina.* sodium azide (100µg/ml) altered antigenic patterns in cv.soymax and reduced charcoal rot disease (Chakraborty and Purkayastha 1987). Common antigenic relationship between susceptible rice cultivar (Jaya) and *Sarocladium orzae* could be altered by the application gibberellic acid (100µg/ml.) and sodium azide (100µg/ml). These chemicals reduced sheath rot disease of rice (Ghosal and Purkayastha, 1987).

Evaluation of antisera raised against pooled mycelial suspensions from five isolates (Pf-1, Pf-2, Pf-3, Pf-10 and Pf-11) representing five physiological races of *Phytophthora fragariae* for detecting the red core disease of strawberries by enzyme linked immunosorbant assay (ELISA) was done by Mohan (1988). Root extracts prepared from alpine strawberry *Fragaria vesca* and *F. ananassa* cv. Cambridge Favourite injected with any of the five isolates studied produced strong reaction in ELISA. In *F.vesca* ELISA-positive material was detectable 6-8 days after inoculation before macroscopic symptoms appeared. The cultivar Red Gauntlet, (resistant to Pf-1, 2 and 3 but susceptible to Pf-10 and II) reflected differential response in ELISA. The absorbance produced by extracts of plants infected with virulent isolates was significantly higher than that obtained with the corresponding extracts of plants inoculated with avirulent isolates. The ELISA test proved valuable in screening certified strawberry stock (Mohan, 1988).

other micro organisms found in potato. *P. infestans* mycelium in potato leaf tissue was readily detected by ELISA using either the plate trapped antigen or F(ab)2 antibody fragment techniques (Harrison *et al.*, 1980). Amount of mycelium in leaf extracts was estimated by comparing the values obtained in ELISA with those for known concentrations of *P. infestans* mycelium.

Cross reactive antigens shared by soybean cultivars and the different strains of *Myrothecium roridum* (M-1, ITCC-1143;ITCC-1409) were analyzed by Ghosh and Purkayastha (1990). Results of immunodiffusion revealed that common antigens were present only between virulent strain of *M.roridum* (M-1) and susceptible host cultivars (DS-74-24-2 and PK-327). No cross reactive antigen was detected in case of resistant cultivars (UPSM-19 and DS-73-16). Common antigenic relationship between soybean and *Colletotrichum dematium* var. *truncata* was also studied by Purkayastha and Banerjee (1990) using immunodiffusion, immunoelectrophoresis and indirect ELISA technique. Cross reactive antigens were detected between susceptible soybean cultivars and the virulent strain of *C. dematium* var. *truncata* but no cross reactive antigen was detected between soybean cultivars and avirulent pathogen (*C. dematium*) or non pathogen (*C. corchori*). Results of immunodiffusion and immunoelectrophoresis showed absence of common antigen between resistant cultivars (UPSM-19) and the pathogen while the results of indirect ELISA indicated the presence of common antigen between the two at a very low level. They compared antigenic patterns of untreated and cloxacillin treated soybean leaves which induced resistance of soybean against anthracnose disease. Disappearance of the antigen from cloxacillin treated leaves of susceptible soybean cultivar "Soymax" was correlated with alteration of disease reaction.

Monoclonal antibody (MAb) raised against haustorial complexes (VB10) isolated from pea leaves infected with the powdery mildew fungus *Erysiphe pisi* recognised a 45KDa N-linked glycoprotein which was specially located in the haustorial plasma membrane. This glycoprotein was clearly distinct from a

previously characterised 62kDa plasma membrane which was also specially located in the haustorial plasma membrane. These antibodies were used, along with MAb VB7 which binds to a major 62kDa glycoprotein in the cell wall and plasma membrane of both haustoria and surface hyphae to label haustoria within epidermal strips from infected pea leaves using indirect immunofluorescence. Results showed that all these glycoproteins recognised by MAbs expressed early in haustorial development (Mackie *et al.*, 1993). Molecular differentiation in the extrahaustorial membrane of pea powdery mildew haustoria at early and late stage development was subsequently focussed by Roberts *et al.* (1993).

Polyclonal antiserum raised against a strain of *Fusarium oxysporum* f. sp. *narcissi* was tested by enzyme-linked immunosorbent assay. Antiserum raised to cell wall fractions gave better recognition than to cytoplasmic fractions. Recognition was equally good in artificially and naturally infected bulbs. Little cross reactivity in bulb tissue was shown by three other bulb-rotting fungi. Nine isolates of *F. oxysporum* f. sp. *narcissi* from a wide geographic area gave similar results. Ten days after inoculation the pathogen was readily detected. in the most susceptible cultivar at points remote from the inoculation site. A direct correlation was observed between positive results in the enzymes linked immunosorbent assay and recovery of the pathogen on selected medium (Linfield, 1993).

Purkayastha and Pradhan (1994) observed that three strains of *Sclerotium rolfsii* were serologically different and their pathogenecities also differ markedly with host cultivars. Virulent strains showed common antigenic relationship with their respective susceptible host cultivars but not resistant cultivars. Antigenic change in a susceptible cv. AK-12-24 after treatment with a systemic fungicide kitazin was also evident. This change in host may be due to inactivation of a suppressor/ inhibitor gene for resistance by the fungicidal treatment. They suggested that the resistance could be induced in susceptible plants if specific antigens are eliminated by suitable treatment.

Chakraborty and Saha (1994) detected cross reactive antigens (CRA) shared between *Camellia sinensis* and *Bipolaris carbonum*. Antigens obtained from tea varieties, isolates of *B. carbonum* and non-pathogens of tea (*Bipolaris tetramera*) were compared by immunodiffusion, immunoelectrophoresis and enzyme-linked immunosorbent assay. 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 detected between isolates of *B. carbonum* and resistant varieties (TV-16, 25 and 26), non pathogens and tea varieties, as well as non pathogens and isolates of *B.carbonum*. Indirect staining of antibodies using fluorescein isothiocyanate (FITC) indicated that in cross sections of leaves (TV-18), the CRA was concentrated mainly around epidermal cells. Treatment of mycelia and conidia of *B. carbonum* with antisera to leaves (TV-18) and indirect staining with FITC indicated the presence of CRA in the young growing hyphal tips and conidia.

Polyclonal antisera were also raised against mycelial suspension of *P theae* (isolate-pt-2) causal agent of grey blight disease and leaf antigens of Teen-Ali-17/1/54 and CP-1 and immunological tests were performed in order to detect CRA shared by the host and 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 FITC also indicated the presence of CRA in the epidermal cells and mesophyll tissue of tea leaves. CRA was evident in the young hyphal tips of the mycelia and on the setulae and appendages of the conidia of *P. theae* (Chakraborty *et al.*, 1995a).

Another serological experiment was performed by Chakraborty *et al.* (1996) by raising polyclonal antisera against leaf antigens to tea varieties (TV-18, Teen Ali 17/1/54 and CP-1) and mycelial antigens of *G. cingulata* (isolate GC-1) separately in white rabbits. CRA was found among the susceptible varieties and *G. cingulata* isolates. Such antigens were not detected between *G. cingulata* and resistant varieties of tea, non pathogens and tea varieties as well as *G. cingulata*

and non-pathogens. In cross section of tea leaves (TV-18), the CRA was found to be concentrated in epidermal cells, mesophyll tissue and vascular elements.

Cross reactive antigens shared by *Fusarium oxysporum* and *Glycine max* were also detected using indirect immunofluorescence test by Chakraborty *et al.* (1997). For this, polyclonal antisera were raised against the mycelial suspension of *F. oxysporum* and root antigen of the susceptible soybean cultivar (UPSM-19). The immunoglobulin (IgG) fraction of those antisera were purified by ammonium sulfate precipitation and DEAE-Sephadex column chromatography. Antigens of susceptible cultivars showed higher absorbance values than resistant cultivars when tested against the purified anti *F. oxysporum* antiserum. Indirect fluorescence tests using FITC indicated that in cross-sections of roots of susceptible cultivars (UPSM-19) CRA were concentrated around xylem elements, endodermis and epidermal cells while in resistant varieties fluorescence was concentrated around epidermal cells.

Immunodetection of teliospores of *Telletia indica,* causal agent of Karnal bunt (KB) of wheat using fluorescent staining test were done by Gupta *et al.* (2000). Polyclonal antibodies were raised against teliospores in New Zealand white rabbits. The indirect immunofluorescence (IIF) test was developed using anti-teliospores serum and binding was monitored by goat-rabbit antibody conjugated to FITC. The standarization of IIF test was carried out by optimization of dilutions of anti-teliospores antibodies, fluorescent probe and exposure time. The teliospores of *T. indica* showed bright green, patchy and ring shaped fluorescence around the teliospore. The spore exhibited uniform distribution in discrete regions of spore probably in spore episporium. Similar fluorescence pattern in the teliospores of KB isolated from infected wheat seeds of cultivars HD 23328,UP 2338, PBW 393, WH 542, as well as RR 21 (susceptible cultivars) respectively, is an indication of the presence of similar antigenic configuration of teliospores. Again, they did not exhibit varition in the expression of teliospore associated molecular pattern during previous and subsequent years of infection.

Polyclonal antiserum raised against *T. indica* also reacted strongly in agglutination reaction with intact teliospores of pantnagar isolate. The wheat grains with different grades of infection could be readily detected by Seed Immunoblot Binding Assay (SIBA). The teliospores of Karnal bunt infected wheat seeds when kept for vigour testing on nitrocellulose paper, formed a coloured imprint after the paper was assayed. The SIBA developed should not only be a better indication of teliospores load on seed but also quality of seed in terms of vigour. The developed immuno detection method apparently proves to be useful in routine monitoring of wheat lots for the presence of Karnal bunt pathogen (Kumar *et al.*, 2000). Enzyme linked immunosorbent assay using PAb raised against *Colletotrichum falcatum* was performed in order to detect pathogen well before the symptom development. When 20 different sugarcane varieties were subjected to ELISA test after pathogen inoculation, it showed a clear variation in disease resistance among them as in field-testing. (Viswanathan *et al.*, 2000).

Immunological detection of *Sphaerostilbe repens*, *Trichoderma viride* and *Trichoderma harzianum* using DAC-ELISA formats have been demonstrated by Chakraborty *et al.* (2000) in order to develop strategies for management of violet root rot of tea. Polyclonal antibody based immunoassay for detecting *Fomes lamaoensis*, causing brown root rot disease of tea has also been developed (Chakraborty *et al.*, 2001a). Eight blood samples were collected and IgG were purified using DEAE cellulose. Immunodiffusion tests were performed in order to check the effectiveness of mycelal antigen preparations of *F. lamaoensis* for raising PAbs. Optimization of PAbs were done using indirect ELISA. Increased activity of PAbs against *F.lamaoensis* could be noticed from second bleedings, which continued upto fourth bledding. Root antigens prepared from healthy and artificially inoculated (with *F.lamaoensis*) tea plants (Teen Ali –17/1/54, TV-18, TV-22, TV-26, TV-27, TV-28, TV-30, S-449, BSS-2) were analysed following DAC-ELISA format. Such format was also used to detect the pathogen in infested soil. Young mycelia of *F. lamaoensis* gave bright fluorescence in indirect immunofluorescence tests using PAbs and FITC-conjugates of goat specific for

rabbit globulin. Such immunological assays developed for detection of *F. lamaoensis* in rhizosphere of tea plantation can enable disease prevention at an early stage.

Immunodiagnostic kits were developed for detection of *Ustulina zonata,* causing charcoal stump rot disease, in the soil and tea root tissues. PAbs were raised separately against mycelial and cell wall antigens prepared from 10-day-old culture of *U. zonata*. Optimization of PAbs were done using indirect ELISA. Two different ELISA formats such as direct antigen coated (DAC) and double antibody sandwich (DAS) were tested to detect the pathogen in soil and artificially inoculated tea root tissues. Indirect immunofluorescence using PAbs and FITC-conjugates of goat specific for rabbit globulin were assessed for their potential to detect mycelia and spores in soil (Chakraborty *et al.*, 2001b)

Serological cross reactivity between *Glomerella cingulata* and *Camellia sinensis* were studied by Chakraborty *et al.* (2002b). PAbs were raised against antigen preparations from mycelia and cell wall of *G.cingulata* (isolate Gc-1), causal agent of brown blight of tea, mycelia of *Fusarium oxysporum* (non pathogen of tea) and leaf antigens of TV-18 and CP-1. CRA was found among the susceptible varieties of tea and isolates of *G. cingulata* (Gc-1, 2 and 3). Such antigens were not detected between resistant varieties of tea and isolates of *G. cingulata* (Gc-1, 2 and 3); non-pathogen (*F. oxysporum*) and tea varieties; isolates of *G. cingulata* and *F. oxysporum* and between non-host (*Glycine max*, *Cicer arietinum* and *Camellia japonicum*) and *G. cingulata.* Antisera raised against cell wall preparations gave better recognition than that against mycelial preparations as observed in ELISA test with antigens of tea leaves of different ages.

(B) Potentiality of Biocontrol fungi for crop management

It was as early as 1934 when Weindling showed that *Trichoderma* had the potentiality to be effective agents for biocontrol. Later, isolation of toxic principle

from the culture filtrate of *Trichoderma* (Weindling and Emerson, 1936; Weindling, 1937, 1941) attracted the attention of plant pathologists to the importance of the genus related to the biological control of the disease. Isolation and identification of an antibiotic substance (Viridin) from *T. viride* by Brian and McGowan (1945) further raised hopes of controlling plant diseases by biological means. Since then, a dramatic increase in research efforts have been observed and several review articles (Papavizas and Lumsden, 1980; Schroth and Hancock, 1981; Kommedahl and Windels, 1981; Papavizas, 1985) have been published considering the use of specific microorganisms for the biocontrol of plant diseases. On the basis of the research findings on detailed aspects of *Trichoderma* species, two species mainly *T. harzianum* and T. viride are well recognised now a days all over the world as biocontrol agents.The present review will focus mainly on the potential for biocontrol of plant diseases by *Trichioderma* with special emphasis on its commercial uses.

Biocontrol by adding large amounts of *T. harzianum* its food base to soil is exemplified by the work of Wells *et al.* (1972). These researches were among the first to report the large-scale use of *Trichoderma* preparations on solid media for field control of *Sclerotium rolfsii* on tomato transplant. Their system, however, required large amounts of organic matter (4,200 kg per hectare) for disease control. Backmen and Rodriguez-Kabana (1975) grew *T. harzianum* on a commercial, insoluble diatomaceous earth granule impregnated with molasses and applied the granules by hand over rows of pea nuts at 112 or 140 kg per hectare 70 and 100 days after planting. At 140 kg per hectare, *T. harzianum,* significantly limited damage caused by *S. rolfsii* and increased yield over a three years period. Control by this method was equivalent to that obtained with PCNB. *Trichoderma* species, especially, *T. harzianum* grown on solid substrate have been tested with varying degree of success against the following diseases; white rot of onion caused by *Sclerotium cepivorum* (Abd.-EIMoity and Shatla, 1981; Abd-EIMoity *et al.*, 1982; Papavizas *et al.*, 1982), cucumber diseases and cotton wilt caused by *Veriticillium dahliae* (Fedorinchik *et al.*, 1975), *Rhizoctonia* damping off and blight of several crops caused by *Sclerotium*

rolfsii (Chet *et al.*, 1982 ; Elad *et al.*, 1980,1981a,b,1982a) and *Rhizoctonia* fruit rot of cucumber (Lewis and Papavizas, 1980).

Grosclaude *et al.* (1973) developed an indigenous method to apply conidia of the antagonist to wounds during cutting by means of special pruning shears. The antagonist applied by this method 48 hours in advance of inoculation with the pathogen protected two years old plum trees. Not only a preventive but also a curative treatment has been reported (Dubos and Richard, 1974) for the control of silver leaf disease with *T. viride* (Corke, 1978). The strain of *T. viride* used in these experiment does not produce antibiotics or enzymes and it is not allergenic (Richard, 1979). Attempts to suppress above ground diseases with *Trichoderma* have not been limited to wound applications. Tronsmo and Dennis (1977) were able to protect strawberry fruit against *Botrytis cinerea* and *Mucor mucedo* by spraying strawberry plants at early flowering, with aqueous suspensions of conidia of *T. viride*. In subsequent studies (Tronsmo and Tstaas, 1980), apple flowers sprayed with conidia of an isolate of *T. harzianum* capable of growing at low temperature had considerably lower incidence of dry eye spot in the field.

A greenhouse method for selecting biological agents to control *Rhizoctonia* rot of beans was developed by Cardoso and Echandi (1990). A soil amendment (SF-1) formulated by Huang and Kuhlman (1991a, b), when added at 1% (w/w) to soil, controlled more than 50% of damping off slash pine seedlings caused by *P. aphanidermatum, R. solani* in fumigated or non fumigated soils in the greenhouse. In soil amended with SF-21, the predominant fungus, *T. harzianum* was stimulated and the colony formation units increased and remained high for more than 50 days. Budge and Whipps (1991) applied glass house trials of *T. harzianum* for the biological control of *Sclerotinia sclerotiorum* in lettuce. Isolates of *Trichoderma* spp. were evaluated with two delivery methods suggested by Roiger and Jeffers (1991). The bioassay developed to evaluate isolates of *Trichoderma* spp. was effective.

The efficacy of antagonist *Trichoderma viride* in controlling the pathogenic activity of *Macrophomina phaseotina,* responsible for the dry root-rot of mung was evaluated by Kheri and Chandra (1991). The antagonist applied as seed coating reduced mortality due to *M. phascolina* from 19% to 8% in mung var T-44 and from 19% to 10% in var Pusa Baisakhi in unsterilized soil under green house conditions. The biocontrol efficacy of the antagonist showed an improvement in sterilized soil. The dry weight of shoots, grains and nodules showed an increase of 31.7%, 16.6% and 100% respectively in T-44 and 27%, 32.1% and 133.3% respectively in Pusa Baisakhi. Biological control of bean root rot disease caused by *Rhizoctonia solani* with *T. harzianum* (TC-11) was done by Liv (1991). Following in vitro dual culture technique and antibiosis bioassay, *T. harzianum* (TC 11) was shown to be strongly antagonistic to *R. solani*. Knudsen *et al.* (1991a, b) controlled various soil borne plant pathogens with the help of biocontrol fungus *T. harzianum.* They applied mycelial biomass of *T. harzianum* in alginate pellets with wheat bran.

Application of *Trichoderma* to seed was suggested as an alternative approach to introducing them into soil (Harman *et al.,* 1981). This method requires smaller amounts of biological material than in-furrow or broadcast applications. Control was achieved with conidia of several ultraviolet induced biotypes of *T. harzianum* (Papavizas *et al.,* 1982) and *T. viride* (Papavizas and Lewis, 1983). Improved yields were obtained in a *Rhizoctonia*-infested seed of corn and soybean treated with *T. harzianum* (Kommedahl *et al.,* 1981).

The use of *T. harzianum* as a seed treatment to control *R. solani* was found to be effective in field conditions (Elad *et al.*, 1982). Seed treatment with *T. viride* and *T. harzianum* was also found to be equally good for controlling damping of disease of tomato caused by *Pythium indicum* (Krishnamoorty and Bhaskaran, 1990), *Macrophomina phaseolina* infection on *Vigna mungo* (Shahzad *et al.,* 1991) and to control of root rot disease caused by *Fusarium solani* and *Rhizoctonia solani* on pea root (Diab *et al.,* 1990). Success of seed treatment has

been observed to depend on the isolates used (Papavizas *et al.*, 1982; Papavizas and Lewis, 1983), the age of the seed inoculant (Kommendahl *et al.*, 1981), the kind of soil and its microbiota (Hader *et al.*, 1984) the inoculum potential of the pathogens in the soil (Wu, 1982). Biological control practices for direct protection of plants from pathogens involve the development of antagonistic microorganisms at the infection court before or after infection takes place. The role of *Trichoderma* as fungal antagonists is known for a long time. The treatment of *Trichoderma* on pruning cuts of fruit trees has prevented infection by canker causing pathogens. Sprays with *Trichoderma* in the field also reduced *Botrytis* rot of strawberries and of grapes at the time of harvest and storage (Agrios, 1988).

Spraying the spore suspension of *T. viride* on to sunflower was able to reduce *Sclerotinia sclerotiorum* rot in field (Wu, 1991). Sesan and Tica (1990) controlled grape vine pathogens (*Botrytis cinerea, Armillariella mellea, Phomopsis viticola*) by the application of *T. viride* on seedlings during their hot bed forcing period. Spraying of emulsion of *T. harzianum* spore to control *Alternaria cassiae* and *A. carassa* on *Cassia obtusifolia* was applied by A,sellen *et al.* (1991). Besides spraying, Tschen (1991) controlled stem rot of Chrysanthemum caused by *Rhizoctonia solani* by the application of *Trichoderma* as a coating material.

The mechanisms proposed in connection with the biocontrol of *T. viride* and *T. harzianum* are presumptive. Suggested mechanism for biocontrol by the two species are antibiosis, lysis, composition, and mycoparasitism (Papavizas and Lumsden, 1980, Ayers and Adams, 1981, Cook and Baker, 1983). Several toxic metabolites are produced *in vitro* by the two antagonists, and there is some evidence that such metabolites are produced in bits of organic matter in soil. *T. viride* produce gliotoxin on seed coats in soil and noninoculated pea seed planted in natural soil containing the antagonist had gliotoxin in the seed coats. Besides, *T. viride* also produce various inhibitory substances (Wu, 1991). Dry root rot of mung bean caused by *Macrophomina phaseolina* was reduced by the

application of biocontrol agent *Trichoderma viride* isolates multiplied in organic substrates, such as coir pith, groundnut shell and press mud as row application in a acid soil condition (Raguchander *et al.*, 1993). Among the organic substrate, groundnut shell medium supported the production of maximum number of chlamydospores, better native *Rhizobium* modulation and higher yield. Sclerotial number and root rot incidence were greatly reduced in ground shell as compared to coir pith and press.

Arisan-Atac *et al.* (1995) characterised eleven strains of *Trichoderma viride,* 2 strains of putative teleomorph *Aypocrea rufa* and 9 of several other *Trichoderma sp.* by random polymorphic DNA amplification (RAPD) finger printing and screened for their ability to antagonize growth of European strains of the chest nut blight causing fungus *Cryphonectria parasitica,* using a dual-culture assay. The best strains were found in the species *T. harzionum, T. parecramosum,* and *T. viride.* A field experiment was conducted by Sankar and Jeyarajan (1996) to manage root rot of sesamum, caused by *Macrophomina, phaseolina,* by seed treatment with antagonists. *Trichoderma harzianum* of *T. viride* significantly reduced the root rot incidence to 10.1% and 12.8% respectively, compared to 60% incidence in the control plots. By seed treatment with *T. harzianum,* plants recorded a rhizosphere population of $35x10^3$ cfu/g. Carbendazim treatment did not increase the rhizosphere and soil population of antagonists. Soil population of *Trichoderma* spp. was maximum in all plots applied with *Trichoderma,* spp. High rhizospere/ soil ratio was recorded due seed treatment with antagonists. Seed treatment with *T. harzianum* significantly increased root length, shoot length, yield and oil content over the control.

Ten isolates of *Trichoderma* spp. were screened by Padmodaya and Reddy (1996) *in vitro* for their efficacy in suppressing the growth of *Fusarium oxysporum* f. sp. *Lycopersici. Trichoderma viride* was found highly inhibitory to *F. oxysporum* f. sp. *lycopersici* in dual culture followed by *T. harzianum* . Studies on production of volatile compounds by *Trichoderma* spp. revealed that *T. viride,*

as effective in reducing radial growth. The same isolates also proved effective in reducing radial growth of *F. oxysporum* f. sp. *lycopersici* in a study on production of non-volatile compounds by *Trichoderma* spp.

T. viride, T. harzianum I and II, *T. hamatum* and *G. virens* were used biocontrol agents to manage the ginger rhizome rot disease caused by *Pythium aphanidermatum* and compared with fungicide mancozeb (Usman *et al.,* 1996). Two years of field trials showed that the isolate *T. harzianum* I was efficient in controlling the disease both in solarised and non-solarised pots. The disease incidence was less and the yield was high in both the years. *T. hamatum* was the second best in both the years. In general, the yield was higher in solarised pots in both the years but significant increase in yield was obtained in the second year only. The weed growth was also suppressed in the solarised plot to an extent of 40%.

Baby and Chandramouli (1996) tested antagonistic potential of *Trichoderma* spp. and *Gliocladium virens* against primary root pathogens of tea viz. *Fomes noxius, Poria hypolaterita, Rosellinia arcuata* and *Armillaria* and *T. viride* that of *Rosellinia. G.virens* colonized all the pathogens fairly well. The antagonists showed moderate to high antibiosis against all pathogens excepting *Rosellinia.G. virens* showed high antibiosis to *Rosillinia.*Production of toxic metabolite(s) was more in *G. virens* than in *Trichoderma.* The effect of biocontrol agents and plant and plant product on *Macrophomina phoseolina* causing charcoal rot of cowpea and other soil microorganisms was studied by Ushamalini *et al.* (1997). Soil application of neem cake @ 150kg/ha and Farmyard manure @ 10t/ha reduced the charcoal rot incidence significantly and increased the yield. Both *Trichoderma viride* and *T. harzianum* recorded a root rot incidence of 17.0% and 17.6% respectively as against 38.3% in control. Seeds soaked in 10% extracts of *Adenocalyma alliaceum* and *Vitex nubundo* showed 98.4 and 97.8% germination compared to 92.3% in control. Neem cake registered the maximum

population of fungi, bacteria, actinomycets and minimum population of *M. phaseolina* both in the shizosphere and non-rhizosphere soil at the initial (20DAS) and later (60DAS) stages of crop growth while, the rhizosphere population of *T. viride* in both stage the crop was maximum in seeds treated with *T. viride* and in the non-rhizosphere region, neem cake and *T. harzianum* recorded the maximum population respectively at 20 and 60 DAS.

T. harzianum caused a great reduction in the infection level of damping-off and root-rot diseases and resulted in increased root weight both in pot and field experiments during two successive growing seasons (Abada, 1994). Eleven strains of *Trichoderma viride*, and 9 of several other *Trichoderma* sp. were characterized by random polymorphic DNA amplification (RAPD) finger printing and screened for their ability to antagonize growth of *Cryphonectria parasitica*, using a dual-culture assay. The best strains were found in *T. harzianum* and *T. viride*. The successful application of these strains against chestnut blight *in vivo* is demonstrated. (Arison-Atac *et al.*, 1995). Zimand *et al.*, (1996) observed that germination and germ-tube elongation of *Botrytis cinerea* on bean leaves were reduced in the presence of *T. harzianum* T39. A reduction of 20 to 50% in germ-tube biomass was observed 20 h after inoculation. This reduction in germination did not result in complete prevention of disease development on the leaves. The production of pectin-degrading enzymes by *B. cinerea* was measured up to 4 days after inoculation. They suggested that *T. harzianum* T39 acts by reducing the enzyme activities of the pathogen.

Hyphal interactions between *T. harzianum* and *Sclerotinia sclerotiorum* were investigated in dual culture and in sterilized soil, by light and scanning electron microscopy. In dual culture, partial degradation of the *S. sclerotiorum* cell wall was observed. In sterile soil, conidia of *T. harzianum* germinated and the developing mycelium made contact with that of *S.sclerotiorum* forming short branches and appressorium-like bodies which aided in holding and penetrating the host cell wall. *T. harzianum* conidia reduced the pre and post-emergence effect of

S. sclerotiorum in cucumber by 69 and 80%, respectively, and in lettuce by 46 and 72%, respectively. Hyphal mycoparasitism, rather than sclerotial parasitism, is suggested to be the mechanism by which *T. harzianum* controls *S. sclerotiorum* under these conditions (Inbar *et al.*, 1996).

Over 100 isolates of *T. harzianum* were obtained from soil samples and from the phylloplane of grape and orange. A sub sample of 48 isolates were tested and found to be antagonistic of *Botrytis cinerea* .The antagonistic activity of *T. harzianum* may be effective if it is integrated with other control practices, and may result in acceptable levels of disease control with reduced levels of pesticide use. (Latorre *et al.*, 1997). Numerous fungi and bacteria, including existing biocontrol strains with known activity against soil borne pathogens as well as isolates collected from the roots and rhizosphere of tomato plants growing in the field were tested for their efficacy in controlling *Fusarium* wilt of tomato. (Larkin and Fravel, 1998). Tomato seedlings were treated with the potential biocontrol agents in the greenhouse and transplanted into pathogen-infested field soil. Isolates of *G. virens* and *T. hamatum,* significantly reduced *Fusarium* wilt compared to disease controls.

Hervas *et al.* (1996) determined whether *Trichoderma harzianum,* applied alone or in combination to other biocontrol agents to chickpea cultivars 'ICCV 4' and 'PV 61' differing in their levels of resistance to Fusarium wilt, could effectively suppress disease caused by the highly virulent race 5 of *Fusarium oxysporum* f. sp. *ciceris*. Seeds of both cultivars were sown in soil amended with the three microbial antagonists, alone or in combination, and 7 days later seedlings were transplanted into soil infested with the pathogen. All three antagonistic microorganisms effectively colonized the roots of both chickpea cultivars, whether alone or incombination, and significantly suppressed *Fusarium* wilt development. In comparison with the control, the incubation period for the disease was delayed on average about 3 days and the final disease severity index and standardized area under the disease progress curve were reduced significantly

between 14 and 33% and 16 and 42%, respectively, by all three microbial antagonists. The extent of disease suppression was higher and more consistent in 'PV 61' than in 'ICCV 4' whether colonized by *B. subtilis*, nonpathogenic *F. oxysporum*, or *T. harzianum*. The combination of *B. subtilis* + *T. harzianum* was effective in suppressing *Fusarium* wilt development but it did not differ significantly from treatments with either of these antagonists alone. In contrast, the combination of *B. subtilis* + nonpathogenic *F. oxysporum* treatment was not effective by either antagonist. Two isolates of *T. harzianum* were tested by Kapat *et al.* (1998) to determine their capacity to reduce the level of hydrolytic enzymes produced by *Botrytis cinerea* both *in vitro* and *in vivo*, and to inhibit infection caused by *B. cinerea*.

Mathew and Gupta (1998) studied potential of seven promising biocontrol agents (BCAs) *Chaetomium globosum, Coniothyrium minitans, Gliocladics virens Laltisaria arvalis Trichoderma hamatum Rhizoctonia solani* Kuhn causing root rot of French bean (*Phaseolus vulgaris* L.) under *in vitro* and glasshouse conditions. *In vitro* evaluation of BCAs by dual-culture method revealed that *T. harzianum* caused maximum inhibition, followed by *T. hamatum, T.viride* and *G. virens*. In pot experiments, *G. virens* and *T. harzianum* proved superior to other antagonists in reducting pre-emergence root rot to 6.7 and 13.3% respectively, as compared to 36.7% in control. *T. harzianum* was also effective to reduce post emergence root rot. Pre-inoculation of antagonists proved to be superior method to check post emergence root rot.

Hazarika and Das (1998) tested isolates of *Trichoderma harzianum, T. viride* and *T. virens* for their potential to suppress *Rhizoctonia solani,* the French bean root rot pathogen, under *in vitro* conditions. All isolates inhibited growth of *R.solani.* Culture filtrate of *T. harzianum* and *T. viride* inhibited mycelial growth and sclerotial germination. Wheat bran substrate supported maximum growth of all isolates followed by farm yard manure and tea. Both *T. harzianum* and *T.*

viride effectively controlled the bean rot disease when they were applied as seed and soil treatment.

Prasad *et al.* (1999) tested fourteen isolates of *Trichoderma* and *Gliocladium* species *in vitro* against *Sclerotium roffsii,* 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. polysporum,* inhibited mycelial growth of the pathogen significantly. Among *Trichoderma* species, *T. harzianum* isolates PDBCTH 2 gave 61.4% inhibition of mycelial growth followed by PDBCTH 8 (55.2%) and PDBCTH 7(54.9%). Complete inhibition of sclerotial germination was obtained with the culture filtrates of *T. harzianums* (PDBCTH 2, 7 and 8) and *T. pseudokoningu.* The three *T. harzianum.* siolates and the *T. viride* isolate (PDBCTV-4) were superior under greenhouse conditon with PDBCTH8 showing maximum disease control (66.8%) followed by PDBCTH7 (66.0%), PDBCTV4 (65.4%), PDBCTH2 (61.6%) and were even superior to the fungicide, captan.

Experiments were conducted to determine the influence of VAM fungi, *Rhizobium* sp. and *Trichoderma harzianum* individually as well as in combinations on the material attributes of *Acacia nilotica* seedlings (Rani *et al.,* 1999). The ability of *Trichoderma harzianum* to control the rotting of pepper (*Capsicum annuum*) plant roots caused by *Phytophthora capsici* was studied (Ahmed *et al.,* 1999). Interaction between the fungi was assessed *in vitro* on three culture media (V8c, Czapek and 2% water agar) and *in vivo* in plants grown in a substrate inoculated with *P. capsici* and *T. harzianum.* Studies on mutual antagonism in vitro showed that *P. capsici* was inhibited by *T. harzianum;* however, the intensity of inhibition differed according to the medium used, being greatest on Czapek. Analysis of the fungal populations in the plant growth substrate showed that *T. harzianum* consistently reduced that of *P. capsici* over time. This reduction in pathogen population was associated with a reduction in root rot between 24 and 76% although plant growth (dry weight) was still reduced

by 21.2-24.7%, compared with the uninoculated control. In the absence of *T. harzianum* with the same pathogen inoculum level, the reduction in dry weight was 59.8–68.6% suggesting that *T. harzianum* reduced the damage.

Tomato is affected by many foliar and root diseases of which the soil borne pathogen, *Pythium aphanidermatum* inflicts considerable damage. Alice and Muthuswamy (1999) conducted an experiment to find the efficacy of biodegraded farmyard manure against the soil borne pathogen. To each 20 kg heap of decomposed farmyard manure and decomposed coir pith 100g *Trichoderma viride* commercial formulation (5g/ kg) were added after adjusting the moisture content to 50% w/w. It was thoroughly mixed and the heap was passed with red earth slurry at the sides. This was incubated for 30 days and was applied at a rate of 5g/ kg of pot soil. The following treatments viz. seed treatment (*T. viride* 4g/ kg of seed) along with soil application of biodegraded decomposed farm yard manure immediately after sowing, seed treatment along with soil application of biodegraded decomposed coir pith seed a week sowing significantly recorded 22.34, 22.34, 21.67 and 23.00% preemergence damping off incidence as against the control which recorded 48.67% disease incidence. Correspondingly there was increase in shoot vigour Index (720.89, 719, 78, 737, 04 and 744.75) and rot vigour Index (303.12, 299.3, 307.84 and 307.50). The control recorded a shoot length of 6.4cm and a root length of 2.9cm with a corresponding 331.59 and 148.85 shoot and root vigour Index. To conclude there is no difference in damping off incidence. Pertaining to the time of application viz., the soil application immediately after sowing and a week before sowing. The seed treatment with *T. viride* (4g/ kg of seed) along with soil application (5g/ kg of soil) of biodegraded decomposed farm yard manure and biodegraded coir pith was ineffective against *Pythium aphanidermatum.*

To test the efficacy of *Trichoderma viride* product in different carrier for root rot control of sunflower, three commonly available carriers viz., Talc, Lignite

and Koline were selected by Mohan and Jayarajan (1999). *T. viride* was multiplied in yeast molasses medium. It was mixed with carrier at the rate of 500ml/kg Kilogram of carrier and this was used as stock culture for seed treatment. Four gram of carrier per kilogram of seeds was used in each treatment. Treated seeds were then sown in earthen pots at the rate of 25 seeds in each pot containing 10 kg of black soil. Seeds treated with captan (@ 4gm/kg seeds) used as comparative check and seeds without any treatment served as control. Before sowing, pods were inoculated with 500g of ten day old *Macrophomina phaseolina* culture multiplied in sand maize medium. Each treatments were replicated four times. The result revealed that seed treatment with *T. viride and Tale mixture* @4g/ kilogram significantly reduced the root rot incidence of sunflower and also recorded maximum yield. Other parameters like. *T. viride* population, R. S. ratio, plant height and root length also optimum in *T. viride* + Talc mixture combination. This was followed by *T. viride* and lignite combination. The potential use of native isolates of *Trichoderma viride* as bicontrol agent demonstrated the antagonistic activity against *Macrophomina phaseolina* intecting rice fallow black gram (Rettinassababdy *et al.,* 1999). Six native isolates of *Trichoderma viride* (TV-1, TV-2, TV-3, TV-4, TV-5 and TV-6) obtained from the rhizosphere regions of rice fallow black gram of Karaikal district, U.T. of Pondichery were screened in *vitro* against *M. phaseolina* by dual culture technique. Among the six different native isolates tested, TV-3 was identified as an efficient antagonist which not only inhibited the growth but also reduced the sclerotial size of *M. phaseolina.* Among eight antagonistic microorganisms tested for their efficacy in suppressing *Rhizoctonia bataticola* under *in vitro* conditions, *Trichoderma viride* and *T. harzianum* overgrew the test fungus (Prashanthi *et al.,* 2000). In pot culture experiments, seed treatment and soil drenching of *T. viride* and *P. fluorescencs* reduced the mortality of seedlings to a maximum extent. However, seed treatment was more effective than soil incorporation with the above bioagents. Growth of *Aspergillus niger* was inhibited maximum by *T.*

koningi, followed by *T. harzianum* and *T. hamatum* in *in vitro* conditions. In pot culture experiments and field trials, seed+soil treatment with *Trichoderma* spp. showed reduction in collar rot disease incidence and improved the growth parameters of groundnut viz. number of seeds per plant, 100 seed weight, shelling percent, harvest index, pot yield, dry shoot and root weight (Rao and Sitaramaiah, 2000).

In another experiment, antagonistic microflora, viz. *Trichoderma viride, Trichoderma harzianum, Gliocladium* virens, *Bacillus subtilis* and *Pseudomonas fluorescens* were evaluated against *Ustulina zonata,* causing charcoal stump rot of tea. All antagonistic microflora were most effective in inhibiting the mycelial growth of *U. zonata* in dual culture. Inhibitory activity of autoclaved culture filtrates was much less as compared to filter sterilized culture filtrates. Inoculation of these antagonists by seedling root dip and soil application in disease sick pots significantly reduced mortality of plants, besides increase in plant growth and dry matter production of tea plants. Maximum reduction in plant mortality and highest plant growth and dry matter production was recorded in *T. harzianum* and *B. subtilis* treated plots (Hazarika *et al.,* 2000).

Influence of talc-based formulations of *Trichoderma viride* and *Pseudomonas fluorescens* on damping-off disease, growth of chilli seedlings and population of *Pythium aphanidermatum* was studied by Manoranjitham *et al.* (2000a, b) under pot culture conditions. Seed treatment with *T. viride* ($4g/Kg^{-1}$) + *P. fluorescens* ($5g\ Kg^{-1}$) showed 7.00 and 12.50% of post emergence damping-off, respectively against 27.50 and 54.75% in control. The treatment also increased the shoot length, root length and dry matter production of chilli seedlings, and reduced the population of *P. aphanidermatum* from $16.75x10^2$ cf U g^{-1} at 20 days after souling compared to $17.50x10^{-2}$ cfu g^{-1} and $17.08\ x10^2$ cfu g^{-1} in control.

Manoranjitham *et al.* (2000a, b) further reported that soil application of *Trichoderma viride* and *Pseudomonas fluorescencs* effectively checked the pre-

emergence and post-emergence damping off of tomato caused by *Pythium aphanidermatun* under pot culture experiments. Talc based formulation of antagonists significantly reduced the soil population of *Pythium* and increased the shoot length, root length and dry matter production of tomato seedlings.

Fungal isolates obtained from the rhizosphere of Wheat (*Tritium estivum*) Tomato (*Lycopersicon esculentum*), Brinjal (*Solanum melanogena*) and Diancha (*Sesbania sesban*) were used to suppress foot and root rot of barley caused by *Sclerotium rolfsii* (Bari *et al.*, 2000). Among, 250 fungal isolates, nine of the *Trichoderma* spp. six of *Fusarium* sp. and one of *Pythium* sp. showed positive ability to suppress the disease in barley. One isolate (TF-24) of *Trichoderma* sp. was most effective in increasing seed germination, growth promotion, and reducing the disease incidence of barley. Fungal isolates of *Trichoderma* sp. showed very strong antibiosis in both solid and liquid media against *S. rolfsii* than others.

Nalathambi *et al.* (2000) isolated some isolates of *Trichoderma* and *P. flourescens* from sugarcane under *in vitro* conditions. They reported that treatment with *P. flourescens* significantly reduced the seedling mortality besides better germination (40-50%), increased root and shoot length and seedling vigour than *Trichoderma* (15-20%) and fungicide application under mist chamber conditions. Association of identical bacterial colonies (10^2g) were isolated from surface sterilized root samples 7 weeks after soil application. Singh and Singh (2000) also observed that a local isolate of *T. harzianum* (ITCC No. 4542) directly attacked and lysed the mycelium and sclerotia of *Sclerotium rolfsii* when they grew the two fungi in dual culture in petriplates and soil. *T. harzianum* and *T. viride* treated sclerotia gave complete inhibition of schlerotial germination after 30 days of incubation in soil. In greenhouse experiment *T. harzianum* applied in the form of wheat bran culture of *S. rolfsii* infested soil gave as high as 85.54 and 83.55% disease control in first and second growth cycle of brinjal seedling respectively.

However, *T. viride* gave 86.18% and 85.63% disease control in first and second growth cycle of brinjal seedlings, respectively. The degree of disease control achieved increased in amount of biocontrol agents applied.

Mycelial and sclerotial growth of *Sclerotinia sclerotiorum* was significantly suppressed by *T. viride in vitro.* Collar rot disease in brinjal could be effectively controlled in pot test with unsterilized soil when the antagonists were applied before, after or simultaneously with *S. sclerotiorum* (Phookan and Challiha, 2000). Antagonistic fungi isolated from the rhizosphere of ginger were evaluated for biocontrol potential *in vitro* and *in vivo* against rhizome rot pathogen by Joseph and Sivaprasad (2000). Two isolates viz. *Trichoderma viride* and *Aspergillus fumigatus* significantly reduced disease incidence and pathogen build up. *T. viride* treated pots recorded disease intensity score of 0.3 and pathogen population of 26 cfu/ 50mg soil as against 7.7 and cfu of control. Further *T. viride* exhibited positive influence on plant growth. Gangopadhyay and Joshi (2000) used *T. harzianum* and *T. viride* in controlling root rot of cotton and chickpea in sick fields. Seed treatment with *Trichoderma* SD@4g 1kg seed provided 40-75% disease control against root rot due to *Macrophomina phaseolina* in these two crops. Eapen *et al.* (2000) isolated *Trichoderma* sp. from root and soil samples of cardamom. *T. harzianum* caused maximum suppression of nematodes, especially in native, non-sterile soil under green house conditions. All the isolates promoted the growth of cardamom seedlings, whether or not the plants were infested with root knot nematodes. A mixture of *Trichoderma* isolates when applied in two sick cardamom nurseries reduced the incidence of rhizome rot disease caused by *Pythium vexans* and *Rhizotonia solani* and root Knot nematode, population significantly.

Products to control soil borne pathogens such as *Sclerotinia, Pythium, Rhizoctonia* and *Fusarium* include *Coniothyrium minitans,* species of Gliocladium, *Trichoderma, Streptomyces* and *Bacillus* and nonpathogenic

Fusarium. Products containing *Trichoderma, Ampelomyces quisqualis, Bacillus* and *Ulocladium* are being developed to control the primary foliar diseases, *Botrytis* and powdery mildew (Paulitz and Belanger, 2001).

T. harzianum and *Alcaligenes* sp. strain AMB8 applied alone or in combination significantly reduced the incidence of *Phytophthora capsici* induced nursery rot disease of black pepper (Anith and Man Mohandas, 2001). The bacterial strains were able to survive on the stem cutting of black pepper, which is used as planting material. Combined inoculation had no effect on the population dynamics of the fungal or bacterial antagonist. The biocontrol agents also improved the root and shoot growth of the plants in the nursery. Eight isolates of *T. harzianum* were isolated from soils of different betelvine plantations of West Bengal on modified *Trichoderma* specific medium (TSM) and were tested by D'souza *et al.* (2001) for their cultural, morphometric characters and antagonistic potential against four major fungal pathogens of betelvine. Seed and seedlings rot complex of soybean caused predominantly by *Rhizoctonia solani*, *Sclerotium rolfsii*, *Macrophomina phaseolina* and *Fusarium* sp. in a major obstacle in increasing soybean production in many countries. It is very difficult to manager these pathogens as their nature of survival is both through the formation of sclerotia, Chlamydospores and saprophytic phase on soil organic matter. Biological control has emerged as an alternatives and promising means for management of such type of diseases. Biocontrol agents like *G. virens* and *T. harzianum* antagonise pathogens by antibiosis, competition, mycoparasitism or other of direct exploitation (Pant and Mukhopadhyay, 2001).

Amongst fungal antagonists tested by Sharma and Sharma (2001), *T. harzianum* and *T. viride* were found most effective in inhibiting mycelial growth of *Dematophora necatrix* in dual culture. Pre-inoculation application of *T. harzianum* and *T. viride* reduced seedling mortality in pot experiment. Disease incidence was significantly reduced when *T. harzianum* and *T. viride* were added naturally infested soil 15 days prior to seed sowing. Arya and Kaushik (2001) studied the efficacy of six species of *Trichoderma*, against the forest tree nursery

damping of fungi, namely *Fusarium oxysporum, Pythium aphanidermatum* and *Rhizoctonia solani* both *in vitro* and *in vivo.* Evaluation of the fungal antagonists by dual culture method revealed that *T. viride* caused maximum inhibition of all the three pathogens followed by *T. harzianum, T. hamatum* and *T. longibrachiatum.*