DISCUSSION

Host parasite interactions are generally initiated in nature by the fungal spores since they come in contact with the host cells at the first instance. Therefore, they play a very good recognition phenomenon. The presence of cross-reactive antigen (CRA) between plant host and their parasites and the concept that these antigens might be involved in determining the degree of compatibility in such interactions have been reviewed earlier by several authors (De Vay *et al.*, 1972; DeVay and Adler, 1976; Kalyansundaram, 1978; Chakraborty, 1988; Purakayastha, 1989).

In the present study root antigens of eight soybean varieties and isolates of *F. graminearum* (Fg1 and Fg2) were cross reacted . Antigens from two non pathogens of soybean, viz. *Glomerella cingulata* and *Pestalotiopsis theae* were also considered for serological comparisons. It is significant to note that in immunodiffusion test susceptible varieties shared the common antigens with both the isolates of *F. graminearum* (Fg1 and Fg2) tested. However, antigenic disparity was noticed in cross-reaction with antigens and antisera of resistant variety and the isolates of the pathogen.

The preparation and treatment of antigens are most important because most antigens are labile and easily denatured. The selections of test animal as well as the amount of antigen for immunization purpose are also important since too much material may reduce antibody formation. Moreover, a number of factors such as age of plant tissue, culture of microbes and methods of extraction of antigen have profound influence on the yield of antigenic substance and this may account for the failure to detect common antigens as suggested by DeVay and Adler (1976).

The present study has established very definitely the importance of cross-reactive antigens between host and pathogen in determining the responses of the host to pathogen. This has also been supported by works of several previous workers (Chakraborty and Purakayastha, 1983; Purakayastha *et al.*, 1991, Chakraborty and Saha, 1994; Chakraborty *et al.*, 1999). It is also important in studies on host parasite relationship to determine the cellular location of the CRA.

For this purpose in this study fluorescence tests were conducted with cross section of tea roots as well as mycelia and conidia of *F. graminearum*. Cross sections of soybean roots were treated with anti- *F. graminearum* followed by staining with FITC conjugated antirabbit globule specific goat antiserum. Bright fluorescence was observed in case of epiblema region, and cortical cell.

Treatment of root sections with *F. graminearum* revealed that the CRA was concentrated mainly around cortical zone. Treatment of mycelia and conidia of *F. graminearum* with homologous antiserum and FITC showed a general fluorescence that was more intense on young hyphae. DeVay *et al.* (1981) determined the tissue and cellular location of major CRA shared by cotton and *F. oxysporum* f. sp. *vasinfectum*. On the basis of strong fluorescence obtained at the epidermis, cortex, endodermis and xylem tissues, they suggested that the CRA determinants in roots have a general distribution. DeVay *et al.* (1981) also used FITC labelled antibodies for races of *P. infestans*, to detect the CRA in potato leaf section. It was also reported by Charkraborty and Saha (1994) that CRA between tea and *B. carbonum* were mainly present in the hyphal tips and in patch like areas on the condia, mycelium and mainly around epidermal cells and mesophyll tissues of the leaf. The cellular location of CRA between *P. theae* and tealeaves was also established by Chakraborty *et al.* (1995).

In the present investigation using antibodies indirectly labelled with fluorescein isothiocyanate (FITC) the location of CRA in cross sections of soybean roots of resistant (UPSM-19) and susceptible (Soymax) cultivars and fungal cells (*F. graminearum)* has been established. Treatment of mycelia and conidia of *F. graminearum* with antiserum to roots of cv. Soymax and using indirect staining with FITC indicated that CRA was mainly present in young hyphal tips and in patch like areas on conidia. The results are also in conformity with the work of DeVay *et al.* (1981a) involving treatment of conidia and mycelia of *Fusarium oxysporum* f. sp. *vasinfectum* with antisera to cotton.

The tissue and cellular location of major CRA shared by cotton and *Fusarium oxysporum* f. sp. *vasinfectum* was determined by DeVay *et al.* (1981a). Cross sections of young cotton roots with antiserum to *F. oxysporum* f. sp. *vasinfectum* and followed by an antirabbit globulin specific goat antiserum FITC conjugate exhibited strong fluorescence at the epidermis and xylem tissues indicating a general distribution of CRA determinants in roots. FITC labelled antibodies for races of *P. infestans* were also used to detect CRA in potato leaf sections (DeVay *et al.*, 1981). In cross section of tea leaves (TV-18), cellular location of CRA shared by *B. carbonum* was evident mainly around epidermal cells (Chakraborty & Saha, 1994).

Polyclonal antibodies were raised against mycelial antigens of *F. graminearum* and these were used for determining the presence of cross reactive antigens (CRA) between soybean varieties and *F. graminearum* as well as for the immunodetection of the pathogen in root tissues and in soil. Since enzyme linked immunosorbent assay (ELISA) has proved to be one of the most sensitive serological techniques, PAbs raised against *F. graminearum* were used in ELISA test for pathogen detection. Since ELISA is a very sensitive technique and non-specific binding interferes with the actual antigen-antibody reaction, initially PAbs were purified and IgG fractions were used in all further tests. Prior to other tests, the sensitivity of assay was optimised and the minimum detectable antigen concentration and optimum IgG concentration were determined in homologous reactions. Positive results were obtained with very low concentration of both antigens and IgG. It was reported by Mohan (1988) that a concentration of *Phytophthora* antigens as low as 2µg/ ml could be detected in indirect ELISA by antiserum raised against pooled mycelial suspensions of five *P. fragariae* races. Chakraborty *et al.* (1996) also reported that antiserum raised against *Pestalotiopsis theae* could detect homologous antigens at 25 µg/ml. Antiserum dilution of upto 1:16000 was effective for detection.

The presence of CRA among *F. graminearum* and soybean varieties was evident in indirect ELISA, using PAb raised against mycelial antigen preparations of *F. graminearum* at a concentration of 40 µg/ml with soybean root antigens at a concentration of 100 µg/ml. Though much difference was not observed in ELISA values among the different varieties Soymax the most susceptible variety exhibited the highest value. Alba & DeVay (1985) also detected CRA in crude and in purified preparations from mycelia of *Phytophthora infestans* with antisera of potato at concentration lower than 50 µm/ml protein using indirect ELISA. The presence of CRA in several host pathogen interaction has also been reported by a number of previous workers. e. g. Soybean and *Myrothecium roridun* (Ghosh and Purakayastha (1990): Groundnut and *Macrophomina phaseolina* (Purakayastha and Pradhan (1994); Tea and *Bipolaris carbonum* (Chakraborty and Saha, 1994); Tea and *Pestalotiopsis theae* (Chakraborty *et al.*, 1995) and Tea and *Glomerella cingulata (*Chakraborty *et al.* (2002). Cross reactivity of the PAb raised against *F. graminearum, T.harzianum* and *T.viride* were tested with other fungal species. Results revealed that PAb of *F. graminearum* reacted to some extent with two entomopathogenic isolates *Beauveria* and *Metarrhizium* species.

CRA was also detected in crude preparations and purified preparations from mycelia of *Phytophthora infestans* (races 4 and 1,2,3,4,7) with antisera of potato cvs. King Edward and Pentland Dell in concentrations lower than 50 µg protein/ ml (Alba & DeVay, 1985) using indirect ELISA. Antiserum raised against *Phytophthora fragariae* detected homologous soluble antigen at protein concentrations as low as 2 µg/ml (Mohan, 1988). Indirect ELISA could also readily detect CRA in semi purified mycelial preparation of *B. carbonum* at concentrations ranging from 5-25 µm/ml with antiserum dilution 1:125. In cross-reaction with antiserum of susceptible tea variety (TV-18) with antigenic preparation from *B. carbonum* (isolate BC-1) higher absorbance value was detected than the reaction with resistant variety (TV-26) of tea (Chakraborty & Saha, 1994). Based on these findings it can be assumed that indirect ELISA may serve as an important technique to detect cross-reactive antigens, even in those

interactions where conventional serological techniques have failed to detect (Johnson, 1962; Caroll *et al.,* 1972*).* Mohan (1989) showed that antisera raised against mycelial suspension of *Phytophthora fragariae* (PfM) reacted strongly with antigens from several *Phytophthora* species. He observed that anti-PfM could not be made specific for *P. fragariae* because it was raised to components shown to be antigenically similar in all *Phytophthora* sp. tested. Harrisen *et al.* (1980) further reported that anit *P. infestans γglobulin* reacted strongly with extract of *P. ervthroseptica* in DAC-ELISA but not with extracts of nine unrelated fungi or a culture of bacterium *Erwinia carotovora,* all of which were saprophytes of pathogens of potato.

With the advent of more sensitive techniques like ELISA, detection of plant pathogens in host tissues is now possible even when the pathogen concentration in host tissues in very low or when visible symptoms have not yet developed. This offers a definite advantage over classical techniques and is thus gaining an importance for pathogen detection purposes. Various formats of ELISA using polyclonal antisera has found wide spread application in plant pathology and are routinely used for detection and identification purposes (Clark, 1981; Chakraborty *et al.,* 1996 and Viswanathan *et al.,* 2000). Viswanathan *et al.* (2000) reported that presence of *Colletotrichum falcatum* in sugarcane tissues could be detected by ELISA. They reported that when twenty different sugarcane varieties were subjected to ELISA test after pathogen inoculation a clear variation in disease resistance was seen. They suggested that this technique could be reliably used to screen sugarcane genotypes for red rot resistance at an early stage. In the present study presence of *F. graminearum* in soybean root tissues could be detected by DAC-ELISA using PAb raised against mycelial antigens. It was observed that PAb of *F. graminearum* could also react with antigens from roots infected with other pathogens showing certain degree of cross reactivity. Since PAbs raised against *F. graminearum* could detect the presence of the pathogen in root tissues, it was decided to determine the efficacy of the PAb in detecting the specific pathogen in the soil. Detection of specific pathogen in soil equally or

more important than detecting the pathogen in the root tissues. Detection of specific pathogens in soil requires very sensitive techniques, which would make it possible to differentiate between the various microorganisms. Use of serological techniques most specifically ELISA are gaining importance in such studies. In the present study, initially antigens prepared from soil collected from various tea estates were tested against PAb of *F. graminearum* by DAC-ELISA. Of the twenty-five soil samples tested four samples showed high A_{405} values while all the other had relatively low values. Thus it was possible to identify these soils as being contaminated with *F. graminearum*. Wakeham and White (1996) reported the ability of polyclonal antisera of *Plasmodiophora brassicae* to detect the presence of the pathogen in soil. In another study Walsh *et al.* (1996) reported serological detection of spore balls of *Spongospora subterranea* and its quantification in soil. They reported that the antiserum could detect about 100 spore ball/gm soil but discrimination of spore ball levels appear to be better for concentration greater than 2000/gm soil. There was a quantifiable relationship between concentration of spore balls and ELISA values. In the present study, using spiked soil, the ELISA values decreased with decrease in concentration of spores. Thus ELISA showed potential for detection of *F. graminearum*, *T. harzianum* and *T. viride* in soil.

Detection of pathogen in host tissues using antibody based immunoflurescent technique has been reported by several previous authors (Warnock, 1973; Hornok and Jagicza, 1973; Reddy and Anantanarayanan, 1984). Dewey *et al.* (1984) suggested, on the basis of immunofluorescence studies that chlamydospores, basidiospores and mycelia of *Phaeolus schweinitzii* contained molecules antigenicaly related to species specific antigens secreted by mycelia grown in liquid culture. They also demonstrated the presence of mycelium and chlamydospores in naturally and artificially infested soil samples, using this technique. *Phytophthora* could be detected in soil by immunofluorescence antibody technique (Watabe, 1990).

The dot immunobinding technique has been found to be rapid and sensitive method for detection of fungal pathogens is a more recent application of this method. Antiserum specificity obtained against fungal pathogen varied greatly in the studies done by Lange *et al.* (1989). The antiserum against *Plasmodium brassicae* used in their study showed no cross reaction with other common rest pathogen (*Pythium ultimum, Rhizoctonia solani and F. oxysporum.* In this study, antigen of mycelia, amended soil, soil from infected plot, healthy and *F. graminearum* inoculated soybean root, mycelial antigen of biocontrol fungi were prepared and tested on nitrocellulose paper against PAbs raised against *F. graminearum*, *T.harzianum*, and *T. viride* using fast red or NBT/ BCIP as substrate. Antigens of homologous source, soil of infected plot showed deep coloured dot. Infected soybean root antigens also showed deep coloured dot when compared to healthy confirming the presence of fungal pathogen. Wakeham and White (1996) got positive detection of soluble components of the spore wall and whole resting spores of *P. brassicae* in PBST.

Complex mixture of antigens can be separated by high-resolution techniques such as sodium dodecyl acrylamide gel electrophoresis using discontinuous buffer systems and two-dimensional techniques. However one separated in this matter, it has been difficult to determine which of the separated species reacted with a given antiserum. Several methods have been developed previously. Towbin *et al.* (1979) overcame these problems by electrophoretically transferring the separated mixture onto nitrocellulose. The PAbs of *F. graminearum*, *T.harzianum* and *T. viride* are very much specific for detection of the pathogen in the soil, infected root tissues and in different isolates of fungi. Walsh *et al.* (1996) also performed Western blotting using the raw serum of *Spongospora subterranean* spore balls.

Consequent to the study on the detection of *F. graminearum* root tissues and soil, experiments were conducted both *in vivo* and *in vitro* for the management of root rot disease. For this purpose *Trichoderma harziamum*

T. viride were selected and experiments were conducted as a biocontrol agents Both inhibited the growth of *F. graminearum in vitro.* There are several reports on the ability of *T. harziamum and T. viride* to inhibit the growth of pathogen under invitro condition. 10 isolates of *Trichoderma* species were screened by Padmodaya and Reddy (1996) in *in vitro* for their efficacy in suppressing the growth of *Fusarium oxysporum* f. sp. *lycopersici, Trichoderma viride* (H) was found highly inhibitory to *F. oxysporum* f. sp. *lycopersici* in dual culture follwwed by *T. harziamum* (A. P.). Studies on production of volatile compounds by *Trichoderma* species revealed that *T. virde* (H), *T. viride* (A. P.) and *Trichoderma* sp. (D) as effective in reducing radial growth of *F. oxysporum* f. sp. *lycopersici* in a study on production of non-volatile compounds by *Trichoderma* spp. Baby and ChandraMouli (1996) tested antagonistic potential of *Trichoderma* spp. and *Gliocladium virens* against primary root pathogens of tea viz *Fomesnoxius, P. hypolaterita, Rosellinia arcuata and Armillaria* and *T. viride* that of *Rosellinia G. virens colonozed all the pathogens excepting Rosellinia. G. virens* showed high antibiosis to *Rsosellnia.* Production of toxic metabolitel(s) was more in *G. virens* than *Trichoderma* Hazarika *et al.* (2000) also tested the antagonistic effect of *Trichoderma harzianum* against *U. zonata,* causing charcoal stump root of tea in dual culture method. Both antagonists were most effective in inhibiting the mycelial growth of *U. zonata.* Assam and Tamil Nadu isolates of *T. harziamum, T. viride* and *T. biride* were tested by Hazarika and Das (1998) for their potential to suppress *Rhizoctonia solani.* Culture filtrate of *T. harziamum and T. virde* inhibited mycelial growth and scolerotial germination. Wheat bran substrate supported maximum growth of all isolates followed by firm yard and tea. Both *T. harziamum and T. viride effectively* controlled the bean rot disease when they were applied as seed and soil treatment. In dual culture of 11 isolates of *T. harziamum* three isolates, viz. T8, T10 and T2 were effective against *Sclerotium rolfsii,* the causual agent of stem rot of groundnut and they overgrew the pathogen up to 92%, 85% an 79% respectively, in *vitro.*

Phookan and Chaliha (2000) reported that the growth of *Sclerotinia sclerotiorum* was significantly suppressed by *Gliocladim virens* and *T. viride* significantly. Amongst fungal antagonists tested by Sharma and Sharma (2001), *Trichoderma harziamum* and *T. viride* were found most effective in inhibiting mycelial growth of *Dematophoranectrix* in dual culture.

T. harziamum and *T. viride* were tested in vivo for their ability to reduce violated root rot intensity. Of the various delivery systems tested for this biocontrol agents, tea waste formulation were found t be most effective. Disease intensity was reduced by both *T. harziamum* and *T. viride* when tested under potted conditions as well as in the field. This was observed in all tested varieties. This research are in conformity with that of Hazarika *et al.* (2000). They reported that planning of tea seedlings after dipping roots in spore suspension of *T. harziamum* reduced 56.6% mortality of plant due to *U. zonata* infection. This was also obtained with *T. viride* and *G. virens.* However, they observed that the reduction of morality of plant increased to 62.2% when *T. harziamum* were applied as soil drenceh.

The role of *T. harziamum* and *T. viride* bio control crops is well established. Sarker and Jayarajan (1996) reported that *Seasamum* caused by *Macrophomina phaseolina* was significant and 12.8% respectively compared to 60% incidence in the control pots Prasad *et al.* (1999) found 3 *T. harziamum* isolates (PDBCTH-2, 7 and 8) and the *T. viride* isolate (PDBCTV-4) were highly efficient in controlling root / collar rot of sunflower caused by *Sclerotium rolfsii. Trichoderma* spp. are common antagonists found in almost all the soils. Many isolates produced volatile and non-volatile antibiotics. The most effective antagonists from literature are species of *T. harziamum, T. longibrachiatum and T. viride.* In *Trichoderma* mycoparasitism is one of the main mechanism(s) involved in biocontrol followed by competition and antibiosis. Excretion of extracellular enzymes viz. cellulase chitinase, etc., exhibited by *Trichoderma* spp. which were found a good source for the lysis of the cell walls of the pathogen.

Efficacy of two isolates of *T. longibrachiatum* against *R. solani* was reported by Sreenivasaprasad and Manibhushanrao (1990a, b).

Studies on the mycoparasitism of the antagonists reveals that the hyphae of *T. longibrachiatum* form small hook/peg like structures pressing on the host, coil loosely around the host hyphae directly or produce small branches that coiled tightly. On the other hand, with *T. virens*, the host hyphae intermingled; certain of the host hyphae cells, which were vacuolated, became empty. Further, the hyperparasite coil around the host hyphae and adhere through small appresoria-like structures which even penetrate them at certain points (*Manibhulhanrao et al.*, 1989b).

Baby (1992) further studied the biocontrol potential of *T. virens* (Tv1) by incorporating into soil the wheat-bran-saw dust (WBSD) preparation (15-d) at 10 to 25 g/kg soil and obtained 10 and 57% protection respectively. Incidentally, isolate Th1 showed strong inhibition to the pathogen *R. solani.* Hence, the quantity of commercial products of *Trichoderma* kg/ha can be determined based on the *R. solani* populations in soil. Recently, Jeyarajan and Nakkeeran (1995) discussed about the exploitation of a commercial product of *Trichoderma.* This product for field use could be safely stored for 75-d at a constant temperature of 20-30°C.

Kumaresan and Manibhushanrao (1991) studied the hyperparasitic potential of *T. longibrachiatum* isolates (Th 1 and Th 2) against *R. solani* to understand the associated mechanism(s) involved in the biocontrol efficacy of the antagonists. The isolate Th 1 showed extensive hyperparasitism leading to wall lysis and the protection against ShB fungus was more in *in vitro* studies, while Th 2 isolate surpassed Th 1 in rendering protection to rice plants under green house conditions. This ability of Th 2 might be due to more aggressive coiling of host hyphae. It is pertinent to mention here that in biocontrol strategy, any interference by direct introduction might not yield the microorganisms with the ecological balance of the soil microflora, might not yield the expected result over a long-term

basis. However, the ecological balance of soil saprophytes can be manipulated by a suitable organic amendment to promote a specific group of soil microflora.

Mycoparasitism of rice ShB fungus, *R. solani* by *Trichoderma* spp. and *Gliocaldium* spp., is well known (Baby and Manibhushanrao, 1996a.b.) Among the many potential antagonistic soil fungi, *G. virens* and *T. longibrachiatum* of late have been used as biocontrol agents as they have been termed as presumptive and potential mycoparasites. Of late, the genus *Gliocladium* was merged with *Trichoderma,* hence *G. virens* is referred as *T. virens* in the subsequent literature.

It is generally opined that combination of two or more systems such as fungal antagonists, organic amendments, PAB and systemic fungicides provide high level control than that could be achieved with each method alone. This would expose the soil saprophyte (ShB fungus) to a 'double / triple barrier' whereby the resistance would protect the fungicide from a tolerant isolate of the pathogen and the fungicide would protect the resistance from novel compatible virulence. While the organic manure will increase the residential or introduced antagonists this will again reduce the level of primary inoculums as well as incidence of ShB disease.

A comprehensive monographic study on Rice Sheath blight disease was published by Manibhushanrao (1995) with emphasis on various methods of integrated control. *Trichoderma* species are known to have greater tolerance to a broad spectrum of fungicides are able to tolerate treated soil more rapidly than other soil competitors and are also effective against a wide spectrum of plant pathogens under various conditions.

As early as 60 years ago, it was opined that *Trichoderma* might serve as a potential biocontrol agent against soil-borne pathogenic fungi (Weindling and Emerson, 1936), and it has become an established fact in the following years (Baker, 1989; Adams, 1990; Lynch *et al.* 1991; and Haran *et al., 1996*) *Trichoderma* spp. are capable of effectively controlling a range of pathogens as well as being rhizospehere competent. They also provide varied levels of biocontrol of important soil-borne pathogens viz. *R. solani, Verticillium* spp.

Phythium spp. (Sivan and Chet, 1986 and Devaki *et al., 1992).* The efficacy of *Trichoderma* spp. as biocontrol agents of groundnut stem rot and root-rot diseases were reported (Sreenivasaprasad and Manibhushanrao, 1993).

In the damping off sugar beet caused by *P. aphanidermatum,* control was augmented by using *T. harzianum* and metalaxyl seed treatment (0.1%) simultaneously. However, the antagonist or metalaxyl alone did not yield the level of control (Mukhopadhyay and Chandra, 1986). When *Rhizoctonia ataticola* infested soil was infested with wild and tolerant strains of *T. harzinum* significantly lower disease incidence occurred to the control and other treatments. Significant reduction was observed under both the conditions (Vyas and Khare, 1986). *Trichoderma* sp. with outstanding ability to adapt to extreme conditions of temperature, soil and moisture and pesticides (fungicides) are used by various scientists in India.

Growth enhancement by the fungal antagonist *Trichoderma* spp. is an added advantage of biocontrol agents. In many of the claims from researchers from India, this potential is exploited and the reasons attributed are either due to the phytosanitizing effect or elimination of minor pathogens and clolonizers or due to growth promotive effect (Bagyaraj *et al.,* 1979, Elavarasan, 1989 and Kumaresan and Manibhushanrao, 1991).

Trichoderma spp. is a potential biocontrol agent for control of soil-borne diseases of small cardamom, the queen of species in India. Cardamom is affected severly by *Phytophthora meadi* causing capsule rot, rhizome rot caused by *Phythium vexans and R. solani. Trichoderma vifide and T. harzianum* proved to be effective under nurseries and in plantations (Joseph Thomas *et al., 1996).* Indian scientists are visualizing the commercial formulations of *Trichoderma* s for control of plant diseases. Coffee husk or tea waste and these two in combinations with farmyard manure supported luxuriant growth *T. viride.*

The biological control of important diseases of crop plants like pearl millet, rice, sunflower, maize and cash crop mulberry caused by *Sclerospora grtaminicola* can

effectively be controlled using *Trichoderma viride, T. harzianum, Chaetomium globosum, Aspergillus niger* and *Bacillus subtilis* (Shishupala, 1988 and Shishupala and Shetty, 1989).

Due to the outstanding performance of *Trichoderma* spp. in the control of broad spectrum of plant pathogens, it is essential to improve the biocontrol potential through strain improvement. In India, a continuous attempt to release improved strains through genetic manupulation, protoplast fusion, DNA transformation, producing mutants etc., are in progress. The development of protoplast fusion technique allows recombination in the progeny with different characteristics from two or more parental strains. *Trichoderma* strains developed through protoplast fusion showed very high biocontrol potency and pesticide resistance. These strains exhibited changes at phenotypic and genotypic levels. Thus, development of fungicide tolerant / resistant strains can be successfully used in association with IPM concept (Viji *et al.*, 1993).

Application of wheat bran saw dust (WBSD) preparation of *Trichoderma* spp. has been useful to control several soil-borne, root-infecting pathogens under field conditions (Baby and Manibhushanrao, 1993). This study should be extended on a priority basis to rice ShB control. A suitable, cheap and freely available substrate is recommended for India marginal farmers. This concept is termed as transferring expertise from lab-to land or reaching the unreached. In another study, Sivan *et al.*, (1984) found that a mixture of peat and wheat bran was a much better substrate. Various kinds of formulations can be made which not only ensure survival but also promote activity of the biocontrol agent with the appropriate substrate (Chet *et al.*, 1979; and Sivan *et al.*, 1984). Though *Trichoderma* spp. especially *T. virens* and *T. harzianum* formulations are available in the market, the commercialisation is not popularised. In India, scientists need to work more to bring the commercial forms of microbial antagonists come out as viable and feasible alternative to bio-pesticides useful to farmers.