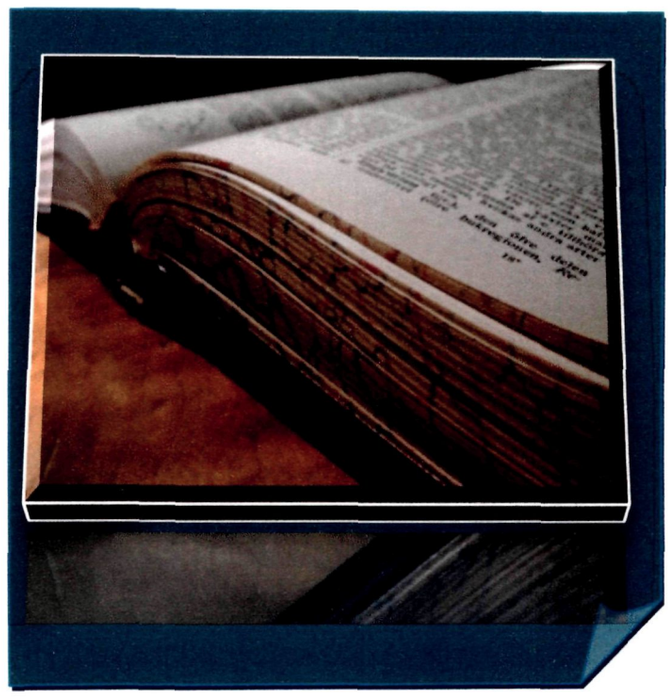
General Discussion

CHAPTER VIII



CHAPTER VIII

General Discussion:

The microorganisms that can grow or activate around plant roots in the rhizosphere (Azcon-Aguilar and Barea, 1992) are ideal for use as biocontrol agents since rhizosphere provides the front line of defense for roots against attack by pathogens (Weller, 1988). During primary infection and also secondary spread on the roots these rhizospheric microorganisms encounter the pathogens.

For the present experiment pathogenicity test was carried out for both pathogen *S. rolfsii* and *M. phaseolina* on groundnut plants, in which isolate of *S. rolfsii* caused wilt and stem rot disease while isolate of *M. phaseolina* caused charcoal or black root rot disease in the test on groundnut plants in these experiments. When groundnut seeds were planted on soil and inoculated with *S. rolfsii*, damping-off and stem rot occurred while *M. phaseolina* inoculation caused occurrence of charcoal or black root rot. Some of the plants showed symptoms of stem rot and black root rot but were upright. The disease was more severe in plants where mycorrhiza was not inoculated. *S. rolfsii* and *M. phaseolina* were re-isolated from the seedling of groundnut and the cultures were identical to original isolate which was used inoculum.

In the present investigation an attempt has been made for the biocontrol of groundnut diseases. For that we have used mycorrhizal fungus *G. fasciculatum* and *T. viride* singly or in combination against fungal pathogens *S. rolfsii* and *M. phaseolina* in groundnut plants. *G. fasciculatum* was used as soil inoculum applied below seeds at the time of plantation. The talc based *T. viride* was treated on the seeds of groundnut before plantation. The groundnut plants defence response in pot trial was observed in terms of disease severity, disease incidence, growth, colonization, physiologically and biochemically. Pathogenicity test of *S. rolfsii* and *M. phaseolina* on groundnut was carried out (as described in CHAPTER IV). The reason for the high mortality was infection caused by the fungal pathogens *S. rolfsii* and *M. phaseolina*. The disease was developed in groundnut plants and caused stem rot, wilting, drying and root-rot.

To fulfill Koch's postulates, dying seedlings and plants were removed at each observation and plated onto PDA media. A fungal species was considered to be pathogenic when its inoculation to groundnut seedlings, using sorghum seed inoculum technique, in the greenhouse resulted in at least one diseased seedling or plant with

the typical symptoms of the diseases. The reisolated fungus was cultured on PDA and colony characteristics were recorded and compared to the original isolates.

Many workers have investigated synergistic effect of VA mycorrhizal fungus and *Trichoderma* species on root pathogens. Study by Harlapur (1988) has showed that combined application of *G. fasciculatum* and *Trichoderma* reduced the severity of foot rot disease of wheat due to *S. rolfsii*. Sreenivasa (1994b) worked on biological control of *S. rolfsii* disease of chilli with combined application of *G. macrocarpum* and *T. harzianum*. Later on study by Sreenivasa (1997) biological ability of VA fungus, *G. macrocarpum* and *T. harzianum* on *S. rolfsii* at two levels of Phosphorous i.e. 75 and 100 percent recommended dose (75 kg/ha) in three varieties of chili (Byadagi, Guntur and Sankeswar) and showed that the percentage production of sclerotial bodies was significantly less in the plants inoculated with both *G. macrocarpum* and *T. harzianum*.

Arbuscular mycorrhizal (AM) symbioses are formed between plants and members of Glomeromycota (Schüßler *et al.*, 2001) in this Glomeromycotan fungus are obligate symbionts and rely on carbon provided by their host plant to complete their life cycle. In return, the fungus provides host plants with improved nutrition, enhanced drought tolerance and increased protection against pathogens. The improvement in nutrition especially P in mycorrhizal roots have been known to play an important role in the reduction of disease severity (Krishna and Bagyaraj, 1983). As arbuscular mycorrhiza (AM) in past have observed to play hinge role in plant protection from many soil-borne plant pathogens than their non-mycorrhizal plants. With raising awareness of possible harmful effects of fungicides on the ecosystem and growing interest in pesticide free agricultural products, biological control now appears to be a promising strategy for managing diseases in a range of crops (David, 2008).

The mycorrhizal plant may provide protection from plant pathogen due to improvement in physiological and biochemical changes in the host and increase in flow of nutrients resulting into greater mechanical strength (Schonbeck, 1979; Auge, 2001). The added surface provided by the external hyphae of endomycorrhizal fungus helps in absorption of phosphorus in mycorrhizal inoculated plants thereby enhancing retrievement of phosphates (Marschner and Dell, 1994). Besides improvement in nutrition of plants by AM fungi, it also reduces harmful effects of pathogen (Borowicz, 2001). Numerous AM fungi have been found to reduce negative effect of soil-borne root pathogen for example root rot disease caused by *Fusarium oxysporum*

f. sp. *Asparagi* and *Helicobasidium mompa* in asparagus (*Asparagus officinales* L.) (Matsubara *et al.,* 2000a; Matsubara *et al.,* 2000b) were reduced by *G. fasciculatum* and *Gigaspora margarita*. Also other mycorrhizal species have been involved in plant growth and disease control such as *G. intraradices* which showed improvement in plant growth as well as protection from nematode infection and multiplication (Bagyaraj *et al.,* 1979). The effect of root-rot caused by pathogen *M. phaseolina* was reduced with the help of *G. intraradices*. Jalali *et al.,* (1990) showed effective management of *M. phaseolina* by mycorrhizal association that caused root-rot in legume such as mungbean. In Pea root rot caused by *Aphanomyces euteiches* was reduced (Akkopru and Demir, 2005) and in tomato *G. intraradices* reduced Fusarium wilt. Plants inoculated with *Glomus* species have been reported to show increase in phenylanine and serine in tomato roots (Suresh, 1980) and these amino acids proved to inhibit nematodes (Reddy, 1974). Newsham *et al.,* (1994) found that AM fungi directly interacted with root pathogen of the winter annual grass *Vulpia cilita* and improved usefulness by interfering with negative effects of the pathogens.

Investigation by Mani and Hepziba (2003) showed that effect of organic amendments and biological control agents viz., *T. viride* and *T. harzianum* was tested against *M. phaseolina* which caused root rot of sunflower for two consecutive seasons. The application of organic amendments such as FYM (12.5 t/ha) showed disease incidence of 35.29 percent as compared to 55.53 percent in the untreated control. Seed treatment with *T. viride* combined with application of neem cake showed 19.18 percent of disease incidence followed by seed treatment with *T. harzianum* in combination resulted in highest yield (822.5 kg/ha) as compared to control (537.50 kg/ha). Dubey (2003) reported that seed treatment with slurry or water mixed spores of *T. viride* and *Gliocladium virens* gave best protection to germinating seeds of urd/mung bean against *Rhizoctonia solani*. Hadar *et al.,* (1984) that showed in seed treatment with spore concentration of *T. hamatum* reduced damping-off caused by *S. rolfsii* on peas and beans. Sreenivasaprasad and Manibhushanrao (1993) found reduction of chicken wilt complex in groundnut by *T. longibrachiatum*. The antagonistic ability of *Trichoderma* isolates is highly variable (Chet *et al.,* 1979), as shown in this study which was observed in his study in which only 11.54 % and 5.77 % of *Trichoderma* isolates tested were effective in controlling *R. solani* and *S. rolfsii* respectively in the bioassay studies. The ability of *Trichoderma* isolates to reduce diseases caused by soil-borne plant pathogens is well documented and it is been

correlated to the antagonistic properties of *Trichoderma*, which may involve mycoparasitism and lysis of pathogenic fungi and/or competition for limiting factors in the rhizosphere mainly iron and carbon (Sivan and Chet, 1986). Shahida *et al.*, (1991) found that *T. harzianum* reduced disease caused by *M. phaseolina*.

Declerck *et al.*, (2002) showed that pathogen decreased the intensity of AM fungal colonization. Similarly, in the present experiment the percent root colonization was found to decrease in pathogen (*S. rolfsii* and *M. phaseolina*) infected mycorrhizal groundnut plant as compared to only mycorrhizal inoculated groundnut plants. The present experiment illustrated that the disease incidence in groundnut plant was found to be higher as the colonization increased after particular growing intervals which can be correlated with the results of Ross (1972); Davis and Menge (1980) who also showed increase in incidence as the colonization due to AM fungi increased.

The disease incidence and severity was observed to be decreased due to treatment by mycorrhiza and *Trichoderma* leading to overall better growth of *Arachis* plants. This beneficial effect may be observed when comparing the mycorrhizal dependency (MD) which showed significant results. The mycorrhizal dependency showed marked increase in AM inoculated *Arachis* plants but it was significantly highest in presence of stress caused by pathogens *S. rolfsii* and *M. phaseolina*. Similar type of results were obtained by Jaizme-Vega *et al.*, (1998) and Rufyikiri *et al.*, (2000a) in which they showed positive attributes of mycorrhizal associations in stressed biotic and abiotic conditions.

In the present experiment we have highlighted use of AM fungi and *T. viride* singly or in combination in groundnut plant. The results showed significant reduction in disease severity and incidence in groundnut plant when treated with microbial bioagents AM fungi and *T. viride* against *S. rolfsii* and *M. phaseolina*. These results are in concurrence with that of Dar *et al.*, (1997) where he found reduction of root rot in bean plants by *G. mosseae*. When plants were colonized by AM fungi, the detrimental effects of many soil-borne plant pathogens can be reduced (Abdel-Fattah, 2000; Yao *et al.*, 2002; Chandanie *et al.*, 2009). *T. viride* was found to be successful in reducing the growth of *M. phaseolina* causing root rot of castor by 84 % (Sundar *et al.*, 1995). Majumdar *et al.*, (1996) found that *Trichoderma* species inhibited growth of *M. phaseolina* that was causing blight in mothbean.

It is well known that *T. harzianum* increases the solubility of phosphorous thereby promoting plant growth. *T. harzianum* is known to inhibit crown and root rot

disease of tomatoes and increase in yield was observed in presence of disease (Ozbay and Newman, 2004b). When groundnut plants were inoculated with *G. fasciculatum* and treated with *T. viride* significantly increased plant growth by increasing number of leaves, shoot and root length, nodule number, fresh and dry weights, pods number and chlorophyll content and all these parameter were more prominent when pathogen was not involved. The growth was observed to be most prominent when both *G. fasciculatum* fungi and *Trichoderma* was involved as compared to single inoculation of AM fungi or *Trichoderma* treatment. Similar types of results were obtained by Abdel-Fattah and Shabana (2002) on *Rhizoctonia* root rot of cowpea and El-Haddad *et al.*, (2004) on white rot of onion. Srinath *et al.*, (2003) found that the height of arbuscular mycorrhiza inoculated *Ficus benjamina* plantlets increased when they were inoculated alongwith either *Bacillus coagulans* or *T. harzianum*. Ozbay and Newman (2004a) reported that shoot height, stem calliper and shoot fresh weight of tomato seedlings increased when inoculated with *T. harzianum*. The enhancement in all the above growth response in mycorrhizal groundnut plant as compared to non-mycorrhizal groundnut plants was due to improvement in photosynthesis, nutrient uptake and plant metabolism brought about by mycorrhizal inoculation (Hayman, 1978). Also, the establishment of mycorrhizal infection can reduce the diseases caused by soil-borne pathogens (Dehne, 1982). Even release of phytohormones by mycorrhizal plants like indole acetic acid (IAA) and cytokinins contributes to increased plant growth (Frankenberger and Arsad, 1995).

The increment in photosynthetic pigments is good parameter for healthy condition of plant as photosynthesis is one of the main physiological processes important for plant growth (Arfan *et al.*, 2007) and highly affected by infection (Radwan *et al.*, 2007). It was found that there was significant increase in content of total chlorophyll when treated singly with *T. viride* or *G. fasciculatum* or in combination of both. The increment occurred by enhancement in the efficacy of photosynthetic apparatus with a better potential for disease resistance and decrease in photophosphorylation rate usually occur after infection (Chandra and Bhatt, 1998). Overall, the total chlorophyll content was higher in mycorrhizal plants than in non-mycorrhizal or diseased groundnut plants. It was observed that there was significant reduction in pigment rate due to infection caused by pathogens, resulting into overall growth inhibition. The decrease in chlorophyll content could be a typical symptom of stress caused by the disease. The increase in cytokinin content prevents degradation of

chlorophyll leading to its prevention and increase in chlorophyll content (Allen, 1981).

Many biochemical and physiological changes are involved in resistance provided by AM colonization which is divisive (Wehner *et al.,* 2009). Such as the activity of both acid phosphatase and alkaline phosphatase was found to be lower in diseased plants as compared to mycorrhiza/*Trichoderma* treated groundnut plants. Activity of acid phosphatase and alkaline phosphatase was more pronounced in mycorrhizal and *Trichoderma* treated groundnut plants. Acid phosphatase is produced in the cell wall and also occurs in cystol of higher plants. Acid phosphatase is important in metabolic function to catalyze phosphate (Heredia, Yen and Sols, 1963) and reduce inorganic phosphate in cells (Banasik *et al.,* 1980; Besford and Syred 1979). The activity of both acid phosphatase (ACP) and alkaline phosphatase (ALP) was found to be significant in mycorrhizal/*Trichoderma* treated plant roots than in non-mycorrhizal plants (Fries *et al.,* 1998; Tararaya and Saito, 1994). Alkaline phosphatase generally emerges during primary mycorrhizal colonization chiefly present in vacuolar compartment (Gianinazzi, 1995). Alkaline phosphatase translocates phosphorous from external hyphae present in form of polyphosphate which are ultimately hydrolysed by alkaline phosphatase. As also put forward by Ezawa *et al.,* (2001) that polyphosphate degradation brought about by alkaline phosphatase in AM colonized roots results into the inorganic phosphate released from arbuscule to root cells.

And the point of pathogen control by same AM fungus (*G. fasciculatum*) on different types of pathogen was not of same level in groundnut plants against plant pathogens *S. rolfsii* and *M. phaseolina* and this difference in the levels of control was also found by Mark and Cassells (1996).

Phenol and Proline:

Phenolic compounds are plant secondary metabolites with no perceptible role in the growth and development of the plant. Accumulation of phenolic compounds is considered to increase the physical and mechanical strength of host cell wall resulting in the inhibition of fungal invasion. Phenolic compounds are considered to be natural antioxidants (Kim, 1997). Phenolic compounds are biosynthesized through the shikimic acid pathway, from which they are produced using intermediates of carbohydrate metabolism (Tomás-Barberán and Espin, 2001). In this experiment the

total phenol and proline content was higher in both the groundnut cultivars with pathogens as compared to healthy ones.

The amount of total phenols and proline was higher when treated with mycorrhiza/*Trichoderma* in single or dual inoculation with pathogen in both groundnut cultivars as compared to control ones. Accumulation of phenols and proline was highest when both mycorrhiza and *Trichoderma* were involved along with the pathogen in both groundnut cultivars. Phenol accumulation was higher because of hinderance in glycolysis by pathogen activity which might have activated the pentose pathway leading to the formation of 4-carbon compounds for their synthesis. Hence, it can be assumed that accumulation of phenols at the infection site may be correlated with the restriction of pathogen development (Heath, 1980). Indications by various researchers have led phenolics as signal in plant development and in plant-microbe interactions (Lynn and Chang, 1990). The first stage of defense mechanism in plants is the rapid accumulation of phenols at the infection site which restricts or slows the growth of the pathogen because of its action as antioxidants, antimicrobial and photoreceptor Lamba *et al.,* (2008).

Study by Selvaraj and Subramanian (1990) have observed increase in phenolic compounds in the host due to the inoculation of VAM fungus. The histochemical study by Krishna (1984) has demonstrated that mycorrhizal roots contain more phenols than their comparable non-mycorrhizal counterparts. The phenol deposition in mycorrhizal tomatoes to encumber the infection and spread of *Fusarium* has been reported by Dehne and Schonbeck 1979b. The increase in total phenol in shoot and root is attributed mainly to general triggering of aromatic biosynthesis (Mahadevan, 1991). Many reports have suggested changes of the cytoplasmic pH in plant tissue (Ojalvo *et al.,* 1987) due to increased phenolic acid content which results into inhibition of pathogen development.

Thus, the presence of higher amount of phenols was credited to provide resistance to disease (Jain and Yadav, 2003; Kushwala and Narain, 2005 and Parashar and Lodha, 2007).

Proline (Pyrrolidine-2-caboxlic acid) is a 5 carbon amino acid known to accumulate in higher plants in response to serious abiotic and biotic stresses (Ghasempour, 2003; Slama, 2006). The accumulated free proline content varies with the degree of stress (Saradhi and Saradhi, 1991). Proline accumulation enables plants

to tolerate stress as this amino acid (1) accumulates in the cytoplasm (Ketchum *et al.*, 1991), (2) lowers the solute potential and (3) balances solute potential in vacuole. The amino acts as scanvenger of ROS prevent induction of programmed cell death by ROS (Chen, 2005). Generally, amino acid content has been regarded to contribute to osmotic adjustment in endophytic-infected grasses (Kavi Kishore, *et al.*, 2005). Yet, it has been reported that during pathogenesis in plants by microorganisms the proline contents increased in many folds in susceptible and resistant cultivars (Raj *et al.*, 1983; Gupta, 2001).

Here, in the present experiment we can conclude that proline accumulation was observed to be higher in diseased groundnut plants as compared to healthy control ones. But proline content was highest in diseased mycorrhizal and *Trichoderma* treated groundnut cultivars as compared to non-mycorrhizal control ones. Our results demonstrate that the proline accumulation was significantly higher when bioagents viz., mycorrhiza and *Trichoderma* were involved in diseased groundnut plants. These results are in agreement with that of Chatterjee and Ghosh (2008) who found higher proline accumulation in diseased tissue which they related to pathological disorderliness.

Protein:

The increased level of proteins was observed in the present experiment in which groundnut plant was treated with AM fungi and *T. viride* against major plant pathogens as compared to control ones. The increase may be ascribed to Gianinazzi-Pearson and Gianinazzi (1995) have demonstrated that new proteins are synthesized in host plant in response to AM colonization. New polypeptides are synthesized during AM infection (Garcia-Garrido *et al.*, 1993; Dumas-Gaudot *et al.*, 1994). The elicitation of host protein synthesis brought about by pathogen penetration in host plant cells helps in restricting the multiplication spread of pathogens in the healthy tissues (Datta, 1999). The increase in protein content has been recognized to stimulate fresh protein synthesis in host plants after infection or to the fungal proteins in mycorrhizal roots (Mathur and Vyas, 1996). Also, presence of pathogensis-related (PR) proteins in plant tissues has been correlated to pathogen or micro-organism resistence by the host (Gnieszka, 2003). Yet, altered pattern of protein synthesis in the plant is not necessarily related to defence response.

Antioxidant enzymes:

The ROS (reactive oxygen species) such as superoxide radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^{\cdot}$) are generally produced in the plants as the result of the metabolic processes that takes place in chloroplast, mitochondria and plasma membrane-linked electron transport system. Although all compartment of the cell are possible sites for $O_2^{\cdot-}$ formation but most important generators of ROS are thought to be the chloroplast, mitochondria and peroxysomes (Fridovich, 1986). Extra ROS accumulation occurs during the the pathogen attacks, wounding, herbivore feeding, UV lights, heavy metals and other stressful condition. Also, it interferes with the electron transport system in the host leading to electron leakage which ultimately results into altered structure of the molecular oxygen. The plant's cells usually try to keep the concentration of ROS to minimum level as they are more reactive than molecular oxygen (O_2) (Wojtaszek, 1997). The sustained accumulation of ROS are known to damage the cell and causes lipid peroxidation, protein denaturation, DNA mutation, molecular disfunction and led to cell death (Hariyadi, 1991). Generally the plants produce many antioxidant and low molecular weight enzymes such as SOD, CAT, PO, ascorbate peroxidase and glutathione reductase etc. They participate in a highly developed detoxification system named the ascorbate-glutathione cycle (Halliwell-Asada cycle) (Mittova, 2000). The SOD converts the superoxide radicals to hydrogen peroxide. Then the CAT and PO reduces the hydrogen peroxide to water and oxygen molecules.

Many defence related enzymes are activated against plant pathogens such as polyphenol oxidase, peroxidase (Dutta and Chatterjee, 2000; Jose *et al.,* 2001) and superoxide dismutase. Peroxidases and polyphenol oxidases are the different types of phenol oxidative enzymes in nature. Pathogen related defence mechanism of polyphenol oxidase (PPO) activity includes; (1) general toxicity of PPO-generated quinones to pathogens and plants cells, accelerating cell death (2) alkylation and reduced bioavailiblity of cellular proteins to the pathogen (3) cross-linking of quinones with protein or other phenolics, forming a physical barrier to pathogens in the cell wall and (4) quinone redox cycling leading to H_2O_2 and other reactive oxygen species, which are known to be important factors in plant pathogen interactions and defense signaling (Li and Steffans, 2002; Raj *et al.,* 2006). Therefore, the present data showed increased polyphenol oxidase and peroxidase activity upon antagonist

treatments or fungal infection in shoot and roots of groundnut plants as compared to control ones which may be a contributing factor in resistance to disease caused by *S. rolfsii* and *M. phaseolina*. In the present experiment the W-51 (Western-51) cultivar showed increased level of polyphenol oxidase and peroxidase activity as compared to susceptible JL-24 (Phule-Pragati) cultivar. Similar type of observation was made by Gogoi *et al.*, 2001 and Raj *et al.*, 2006 where the level of both total phenol and PPO were naturally high in resistant varieties.

Peroxidases are generally hemeproteins which are produced mainly by a number of micro-organisms and plant sources, which catalyze reactions in the presence of hydrogen peroxide (Duran and Esposito, 2000). Increase in peroxidase activity upon infection might be required for an additional deposition of lignin around the lesions induced by pathogen. Increased peroxidase, localized mainly in the vacuole and cell wall have been conclusively documented in VAM-positive plants (Morandi *et al.*, 1984). Peroxidase is considered to be a key enzyme in the biosynthesis of lignin and other oxidized phenols (Bruce and West, 1989). Involvement of peroxidase activity in plant disease resistance has been well documented in several plant patho-systems (Tornero *et al.*, 2002; Martin *et al.*, 2003; Carvalho *et al.*, 2006). Peroxidase was found to be one of the most first enzymes responding and providing fast defence against plant pathogens (Sulman *et al.*, 2001). Plants on infection with plant pathogens led to initiation of peroxidase activity and higher activity was observed in resistant cultivars than the susceptible ones (Mydlarz and Harvell, 2006).

And PPOs are oxidoreductases that catalyze oxidation of phenolics compounds. They catalyze the transformation of large number of phenolics and non-phenolic aromatic compounds (Duran *et al.*, 2002). The present data showed increased peroxidase and polyphenol oxidase activities in both shoot and roots of groundnut plants which may be due to involvement of mycorrhizal fungus, or due to infection leading to triggering of biochemical activities, or both. The activity of peroxidase enzyme in shoot and roots of mycorrhizal plants is enhanced due to the activation, solubilization or *de novo* synthesis of enzymes or their production by the invading mycorrhizal fungus (Spanu and Bonfante-Fasolo, 1988). Peroxidase and polyphenol oxidase mediate the oxidation of phenols and oxidized phenols are highly toxic to the pathogen (Sequeira, 1983). PO and PPO catalyse the oxidation of phenolic compounds through a PPO-PO-H_2O system (Srivastava, 1987). A number of

correlations have been found between PPO and resistance response (Velazhahan, 1994). Peroxidase itself was found to be inhibiting the spore germination and mycelia growth of certain fungi (Joseph *et al., 1998*). The study by Mohan *et al., (1993b)* reported that the higher activity of PO and PPO in the infected tomato leaves might due to their participation in the oxidation of phenolic residues into cell wall polymers in the pathogen-infected cell. Significant levels of peroxidase and polyphenyl oxidase and total phenolics was recorded when Chick pea was pretreated with rhizobium (Arfaoui, 2005). Asha and Kannabiran (2001a) found that the higher PO and PPO activity in the chilli leaves infected with *C. capsici* was mainly due to the enhanced respiratory rate induced by the pathogen activity. Honty *et al.,* (2005) found higher activity of PPO in pear fruit infected with pathogen *Erwinia amylovora.* Thus, significant increase in the antioxidant enzyme activity of SOD, PO and PPO was found in the pathogen infected groundnut plants in the present experiment.

Superoxide dismutases (SODs) are a ubiquitous family of enzymes that can efficiently catalyze the dismutation of superoxide anions. Within a cell, they are the first and most important line of antioxidant enzyme defence systems against reactive oxygen species (ROS) (Igor, 2002). SOD is found in virtually all oxygen-consuming organisms. Deletions or mutations of SOD genes can lead to severe biological disorders (Rosen, 1993).

In our experiments, biochemical observation revealed significant increase in SOD and PO activity when treated with *G. fasciculatum* and *T. viride* singly or in combination with/without pathogens *S. rolfsii* and *M. phaseolina* as compared to control ones. The treatments generally induced systemic resistance in the host cell, which in turn enhanced activation of these enzymes in the conversion of reactive oxygen species or radicals to water in order to reduce the infection. Thus, the rapid conversion reduced the severity of the infection caused by the pathogens *S. rolfsii and M. phaseolina.*

Similar type of finding was made by Naffaa *et al.,* (1999) in perennial ryegrass infected with endophytic bacteria, Zhang *et al.,* (1996) in anthracnose disease of cucumber, Sreedhara *et al.,* (1995) in pearl millet infected with *Sclerospora* sp. Kwon and Anderson (2001) reported that the enhanced SOD activity in the wheat leaves infected with *Fusarium* like fungus isolate. The fungi generate hydrogen peroxide as part of its weaponry to enhance the penetration process into the host cell. Baker and Orlandi (1995) explained that the accumulation of active oxygen species in the plant

cell during interactions with potential pathogens affect many cellular processes (proteins, lipid, polysaccharides and nucleic acids) that are involved in the plant/pathogen interaction. Yim *et al.,* (1990) reported that the elevated concentration of SOD in the infected plant cell generally induced disfunction of all metabolic activities and led to cell death. Levine *et al.,* (1994) and Jabs *et al.,* (1996) concluded that hydrogen peroxide produced as the result of pathogen activity in the plant-pathogen activity direct cause for cellular death of the infected tissue. Bowler *et al.,* (1992) explained that the increased SOD activity in a susceptible host infected with a virulent pathogen was mainly responsible for the detoxification of the oxyradicals. Rusterucci *et al.,* (1996) brought out the fact that the elicitin (capsicein and cryptogein)-treated *Nicotiana* cell exhibited increased activity of active oxygen species. This was mainly due to their participation in the detoxification of reactive oxygen species.

The enhanced activity of SOD and PO recorded in the treated cells generally restricted the pathogen activity. Schinkel *et al.,* (2001) brought out the fact that the main function of SOD was to scavenge the superoxide anion radicals, generated in various physiological processes and to prevent the oxidation of biological molecules.

Howell *et al.,* (2000) found out that *T. virens*-treated cotton seeds induced the production of terpenoid synthesis as the results of ISR. This compound suppressed the enzyme activity of *R. solani*. Similar report on the induced systemic resistance of SR in *T harzianum*-treated host cell which inhibited the enzyme activity of the fungal pathogens was made by De Meyer *et al.,* (1998). Similar findings on the induction of SR in *T. harzianum*-treated host plant against fungal pathogen activity was made by Yedidia *et al.,* (1999) in *Cucumis sativas*; Zimand *et al.,* (1996) against *B. cinerea.*