Discussion

In nature, plants generally have a versatile multi-component defense adequately equipped to provide them protection against most of their potential pathogens; only a few of them can overcome this defense and cause disease (Sinha, 1995). Varieties within a host species are resistant when they possess one or more resistant gene(s) and susceptible when they lack any such gene. The spatial and temporal deployment of plant defense responses involves the complex interplay of signal events often resulting in superimposition of signaling processes (Graham and Graham, 1996). In a host-pathogen interaction, a potential pathogen may recognize features of a plant which signal the suitability of that plant for parasitism. On the otherhand, the potential host may be able to detect or recognize the potential fungal pathogen or non-self and use the initial act of recognition to trigger a range of induced resistance mechanisms (Callow, 1982 & 1983; Purkayastha, 1994). Every plant possesses a diverse array of resistance mechanisms operating at different levels and successful infection is dependent on the ability of pathogen to avoid or overcome each in turn.

In the present study, at the onset, pathogenicity test of *Fusarium oxysporum* was carried out on ten cultivars of soybean. Of the ten cultivars tested, Soymax was the most resistant followed by Bragg while JS-2 and UPSM-19 were most susceptible. Differences in pathogenicity of *Macrophomina phaseolina* to different cultivars of soybean have been reported by previous workers (Agarwal et al 1973; Gangopadhay, *et al.* 1973. Oswald and Wyllie 1973; Purkayastha *et al.* 1981). It has been reported that most fungal and viral pathogens of soybean seedlings, plants, pods and seeds have an asymptomatic or latent period after infection or colonization (Sinclair, 1991). Of the reported diseases of soybean atleast 10-15, including *F. oxysporum* are known to cause latent infection. It has been shown by Rizvi and Yang (1996) that soybean seedlings contain number of fungal flora, some of which are pathogenic and others are non-pathogenic. Percentages of major fungal taxa isolated from soybean seedlings were- *Rhizoctonia solani* 27.5%, *Fusarium* sp 11.9%, *Pythium* sp and *Phytophthora sojae* cumulatively 60.5%. Repeated pathogenicity tests confirmed that *Pythium* sp, *Phytophthora sojae* and R. *solani* were the major causal fungi associated wit the seedling disease complex of tested soybean.

Deposition of pathogen inoculum on the host surface is followed by spore germination, penetration and growth of mycelia within the host tissue. This depends on a number of factors which may be environmental or of host origin. In view of this, the effect of some of the important factors on mycelial growth of *F. oxysporum* and bacterial growth of *B. japonicum* have been investigated *invitro. Maximum* mycelial growth of *F. oxysporum* occurred at an incubation period of 9 days, p^H 5 with yeast extract as nitrogen and dextrose as carbon source. Since the nature of the fungal spore wall is important in the initial recognition and subsequent infection processes, in the present study the nature of conidial wall component of *F. oxysporum* was determined by agglutination test. Responses of conidia to different lectins revealed that the surface mainly contained L-D glucopyranoside and N-acetyl L-D galactosaminyl residues. Cristinzio et al (1988) reported that the surface of conidial wall of *F. solani* and *F. oxysporum* contained L-D mannosyl and L-D galactosaminyl residues while these were not found to occur in the outermost layer of the conidial wall of *F. culmorum, F. sambucinum, F. graminearum, F. aveneceum, F. moniliformae* or *F. xylariodes.* On the basis of studies with FITC-conjugated lectins Mercure *et al.* (1995) reported that the material released from conidia of *Colletotrichum graminicola* was a glycoprotein and contained glucose and or mannose. In case of *B. japonicum* optimum incubation period was 8 days, pH was 7 and maximum growth occurred with yeast extract and mannitol as nitrogen and carbon sources, respectively.

As *B. japonicum* is a usual component of soybean rhizosphere, experiments were conducted to determine the effect of seed bacterization with *B. japonicum* followed by growth of plants in *F. oxysporum* infested soil on disease development. Results clearly indicated that preinoculation with *B. japonicum* reduced disease intensity significantly. Such results have also been obtained by several previous workers. Tu (1978) observed that the development of root rot disease of soybean caused by *Phytophthora megasperma* decreased with increasing rhizobial population in the soil. Tu demonstrated that rhizobia could colonize hyphae particularly at their tips. Savada (1982) reported that as a result of interaction of *Rhizobium melilotii* and *Fusarium oxysporum* in the rhizosphere of alfa-alfa both root rot and nodulation was decreased. Similar findings were also reported by Chakraborty & Chakraborty

(1989) in case of *R. leguminosarum* and *F. solani, sp. pisi* on pea. Fully sporulated culture of *Bacillus cereus* and sterilized filtrates of this culture were effective in protecting alfa-alfa seedlings against damping off (Handelsman et. al 1990). A cell free culture filtrate of *B. subtilis* was also reported to significantly reduce disease intensity on alfa alfa seedlings from 56% to 16%, although treatment of seedlings with washed cell suspension had no influence on disease (Douville and Boland, 1992). Suspensions from washed or non-washed *Saccharomyces cerevisiae* cells and filtrates of the suspension reduced the development of *Colletotrichum graminicola* as well as the expression of anthracnose on maize leaf when they were previously or concomitantly treated with these preparations (Dasilva and Pascholati 1992). The authors attributed the reduction of the development of *C. graminicola* and disease expression on the leaves by filtrates of cell suspension of *S. cerevisiae* to a thermolabile substance or complex of substances released from the cells into the filtrates. Crop improvement and disease suppression by a *Bacillus* sp. from pea rhizosphere was also reported by Kumar (1996). Rupe *et al.* (1996) isolated fungi from the rhizosphere and rhizoplane of healthy or mildly diseased plants collected in areas of fields severely affected by sudden death syndrome of soybean (SDS) caused by *Fusarium solani.* Of the 151 fungi evaluated for control of this disease in greenhouse test, 46 demonstrated control activity. None of these isolates had *invitro* antibiosis activity at either high or low nutrient levels. Predominent fungi demonstrating biological control activity were strains of *F. solani* and *F. oxysporum.* In the present study, nodulation was also reduced to some extent by *F. oxysporum.* Treatment of the soil with *T. harzianum* prior to infestation with *F. oxysporum* also gave significant reduction in disease. Further, joint inoculation with *T. harzianum* and *B. japonicum* (in the soil and seed respectively) reduced disease intensity to an even greater degree indicating that neither *T. harzianum* nor *B. japonicum* inhibited the activity of the other. Role of *Bradyrhizobium japonicum* and *Trichoderma* sp. in the control of root rot of soybean has also been studied by Ehteshamul-Haque and Ghaffar (1995). *T. harzianum, T. viride, T. hamatum, T. koningi* and *T. pseudokoningi* significantly controlled the infection of 30 day old seedlings by *Macrophomina phaseolina, Rhizoctonia solani* and *Fusarium* sp. In 60 day old plants *Trichoderma* sp. and *B. japonicum* inhibited the growth of *R. solani* and *Fusarium* sp. whereas the use of *B. japonicum* with other species of

Trichoderma controlled the infection by *M. phaseolina*. Novikova et al (1995) also reported that application of mixture of alirin B and alirin S (biopreparations) reduced severity of cucumber root rot and also stimulated plant developmt and resulted in yield increase. The application of biopreparation shifted the ratio between antagonistic and phytopathogenic microbes in soil and led to the increase in antagonistic species.

Reduction of disease intensity by a particular biocontrol agent may be due to antagonistic effect of the biocontrol agent which may directly inhibit the growth of the pathogen *invivo*. On the other hand the biocontrol agent may induce some biochemical changes in the host which trigger off defense responses. In the present study therefore, attempts were made to determine the mechanism of action of *B. japonicum* and *T. harzianum* which led to reduction in disease severity. At first, interaction studies were conducted *invitro* involving the three microorganisms. Results clearly indicated tht *T. harzianum* was antagonistic to *F. oxysporum* and inhibited its growth significantly while *B. japonicum* in no way inhibited the growth of *F. oxysporum*. The results obtained are in confirmity with those of several previous workers. Cubeta et al (1985) reported that when a *Bacillus subtilis* isolate from soybean was tested for antagonism against 26 fungi commonly associated with soybean it showed fungicidal activity against *Penicillin* sp and fungistatic to all others. Autoclaved filtrates of *B. subtilis* culture inhibited growth and stroma formation of *Phomopsis* sp. El-Abyad *et. al* (1993) reported that 80% concentration of culture filtrate of *Streptomyces pulcher* or *S. canescens* significantly inhibited spore germination, mycelial growth and sporulation of *Fusarium oxysporum* f. sp *lycopercisii, Verticillium albo-atrum* and *Alternaria solani*. In the present study also both autoclaved and nonautoclaved culturate filtrate of *T. harzianum* reduced the growth and sporulation of *F. oxysporum*. Hessenmuller and Zeller (1996) reported that 50 antagonistic bacterial isolates out of the genera *Agrobacterium, Bacillus, Enterobactor* and *Pseudomonas* inhibited the mycelial growth of *Phytophthora* sp. in dual cultures. The most inhibitory activity with a reduction of 68% was shown by an isolate from *B. licheniforms against* P. *cactorum*. 2-4 diacetyl phloroglucinol a secondary metabolite produced by *Pseudomonas fluorescens* - F113 was capable of protecting sugarbeet against the causal agent of damping off, *Pythium ultimum*. *Stenotrophomonas*

maltophilia strain W81(P) produced chitinase and protease enzymes and was capable of conferring plant protection against the disease causing activity of *Pythium ultimum invitro.* On the otherhand reports are also available of biocontrol activity where no inhibition in growth was observed (Chakraborty & Chakraborty, 1989; Rupe et al, 1996).Bertagnolli(1996.) reported that cell free culture filtrates of the fungal plant pathogen *Rhizoctonia solani* isolate 2B12 inhibited the growth of the biocontrol agent *Bacillus megatarium* and *Trichoderma harzianum by secretion* of endoprotease, exochitinase, glucanase and phospholipases. In the present study, *F. oxysporum* and *T. harzianum* both inhibited the growth of *B. japonicum* to a certain extent *invitro.*

It was established from the results of the present investigation that disease reduction by two tested microorganisms - *B. japonicum* and *T. harzianum,* were achieved probably by two different mechanisms. *T. harzianum* inhibited the growth of *F. oxysporum* and the observed disease reduction was by its direct antagonistic effect on *F. oxysporum. T. harzianum* is known to have antagonistic effect on plant pathogenic fungi and results of all the previous and present study taken together pointed to antagonism as the most probable mechanism. Hence no further tests were carried on with *T. harzianum. B. japonicum* on the other hand did not have any antagonistic effect against *F. oxysporum* and several further experiments were designed and carried out to determine the mechanism by which *B. japonicum* reduced disease intensity.

Phytoalexin production is one of the most extensively studied inducible defense responses. These constitute a chemically heterogenous group of substances belonging to various classes of natural products. Among these are isoflavonoids, sesquiterpenoids, polyacetylenes as stilbenoids (Ebel and Grisebach,1988). The major phytoalexin accumulating in soybean root infection site is the substituted pterocarpen glyceollin and some of its other isomers glyceollin II and III. Phenylalanine ammonia lyase is one of the early key enzymes in the biosynthesis of glyceollin. It has been shown that application of R-(1 amino 2 phenylethyl) phosphonic acid which is an inhibitor of phenylalanine ammonia lyase to soybean root infection system gave rise to complete loss of resistance of a soybean cultivar to a normally incompatible *Phytophthora megarperma* Race 1 (Waldmueller and Grisebach,1987). Considering the above, in the present investigation, changes in the activity of

phenyl alanine ammonia lyase (PAL) and accumulation of glyceollin after disease reduction by *B. japonicum* was determined. Peroxidase is another enzyme related to induced resistance in several cases (Parent and Asselin 1984; Kuc, 1985). Peroxidase is reported to have an important function in secondary cell wall biosynthesis by polymerizing hydroxy and methoxy cinnamic alcohols into lignin and forming rigid cross links between cellulose, pectin, hydroxyproline rich glycoproteins (HRGP) and lignin (Grisebach,1981). Lignification, systemic accumulation of HRGP and cell wall apposition are associated with induced resistance to blue mold in tobacco (Ye *et al.* 1990) Hence, in the present investigation peroxidase was also selected as one of the enzymes which could be involved in induction of resistance.

Activity of both PAL and peroxidase were assayed in four sets of plants consisting of healthy,*B japonicum* inoculated, *F. oxysporum* inoculated and *B. japoniucm* + *F. oxysporum* inoculated of a resistant (Soymax) and a susceptible (JS-2) cultivar. PAL activity was higher in the resistant cultivar after inoculation with *F. oxysporum,* but reached a peak in both cultivars after 16 h. of inoculation with F. oxysporum. A significant observation here was that inoculation with *B. japonicum* and *F. oxysporum* increased PAL activity greatly in both the cultivars. Thus the activity of this enzyme seemed to be related to the observed disease reduction by *B. japonicum.* On the other hand the activity of peroxidase did not increae in treatments involving joint inoculation with *B. japonicum* and *F. oxysporum.* In relation to healthy control however, the activity of peroxidase increased significantly when inoculated with *F. oxysporum* in both the cultivars; maximum activity was obtained after 12h of inoculation with *F. oxysporum.* Results of the present investigation therefore clearly bring out certain interesting facts. Firstly PAL seems to be involved in disease resistance mechanism, since its activity was higher in case of incompatible reaction as also when disease resistance was induced by preinoculation with *B. japonicum.* Secondly peroxidase did not seem to be involved in specific compatible / incompatible reaction but rather it seemed to accumulate as a general result of infection.

Works of several previous authors have also demonstrated a definite role of PAL in host resistance. (Bhattacharyya & Ward, 1986; Bonhoff *et al.*, 1986; Southerton and Deverall, 1990; Chakraborty *et al.* 1993; Shiraishi *et al.* 1995; Edens *et al* 1995). Bhattacharyya and Ward (1987) reported that PAL

activity of soybean was enhanced in the first few hours following inoculation in the resistant but not in the susceptible response of soybean hypocotyls to *P. megasperma* f. sp glycinea. Considering that PAL is an important enzyme in the biosynthesis not only of glyceollins but also of lignins, phenolic compounds in general and melanins, all of which have been associated with resistant responses in various host plants the authors suggested that activity of PAL could be a useful indicator of the activation of defence related responses. They also demonstrated that induction of susceptibility to the pathogen by changes in temperature conditions was associated with the suppression of PAL activity. Esnault et al. (1987) suggested that production of mRNAS for enzymes leading to phenyl propanoid biosynthesis in soybean hypocotyls was an early response to infection with an incompatible but not with a compatible race of *P. megasperma* f. sp. glycinea. They postulated that the biosynthetic steps controlled by PAL and CHS (chalcone synthase) are relatively remote from those involed in the final elaboration of the glyceollins. Cuypers *et. al.* (1988) also demonstrated that the timing of PAL mRNA accumulation in potato differed markedly between two types of interaction, compatible and incompatible races of *Phytophthora infestans.* A marked increase in the accumulation of PAL mRNA was observed 3 hour after inoculation of the incompatible race at the infection sites where as it was 6 hour after inoculation of the compatible race. It was also reported by Yamada et al. (1989) that treatment of etiolated pea epicotyl tissue with elicitor activated the accumulation of PAL and CHS mRNAS within 1 hour, followed by an increase in PAL activity and pisatin biosynthesis. Concomitant presence of suppressor with elicitor resulted in delay of this host defence reaction.

Southerton & Deverall (1990) have shown that in the wheat - rust (*Puccinia recondita f. sp. tritici*) interaction increase in PAL levels that could be associated with resistance expression occur between 16 and 20 h after inoculation when effects upon the fungus were first seen and when resistant host cell began to collapse. They suggested that PAL may play a more regulatory role in wheat line as in a number of other plants. Similar results have also been reported by Chakraborty *et al* (1993) in *Brassica napus* inoculated with different strains of *Leptospharia maculans.* They demonstrated that a weakly virulent strain elicited more PAL activity as early as 12 h after

inoculation in the resistant cultivar. While PAL activity decreased after 2 days in the susceptible cultivars, in the moderately resistant cuitivar it attained a plateau and did not show any decrease even 3 days following infection. On the basis of this the authors suggested that the maintenance of PAL activity at a high level for a considerable period may be one of the factors responsible for the incompatibility reaction. Shirashi et al. (1995) on the other hand demonstrated increased PAL activity in the barley leaves after inoculation with the powdery mildew pathogen *Erysiphe graminis f.* sp. *hordei* in both resistant and susceptible cultivars. They suggested that the response was not specific and was not a reflection of the resistantce or susceptibility of the cultivar to the pathogen. Elevation of PAL levels by the host was considered to be the direct response to attempted penetration by the fungi.

In the present study, peroxidase increased as a result of infection but not during induced resistance. Activity of peroxidase was greater in the susceptible cultivar than in the resistant one. Akhtar and Garraway (1990) observed an increased peroxidase activity in the susceptible isoline compared with the resistant one when both were treated with sodium bisulphite prior to inoculation with the pathogen *B. maydis*. The sodium bisulfite-induced increase in peroxidase activity persisted even when leaves pretreated with sodium bisulfite were inoculated with *B. maydis* race T and subsequently incubated for 48 h in the dark at 28°C. Concomitant with increased peroxidase activity due to sodium bisulfite, increased sporulaion of *B. maydis* on maize leaves and increased electrolyte leakage was also observed. These results therefore support the finding that increased peroxidase activity can be correlated with increased infection. On the otherhand there are also reports of increased peroxidase activity due to induction of disease resistance. Ye et al (1990) reported that stem infection of tobacco cultivars with *Peronospora tabacina* or leaf inoculations with tobacco mosaic virus induced systemic resistance to both pathogens with a simultaneous increase in peroxidase activity. They further observed enhanced PR proteins as well as peroxidase, β-1,3 glucanase and chitinase activities in induced plants (Ye et al, 1990a). Irving & Kuc (1990) also obtained increased activities of peroxidase and chitinase in plants sprayed with K_2HPO_4 prior to inoculation with *Colletotrichum lagenarium*. This treatment also induced systemic resistance as well as increased chitinase activity. They

suggested that systemic induced resistance resembled passive resistance as it relied upon increased levels of enzymes associated with plant defense in a manner similar to physical barriers.

Subsequent to studies on enzyme responses in soybean, accumulation of glyceollin was determined in plants following different treatments already described. Significant differences in glyceollin level were obtained between susceptible and resistant cultivars inoculated with the pathogen. Increased glyceollin levels were also obtained when resistance was induced due to preinoculation with *B. japonicum*. A number of previous workers have presented conclusive elidence that resistant cultivars of different host species produce more phytoalexins in response to fungal infection than the susceptible ones. (Ingham *et al* 1981; Tegtmeier and Van Etten 1982, Kumar and Sridhar 1984, Rouxel et al 1989; Abazkhodjaev *et al* 1995; Zeringeu 1995, Essenberg and Pierce 1995.

Of the different host pathogen interactions studied till date soybean-*Phytophthora megasperma* has perhaps been the most widely studied (Keen *et al* 1971; Bhattacharyya and Ward 1986, 1987 and 1988; Ebel and Grisebach 1988, Ward, 1989; Yoshikawa 1995). Similar results have also been obtained in soybean with other pathogens (Kaplan *et al* 1980; Purkayastha *et al*. 1981; Purkayastha & Chakraborty 1983; Long *et at*. 1985 ; Chakraborty & Purkayastha 1987). In spite of extensive work on the role of phytoalexins in host resistance relatively little work has been done on their role in induced resistance. Purkayastha et at. (1981) reported that induced resistance of soybean due to *B. japonicum* could not be correlated with increased glyceollin accumulation. Further, they attributed the observed disease reduction to rhizobitoxin produced by *R. japonicum* (Chakraborty & Purkayastha, 1984). Contrary to this observation Chakraborty & Chakraborty (1989) obtained increased phytoalexin (4-hydroxy 2.3.9 trimethoxy pterocarpan) in pea where resistance was induced by *R. leguminosearum* against *Fusarium solani* f. sp *pisi*. These results are in agreement with the present findings. Mithoefer *et al*. (1996) reported that fungal β.glucan induced glyceollin synthesis in soybean was suppressed by cyclic 1,3-1, 6-β glucans from *B. japonicum*. They have demonstrated that both the fungal and the bacterial β-glucans are ligands of β glucan binding site which are putative receptors for the elicitor signal compounds in soybean roots.

Whereas the fungal β glucans stimulated phytoalexin synthesis even at low concentrations, the bacterial glucans appear to be inactive even at relatively high concentration. Competition studies indicated that increasing concentrations of the bacterial glucans progressively inhibited stimulation of phytoalexin synthesis.

In the present investigation after having established the role of PAL, peroxidase and glyceollin in induced resistance, further experiments were conducted on changes in proteins and serological relationships between soybean and *F. oxysporum*. Two cultivars, one resistant (Soymax) and the other susceptible (UPSM-19) were selected and studies were conducted not only in the roots but also seeds as *F. oxysporum* in many cases is present as a latent infection in soybean seeds. Results revealed that protein contents did not differ significantly in the two cultivars but the seeds had very high protein content as compared to the roots. Inoculation with the pathogen led to increase in the protein content in both roots and seeds. In roots, inoculation with *B. japonicum* increased protein content but this could not be correlated to induced resistance. Analysis of root protein by SDS-PAGE revealed the presence of some extra bands in *B. japonicum* inoculated roots but no major difference were obtained after induction of resistance. In case of seed proteins infection led to absence of many of the common bands. In this case therefore even though protein content increased after inoculation the number of protein bands decreased. Zhang and Smith (1996) reported that inoculation of soybean with genistein preincubated *B. japonicum* increased soybean protein content and dry matter yeild under short season conditions. Velicheti et al (1992) observed increased quantity of lipoxygenases and soybean seed lectin in the seed coat of soybean infected with *Phomopsis longicolla*. Higher levels of lectins were observed in cultivars of soybean resistant to *Phytophthora sojae*.

Involvement of antigens in disease reaction of plants has also been reported where antigenic similarity in susceptible / compatible host parasite interactions have been obtained. Purkayastha (1995) suggested that phytoalexins and plant antigens together could be involved in disease reaction and that production of phytoalexins depends on the interactionof host parasite proteins or specific antigens. Hence in the present study investigations on the serological relationship between *F. oxysporum* and soybean cultivars were

next determined. Results of immunodiffusion, immunoelectrophoresis and ELISA clearly revealed more antigenic similarity between susceptible cultivars and the pathogen or the presence of cross reactive antigens in the susceptible host-pathogen interaction. Several earlier studies have also implicated the importance of common antigens in host pahtogen compatibility. The occurrence of CRA and their involvement in various host parasite combinations have been demonstrated by a number of previous authors (Alba *et al* 1983; Chakraborty & Purkayastha 1983; Alba & Devay 1985; Purkayastha & Banerjee 1986; Ghosh and Purkayastha 1990; Purkayastha & Pradhan 1994; Chakraborty & Saha 1994 and Chakraborty *et al.* 1995). Among all available serological techniques for detection of CRA, enzyme linked immunoserbent assay is probably the most sensitive. In the present study antisera were raised against antigenic preparation of *F. oxysporum* and soybean roots which were initially purified and IgG fraction was used in all further tests. This was necessary to minimise non specific binding which may interfere with the actual antigen-antibody reaction. At the onset the sensitivity of the assay was optimized. ELISA readily detected CRA in antigen preparations of *F. oxysporum* at a concentration of 5 μg / ml with 1:125 antiserum dilution. Among the ten soybean cultivars tested high absorbance values were obtained in reaction of anti *F.oxysporum* antiserum with antigenic preparations from susceptible cultivars. In order to confirm that the observed cross reactivity between *F. oxysporum* and susceptible cultivar was specific, antigen preparations from non host (*C. sinensis*) and non-pathogen (*G. cingulata)* were also assayed with antisera of *F. oxysporum* and susceptible soybean cultivar (UPSM-19).None of the above reactions showed any reactivity to ELISA.

Differential response of the different cultivars of soybean to *F. oxysporum* has been established by the pathogenicity test and cross reactivity test. Following this, the ability of the antiserum of *F. oxysporum* to detect the pathogen in infected root was tested in ELISA. ELISA could detect infection in both susceptible and resistant cultivars. Pathogen detection in the host tissue by ELISA has also been reported by a number of previous workers (Mohan, 1988 ; McDonald, 1990; Lyons and White, 1992; Linfield, 1993 : Chakraborty *et al.* 1996). Since significant differences were obtained in ELISA between healthy and infected extracts, in the next phase ELISA were performed

with root antigens after inoculation with *F. oxysporum, B. japonicum* or *F. oxysporum* + *B. japonicum.* Significant differences were obtained between healthy and *F. oxysporum* or *B. japonicum* + *F. oxysporum* inoculated root antigens but not beteen healthy and *B. japonicum* inoculated extracts. ELISA values in *B. japonicum* + *F. oxysporum* antigens were lower than those with *F. oxysporum* alone. Thus reduction in disease due to *B. japonicum* inoculations reduced the ELISA *reactivity.* Antiserum raised against *F. oxysporum* could not detect *B. japonicum.* Results were similar in both the cultivars.

It is also important in studies on host parasite relationship to determine the cellular location of cross reactive antigens which are involved in host sesceptibility / compatibilty with the pathogen. For this, in the present investigation fluorescence tests were conducted with sections of soybean roots as well as mycelia, conidia and chlamydospore of *F. oxysporum.* Cross sections of soybean roots were treated either with anti-*F. oxysporum* or anti UPSM-19 antiserum followed by staining with FITC conjugated anti rabbit globulin specific goat antiserum. In sections of suceptible cultivar (UPSM-19), bright fluorescence developed which was concentrated mainly around epidermal cells, the endodermis and xylem elements and was distributed throughout the cortial tissues. In roots of cv Soymax, fluorescence was noticed only in the epidermal cells and cortical tissues. Treatment of mycelia, conidia and chlamydospores with homologous antiserum and FITC showed a general fluorescence. 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 detrminants in roots have a general distribution. The cellular location of CRA between tea and *Bipolaris carbonum* and tea and *P. theae* have also been established (Chakraborty and Saha, 1994; Chakraborty *et al.*,1995).

Detection of pathogen in host tissues using antibody based immunofluorescent technique also been reported by several previous authors (Dewey *et al,* 1984; Watabe, 1990; Wakeham and White, 1996). Troxler *et al.* (1997) used immunofluorescence microscopy to investigate the colonization of tobacco roots by the biocontrol agent *Pseudomonas fluorescens* CHAO and its physical relationship with the black root rot fungus *Thielaviopsis basicola.*

The pseudomonad delayed colonization of the interior of tobacco roots by *T. basicola.*

It can be generalized from the aforesaid considerations that host-pathogen interaction is a complicated process, and cannot be viewed in isolation, since in nature, disease is never the outcome of a single host-pathogen interaction but rather the cumulative effects of a number of interactions. Detailed studies on the interaction between the rhizosphere microorganisms of soybean - *B. japonicum, T. harzianum* and *F. oxysporum,* the former two beneficial and the latter, a pathogen have thrown some light on the complicated mechanisim. It has been established that for effective induced resistance to occur, several non-specific host defense responses may be activated. As with other host pathogen systems, resistance has been correlated with increased PAL activity and glyceollin content, but no role for peroxidase in induced resistance has been established. It is also evident that biochemical changes in soybean are closely related to changes in antigenic relationships between host and pathogen. No direct evidence for correlation of biochemical defense responses and serological changes have yet been forthcoming, but it has been suggested that if cross reactive antigens have a functional role other than in recognation phenomena, it probably will be found in the infection process and be subject to the over riding effects of substances such as phytoalexins or other inhibitory substance already present in host tissues or induced by parasitic microorganisms (DeVay *et al.* 1981). Thus it can be conclusively stated that the final resistance / susceptibility of host to a pathogen cannot be attributed to a single factor, but the outcome of a number of responses occurring at different cellular levels.