CHAPTER-II

Review of Literature
REVIEW OF LITERATURE

Coleus forskohlii (Willd.) Briq. [syn. Coleus barbatus (Andr.) Benth.] is an important medicinal plant which comes into prominence by virtue of having its presence of terpenoids and phenolics. The swollen primary roots (tubers) are found to be rich source of forskolin. Because of continuous collection of tubers from its wild sources, the plant has been included in the list of endangered species. Apart from this anthropological stresses, the plants are now facing continuously to different types of pathogenic attack, among which the wilt disease caused by Fusarium oxysporum is most important one, occurring in severe form thereby posing a threat to its propagation and cultivation. Literature pertaining to the fusarial wilt of Coleus and its associated management aspects has thoroughly been reviewed and presented to the following outlines:

I. Reports pertaining to the occurrence of the disease.
II. Antioxidant properties of the plant.
III. Antimicrobial activity of the plant.
IV. Histochemical localization of forskolin vis-a-vis phytochemical constituents in Coleus plant.
V. Factors affecting in vitro growth of the pathogen.
VI. Rhizosphere and phyllosphere mycoflora of Coleus plants against the pathogen and selection of potential antagonistic fungi to control the test pathogen under in vitro and in vivo condition.
VII. Colonization and diversity of mycorrhizal (VAM or AM) fungi in rhizosphere soil and roots of Coleus plant.
VIII. Mycoparasitic action of the antagonists in terms of their degree of hydrolytic enzyme production.
IX. Siderophore mediated completion between the antagonists and the pathogen.
X. Efficacy of phytoextracts against the pathogen.
XI. Evaluation of fungicides and antibiotic against the pathogen.
XII. Soil solarization in reducing disease incidence.
XIII. Implication of integrated disease management strategy towards the control of the disease.
XIV. *Glomus fasciculatum* in defense responses to fusarial wilt of *Coleus forskohlii*.

XV. Host defense enzymes during pathogenesis.

XVI. Exploitation of VAM fungus towards the host resistance and yield.

Reports pertaining to the occurrence of the disease

Several fungal diseases are reported on *Coleus* plant in our country. The crop is susceptible to attack by many diseases *viz.*, leaf spot caused by *Botryodiplodia theobromae* (Ramaprasad, 2005), stem blight caused by *Phytophthora nicotianae* var. *nicotianae* (Ramaprasad, 2005) and leaf blight caused by *Rhizoctonia solani* has been reported (Shukla *et al.*, 1993). Kamalakannan (2003) reported the occurrence of pathogens like *Rhizoctonia solani* and *Macrophomina phaseolina* in root rot of *Coleus forskohlii*. Among these, the plant suffers tremendously from a wilt pathogen, *Fusarium oxysporum* (Khatun and Chatterjee, 2011) that result in tremendous economic loss to our country. Most of the vascular wilt causing Fusaria belong to the species *Fusarium oxysporum* and different host plants are attacked by special forms or races of the fungus (Sharma and Bohra, 2003; Singh *et al.*, 2005; Groenewald, 2005; Tsrorlakhhim *et al.*, 2007). *F. oxysporum* is an anamorphic species that includes numerous pathogenic strains causing wilt and root rot diseases of a broad range of host plants having agricultural and ornamental values (Appel and Gordon, 1996). Recorbet *et al.* (2003) conducted a field survey where they recorded that *F. oxysporum* causes wilt in about 80 botanical species.

Antioxidant properties of *Coleus forskohlii*

Plants and plant-derived products are part of the healthcare system since ancient human civilization. Herbal medicines have been used for many years (Kareru *et al.*, 2007). Generally, they use plants for nourishment and medical purposes (Cakilcioglu and Turkoglu, 2010). As in the case in the other countries of the world, in recent years, the plants used traditionally for curative purposes have attracted the attention of researchers (Saya *et al.*, 2000; Etkin and Elisabetsky, 2005; KargIoglu *et al.*, 2008; Ugurlu and Seçmen, 2008; Pieroni and Giusti, 2009; Leonti, 2011). Oxidative stress can arise from an imbalance between the generation and elimination of reactive oxygen species (ROS), leading to excess ROS levels, that inflicts indiscriminate damage to virtually all biomolecules, leading, in turn, to various diseases and cell death (Scandalios, 2005). Reactive species can be eliminated by a number of enzymatic and non-enzymatic antioxidant defence mechanisms (Boullier *et al.*, 2001). Despite the presence of the antioxidant defence system in the cell to counteract oxidation from ROS, radical-related damage
of DNA and proteins has been proposed to play a key role in the development of age dependent
diseases such as cancer, atherosclerosis, arthritis, Alzheimer’s disease, other neurodegenerative
disorder and other such conditions (Collins, 2005). The use of traditional medicine is wide
spread and plants are still a large source of natural antioxidants that might serve as leads for the
development of novel drugs (Linn and Huang, 2002).

Hence, the correct balance between the oxidants and antioxidants in the body is crucial. Free
radicals are generated by normal metabolic processes. There are also natural substances in the
body with antioxidant activity such as peroxidases, catalase and glutathione. Exogenous sources
of antioxidants from plant materials are deemed important to augment endogenous antioxidant
sources (Olarte et al., 2010; Ganu et al., 2010). Most exogenous antioxidants are obtained from
dietary sources and food supplements.

Crude extracts of the various parts (leaves, fruits, roots, stem and trunk bark) of *Garcina
atroviridis* showed strong antioxidant activity exceeding that of the standard vitamin E (Mackeen
et al., 2000). Aqueous extracts from the different parts of the four medicinal plants, *Momordica
charantia*, *Glycyrrhiza glabra*, *Acacia catechu* and *Terminalia chebula* were found to be rich
sources of enzymatic and non-enzymatic antioxidants (Naik et al., 2005). The roots, stem, leaves
and tubers of *Withania somnifera* and *Coleus forskohlii* were found to be rich sources of both
enzymatic and non-enzymatic antioxidants (Sumathi and Padma, 2008, Khatun et al., 2011b).

**Antimicrobial activity of the plant**

The use of higher plants and their preparations to treat infectious diseases is an age-old practice
and in the past possibly the only method available. However, the systematic study of higher
plants for detecting antimicrobial activity is of comparatively recent origin (Skinner, 1955).
These investigations have been triggered by the emergence and spread of antibiotic
resistant microorganisms causing the effective life-span of existing antibiotics limited (Cowan,
1999). Hence, the plant kingdom is being screened for newer and effective chemotherapeutic
agents. Higher plants can serve both as potential antimicrobial crude drugs as well as a source of
new anti-infective agents (Rios and Reico, 2005). Several plants of the genus *Zanthoxylum* have
been studied and different antimicrobial effects have been reported among them (da-Silva et al.,
2006). Yao et al., (2005) found that the ethanol extract from root of Chinese *Z. nitidum* had
moderate antibacterial activity against oral Gram-positive bacteria. The *in vitro* antibacterial
activity of aqueous and ethanol extracts from the stem bark and root of *Zanthoxylum nitidum*
(Roxb.) DC (Rutaceae), growing in north-east India was evaluated against five Gram-positive bacteria including *Staphylococcus aureus, Streptococcus faecalis, Bacillus cereus, Sarcina lutea, Bacillus subtilis* and two Gram-negative bacteria, *Klebsiella pneumoniae, and Escherichia coli*; using disk diffusion method followed by determination of minimum inhibitory concentrations (MIC) by broth dilution method, against sensitive bacteria (Bhattacharya et al., 2009). Plants have been formed the basis of natural pesticides, that make excellent leads for new pesticide development (Newman et al., 2000). The potential of higher plants as a source of new drugs is still largely unexplored. Hence, last decade witnessed an increase in the investigation on plants as a source of new biomolecules for human disease management (Grierson and Afolayan, 1999). Traditionally plants have been well exploited by man for the treatment of human diseases, Ayurveda is a good example, but not much information is available on the exploitation of plant wealth for the management of plant diseases, especially against phytopathogenic fungi. Fungi cause severe damage to stored food commodities. Among different species of fungi *Aspergillus* sp., *Fusarium* sp. and *Penicillium* sp. are associated with heavy loss of grains, fruits, vegetables and other plant products during picking, transit and storage rendering them unfit for human consumption by producing mycotoxins and affecting their nutritive value (Miller, 1995; Janardhana et al., 1999; Galvano et al., 2001). Many seed borne fungi, which cause severe damage to stored food commodities, were generally managed by synthetic chemicals, which were considered both efficient and effective. The continuous use of these synthetic fungicides started unraveling nonbiodegradability and known to have residual toxicity to cause pollution (Pimentel and Levitan, 1986). Pesticide pollution of soil and water bodies is well documented (Nostro et al., 2000). Hence in recent time application of plant metabolites for plant disease management has become important viable component of Integrated Pest Management, as plant metabolites are eco-friendly. The aqueous and different solvent extracts viz., petroleum ether, benzene, chloroform, methanol and ethanol extracts and isolated constituents of *Mimusops elengi* L. (Sapotaceae) was screened in vitro for antifungal activity, by poisoned food technique against wide array of seed borne phytopathogenic fungi (Satish et al., 2008). In vitro antimicrobial activity of plant extracts (*Allium sativum, Zingiber officinale, Caryophyllum aromaticus, Cymbopogon citratus, Mikania glomerata and Psidium guajava*) extracts against Gram-positive and Gram-negative strains of bacteria isolated from human infections has been reported (Ushimaru et al., 2007). The methanol leaf extracts of *Acacia nilotica, Sida cordifolia, Tinospora*
cordifolia, Withania somnifera and Ziziphus mauritiana showed significant antibacterial activity against *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas fluorescens*, *Staphylococcus aureus* and *Xanthomonas axonopodis* pv. *malvacearum* and antifungal activity against *Aspergillus flavus*, *Dreschlera turcica* and *Fusarium verticillioides* as compared to root/bark extracts (Mahesh and Satish, 2008). Antimicrobial activity of some medicinal plants is reported by previous authors (Janovska et al., 2003; Bamola et al., 2008; Kanchana and Ramanathan, 2008; Philip et al., 2009).

**Histochemical localization of forskolin vis-a-vis phytochemical constituents in Coleus plant**

Throughout history, humans have derived many uses and benefits from the plants found in their own region. Initially, wild plants were collected from their natural habitat, followed by the cultivation of those that were used most commonly (Akan et al., 2008). Today the value of the plants is acknowledged and a number of studies are conducted on the plants. There is a growing body of research particularly concentrating on taxonomy, ethnobotanics, plant morphology, anatomy and plant chemistry (Kıvcak et al., 2009; Cabi et al., 2010; Duran et al., 2010; Köyuncu et al., 2010; Bani et al., 2011; Cakilcioglu and Civelek, 2011; Jabeen and Aslam, 2011; Korkmaz and Ozcelik, 2011; Ozudogru et al., 2011; Cakilcioglu et al., 2011; Cakilcioglu and Khatun, 2011). Microscopic structure is long established as providing the most useful and reliable criterion in spite of chemical methods of analysis; especially chromatography which now is accepted as a standard technique for the identification of many vegetable materials. Microscopy also has the advantage of requiring only small quantities of the material and once the method is established, a conclusion as to whether or not the sample is genuine can be reached very quickly. Abraham et al. (1988) found that the transverse section of the root of *C. forskohlii* shows the presence of cytoplasmic vesicles containing terpenoids; which has further been confirmed (Narayan et al., 2002). From histochemical analysis forskolin has been reported to be present in the cells of cork, cortex, medullary rays and xylem in roots and tuber of *C. forskohlii* (Khatun et al., 2011c). Histochemical observations and analyses of leaves of *C. forskohlii* showed the presence of forskolin in the cells of palisade parenchyma, spongy parenchyma and in glandular trichomes of leaf in both the upper and lower epidermis (Khatun et al., 2011d). Qualitative analysis showed that except alkaloids; terpenoids, tannins, flavonoids, phlobatannins, saponins
and cardiac glycosides are present in both the roots and tubers of *C. forskohlii* (Khatun et al., 2011c).

**Factors affecting in vitro growth of the pathogen**

Fungi are very sensitive to the environmental factors and their growth is greatly influenced by the medium or by the substratum on which they grow, pH of the medium and incubation temperature might also be the determining factor.

**Requirement of media**

Fungi and all other living organisms, require basic nutrients for the sustenance of life. Nutrition governs the vital processes in fungi due to their inability to synthesize their own food (Srivastava, 1970). Fungi are therefore, dependent on the medium or the substrate for the elements and compounds they require for their nutrition (Mehrotra, 1988). Although all the fungi have the same basic requirements but there is diversity as to the use of organic and inorganic compounds and there is not a single specific medium or substrate that can universally be suited to all the fungi. As such media, having varied compositions exert considerable influence on the growth of the fungi by supplying them the necessary energy. Jeyalakshmi et al. (2001) determined the aggressiveness of *Fusarium pallidosoreum* cultured on different substrate media and found that potato dextrose and potato sucrose media were most effective for mycelia growth of the fungus. Prenutritional status of culture media has been shown to increase the pathogenicity of many imperfect fungi including *Fusarium* spp. (El-Fakhouri et al., 1995). Among the media tested for growth and sporulation of *Fusarium chlamydosporum*, maximum growth was obtained on potato dextrose agar and rose Bengal agar while the least growth was observed on host extract agar (Shyla, 1998). Desai (1982) reported that, *Fusarium moniliforme* was able to grow very well in Czapek’s-Dox broth while, the least growth was recorded in Elliott’s broth. Richard’s medium was found to be best suited medium for the growth of *Fusarium udum* the causal agent of *Fusarium* wilt on pigeon pea (Sataraddi et al., 2003). Brannon (1923) observed that, glucose and fructose were utilized equally by *Fusarium* spp. for tissue formation, when grown on Czapek’s modified solution. Moore (1924) reported that, the weight of mycelium varied with the source of sugars. Moore and Chupp (1952) working with *Fusarium oxysporum* f. sp. *lycopersici*, *Fusarium conglutinans* and *Fusarium oxysporum* f. sp. *niveum* reported that, all the three were able to utilize a number of carbon sources and hydrolyzed starch. Sowmya (1993) studied the effect of carbon sources on growth of *Fusarium*
Fusarium oxysporum f. sp. cubense. Maximum growth was noticed in medium with glucose as carbon sources while, Sataraddi et al. (2003) reported that the maximum growth of Fusarium udum was obtained on media with mannitol followed by fructose as carbon sources. Singh et al. (2005) reported that F.oxysporum f.sp. lini grew well in linseed and maize meal media. The biomass production of F.equiseti was highest in modified Czapek Dox Broth (Dirak and Ozcelk, 2001). F.oxysporum f. sp.lini when grow as static surface culture in the best synthetic medium containing 15% glucose and 0.4% ammonium as sole carbon and nitrogen sources respectively, it produces biomass of high protein and oil content (Joshi and Mathur, 2006).

**pH requirement of the fungus**

Most of the fungi are very much sensitive with regard to the pH of the medium, while some others can tolerate a wider range of acidity or alkalinity. However, all have some particular reaction, at which they obtain their optimal growth. Experimental evidences generally indicated that they are more tolerant to acidic pH than to basic pH. Tandon (1961) observed that most fungi grow at pH between 4 and 8, although there are some exceptions, which have either a narrower or a wider range of pH tolerance.

The pH of soil is important in the occurrence and severity of plant diseases caused by certain soil borne pathogens. *Fusarium oxysporum* f. sp. lini grew well at pH 5.5 (Singh et al., 2005) or at pH 5.9 (Joshi and Mathur, 2006). *Fusarium oxysporum* f. sp. niveum could grow well on wide range of pH varying from 3.2 to 8.3 with the optimum lying between pH 5.0 and 6.5. As the pH increased or decreased from the optimum, the rate and amount of growth as well as the extent of sporulation gradually decreased (Jhamaria, 1972). Marras et al. (1981) reported that the optimum pH for *Fusarium roseum* var. avenaceum was 7.0. Jadhav et al. (2000) reported that, the fungus *Fusarium chlamydosporum* recorded maximum growth at pH 6.5 which was followed by pH 6.0 and 5.5. Rawal et al. (2003) noticed that, the pH 6.5 favoured maximum growth and sporulation of *Fusarium* spp. The maximum growth of *Fusarium chlamydosporum* was noticed at a pH level of 7.0 which was significant over other pH levels (Ramaprasad, 2005). Brinjal wilt pathogen *F.solani* showed the optimum growth at pH level 7.4 with a tendency of less growth on either side of more acidic or less less acidic (Chakraborty, 2005). Maximum biomass production of the *Fusarium oxysporum* was recorded at pH 7.2 followed by pH 7.4 (Ojha, 2008).

**Temperature requirement of the fungus**

Temperature is one of the cardinal environmental factors, which determine the distribution of fungi in different ecological niches (Dix and Webster, 1995; Magan, 1997). It exerts an influence on fungi largely on enzyme-catalysed reactions. Temperature affects the number of spores released in a given time period (Sharma, 2006). The temperature requirement of fungi may vary considerably not only from one species to another but from one strain to another of the same species. Every fungus has minimum, maximum and optimum temperature for spore germination (Pathak et al., 2003).

The temperature is one of the major limiting factors on distribution and occurrence of *Fusarium* species in different vegetation. Temperature of 30°C was found to be optimum for fungal growth (Gul et al., 2005). Singh et al., (2005) reported that *F. oxysporum* f.sp. *lini* requires an optimum temperature of 25°C±2°C for growth and sporulation. The biomass production of *F. equiseti* was maximum at 25°C (Dirak and Ozcelk, 2001). Joshi and Mathur (2006) studied the effect of growth temperature on physico-chemical characteristics of fungal oil and they showed that when *F. oxysporum* f.sp.*lini* grown as static surface culture in synthetic basal medium, the fungus produced high protein and oil at 28°C±1°C. Alberts et al. (1990) noted that growth rate of *F. moniliforme* was higher at 25°C than at 20°C and maximal yield of mycotoxin fumonisin B₁ was detected at 25°C.

**Seasonal variation of VAM colonization in roots of Coleus forskohlii**

VAM fungi colonize the majority of herbaceous plants roots in natural ecosystems all over the world (Allen et al., 1995). There exist seven groups of mycorrhizal fungi which form a living bridge between the roots and the soil in most ecosystems (Brundrett, 2004; Mehrotra, 2005). Mycorrhiza fungal diversity determining the plant biodiversity and ecosystem viability and productivity (Hart and Klironomos, 2002; Chaudhri, 2005) and thereby influencing the plant communities by affecting species richness (Gange et al., 1990) or species evenness (O’ Connor et al., 2002) have been well documented.

VAM fungi are known to improve the nutritional status, growth and development of plants, protect plants against root pathogens (Vyas and Shukla, 2005) and offer resistance to drought (Auge and Moore, 2005) and salinity (Rabie et al., 2005). Though these fungi are not host specific, recent studies have clearly brought out host preference in AM fungi thus emphasizing the need for selecting efficient AM fungi for inoculating a particular host. Host preference has
been reported in a few medicinal plant species like *Phyllanthus amarus* and *Withania somnifera* (Earanna, 2001), *Coleus forskohlii* (Gracy and Bagyaraj, 2005), *Andrographis paniculata* (Chiromel and Patil, 2006), medicinal plant of Apocynaceae and Asclepiadaceae (Swarupa Rani and Manoharachary, 2007). These fungi form unique structures namely vesicles, arbuscules and hyphae inside the root cortices while hyphae bearing spores/sporocarps and sometimes extrametrical vesicles are present outside the root. Seasonal effects also influence the establishment of plant under field conditions, depending on the efficiency of the indigenous VAM fungi (Lugo and Cabello, 2001; Sharma et al., 2005; Khatun and Chatterjee, 2008).

**Rhizosphere and phylloshere mycoflora of Coleus plants and their antagonism to the pathogen**

Soil borne plant pathogen is posing a great problem towards strategic management of the disease. Soil borne diseases are difficult to control and seed treatment with fungicides does not protect the crops for long periods. Continuous use of same fungicide results in the development of fungicide resistant strains of the pathogen and soil application of fungicide is expensive and also deleterious for associated soil microbiota (Bunker and Mathur, 2001). As an alternative to chemical fungicide in recent times biocontrol agents are gaining impotance in the management of the plant pathogens (Manoranjitham et al., 2002). Biological management of soil borne diseases is increasingly gaining stature as a possible practical and safe approach (Patel and Anahosur, 2001).

For practical biocontrol, use of local isolates of biocontrol agents recovered from naturally disease suppressive soils during the active crop growth is preferable over the aliens ones (Vyas and Mathur, 2002). Dominance of specific rhizosphere organisms were found to arrest the growth of pathogenic fungi as they exhibit strong antagonism (Shah et al., 2005). It has been suggested that microorganisms isolated from the root or rhizosphere of a specific crop may be better adapted to that crop and may provide better control of diseases than organisms originally isolated from other plant species (Cook, 1993). Such plant associated microorganisms may make better biocontrol agents because they are already closely associated with and adapted to the plant or plant part as well as the particular environmental conditions in which they must function.
The screening of such locally adapted strains has yielded improved biocontrol in some cases (Cook, 1993).

The microorganisms employed in biological control of plant pathogens are termed as antagonists. An antagonist is a microorganism that adversely affects another i.e. the target pathogen growing in association with it (Baker and Cook, 1974). Antagonism is the balanced wheel of nature, which operates through competition, parasitism and antibiosis (Chakraborty, 2005; Ojha, 2008). Fungi have got maximum attention as antagonists probably because of the ease in handling and identification compared to other microbes. The use of fungal bioagents against the pathogen has been viewed as an alternative disease management strategy and of the several fungal antagonists tested, *Trichoderma* spp. were extensively explored for the control of soil plant pathogens (Bandyopadhyay et al., 2001; Khan and Sinha, 2005).

*Trichoderma* spp. are fungi that are present nearly all agricultural soils and in other environments such as decaying woods. *Trichoderma* represent the most thoroughly studied fungus which exerts the antagonistic properties towards soil borne plant pathogens. *Trichoderma* strains grow rapidly when inoculated in the soil, because they are naturally resistant to many toxic compounds including herbicides, fungicides and pesticides such as DDT, and phenolic compounds (Chet et al., 1997). *Trichoderma* species can act as biocontrol agents through different synergistic mechanisms (Hermosa et al., 2000). *Trichoderma* plant pathogen interaction suggests competition for nutrients and space (Simon and Sivasithamparan, 1989; Ozbay and Newman, 2004; Sharma and Sain, 2005), inactivation of the enzymes produced by the pathogens (Ozbay and Newman, 2004) modifying the environmental conditions (Benitez et al., 2004), production of inhibitory soluble metabolites (Dennis and Webster, 1971a), mycoparasitism or hyperparasitism involving production of lytic enzymes (Chet et al., 1981; Elad et al., 1982b; Chet, 1990; Haran et al., 1996; Ozbay and Newman, 2004; Chakraborty, 2005), promotion of plant growth and plant defensive mechanisms leading to induced resistant in the host (Benitez et al., 2004; Ozbay and Newman, 2004) and production of siderophores which play a significant role in the antagonism of other fungi (Anke et al., 1991; Srinivasan et al., 1992; Sharma and Sain, 2005). Mechanism of antagonism by employing *Trichoderma harzianum* involving competition, lysis and hyperparasitism has been evidenced from the works of Goodwin-Egein et al. (2001). *Trichoderma* spp. produce antifungal substances and act as mycoparasite and lyse the
pathogenic fungi by secreting hydrolytic enzymes (Baker, 1987; Zaldivar et al., 2001; Shah et al., 2005). TmkA, a mitogen activated protein kinase (MAPK) of *T. virens* is involved in biocontrol properties (Mukerjee et al., 2003) and is involved in induction of plant systemic resistance (Viterbo et al., 2005). Mukherjee et al. (2007) noted cAMP signalling is involved in growth, germination, mycoparasitism and secondary metabolism in *T.virens*.

**Competition**

*Trichoderma* has a superior capacity of mobilization and taking up soil nutrients compared to other microorganisms (Benitez et al., 2004). The efficient use of available nutrients is based on the ability of *Trichoderma* to obtain ATP from the metabolism of different sugars, such as those derived from polymers wide-spread in fungal environments; cellulose, glucan and chitin among others, all of them rendering glucose (Chet et al., 1997). The key components of glucose metabolism include assimilation of enzymes and permeases, together with proteins involved in membrane and cell wall modifications. While the role of glucose transport system remains to be discovered, its efficiency may be crucial in competition (Donzelli et al., 2001), as supported by the isolation of a high-affinity glucose transporter, Gtt1, in *T.harzianum* CECT 24/3 (Benitez et al., 2004). This strain is present in environments very poor in nutrients, and it relies on extracellular hydrolases for survival. Gtt1 is only expressed at very low glucose concentrations i.e. when sugar transport is expected to be limiting in nutrient competition (Delgado-Jarana et al., 2003). *T.harzianum* controls *Fusarium oxysporum* by competing for both rhizosphere colonization and nutrients, with biocontrol becoming more effective as the nutrient concentration decreases (Tjamos et al., 1992).

**Stimulation of plant resistance and plant defense mechanisms**

*Trichoderma* strains are always associated with plant roots and root ecosystem. Some authors have defined *Trichoderma* strains as plant symbiont opportunistic avirulent organisms, able to colonize plant roots by mechanisms similar to those of mycorrizal fungi and to produce compounds that stimulate growth and plant defense mechanisms (Harman et al., 2004). Root colonization by *Trichodema* strains frequently enhances root growth and development, crop productivity, resistance to abiotic stresses and the uptake, and use of nutrients (Arora et al., 1992). Together with the synthesis of stimulation of phytohormone production, most
Trichoderma strains acidify their surrounding environment by secreting organic acids, such as gluconic, citric or fumaric acid (Gomez-Alarcon and de la Torre, 1994). These organic acids result from the metabolism of other carbon sources, mainly glucose, and, in turn, are able to solubilise phosphates, micronutrients and mineral cations including iron, manganese and magnesium (Harman et al., 2004). Plants react against fungal invasion by synthesizing and accumulating phytoalexins, flavonoids and terpenoids, phenolic derivatives, aglycones and other antimicrobial compounds. Trichoderma strains are generally more resistant to these compounds than most of the fungi and their ability to colonize plant roots strongly depends on the capacity of each strain to tolerate them. This resistance is considered an essential requirement for plant colonization, has been associated with the presence of ABC transport systems in Trichoderma strains (Harman et al., 2004).

The intensity of pectolytic enzyme activity particularly the polygalacturonase activity has been reported to be reduced in the presence of a strain of Trichoderma (Urbanek et al., 1991; Zimand et al., 1996). This may be due to accumulation of the larger molecules of oligogalacturonides and elicitation of the defence mechanism of the host plants as result of which the severity of the development of disease is being slowed down (Urbanek et al., 1991).

The addition of Trichoderma metabolites that may act as elicitors of plant resistance or the expression in transgenic plants of genes whose products act as elicitors, also result in the synthesis of phytoalexins, PR proteins and othert compounds and in an increase in resistance against several plant pathogens, including fungi and bacteria (Elad et al., 2000; Dana et al., 2001). Barley expressing Trichoderma atroviride endochitinase Ech 42 showed increased resistance towards Fusarium infection (McIntyre et al., 2004). Trichoderma strains are able to modify external pH of rhizosphere and to adapt their own metabolism to the surrounding growth conditions would consequently reduce the virulence of phytopathogens because most pathogenecity factors could not be synthesized (Benitez et al., 2004).

Mycoparasitism

Mycoparasitism, the direct attack of one fungus on another, is a very complex process that involves sequential events, including recognition, attack and subsequent penetration and killing of the host. This is followed by the growth of the mycoparasite on the host fungal content (Chet
et al., 1981; Elad et al., 1982b; Desai and Schlosser, 1999; Benitez et al., 2004; Chakraborty, 2005). *Trichoderma* spp. may exert direct biocontrol by parasitizing a range of fungi, detecting other fungi and growing towards them. Mycoparasitism of plant pathogenic fungi by *Trichoderma* isolates has been well researched and is widely considered to be a major contributing factor to the biocontrol of *Trichoderma* spp. of a range of commercially important plant diseases (Harman et al., 1981). Mycoparasitism of *T. lignorum*, *T. viride* has been reported on the fungi causing diseases of plants particularly the soil borne diseases of plants particularly the soil borne diseases (Jee et al., 1987; Ozbay and Newman, 2004).

Mycoparasitism involves morphological changes, such as coiling and formation of appressorium like structures, which serve to penetrate the host and contain concentrations of osmotic solutes such as glycerol (McIntyre et al., 2004). *Trichoderma* attaches to the pathogen with cell wall carbohydrates that bind to pathogen lectins. Once *Trichoderma* is attached, it coils around the pathogen and forms appressoria. The following step consists of the production of cell wall degrading enzymes and peptaibols (Howell, 2003) that facilitate both entry of *Trichoderma* hypha in the lumen of the parasitized fungus and assimilation of the cell-wall content. Dubey and Suresh (2006) observed the principle mechanism of mycoparasitism of *Trichoderma* strains as coiling around the *Fusarium oxysporum* hyphae and lysis. Investigation on the responsible signal transduction pathways of *T. atroviride* during mycoparasitism has led to isolation of key components of the cAMP and MAP kinase signalling pathways, such as α-subunits and G-protein (G-α), which control extracellular enzyme, antibiotic production and coiling around host hypha (McIntyre et al., 2004).

**Antibiosis**

Antibiosis occurs during interactions involving low-molecular weight diffusible compounds or antibiotics produced by *Trichoderma* strains that inhibit the growth of other microorganisms. The culture filtrate of *Trichoderma* spp. contains some kinds of antibiotics or enzymes that are responsible for the inhibition or suppression of the vegetative growth and spore germination of pathogenic fungi (Dutta, 1998; Zaldivar et al., 2001; Chakraborty, 2005; Ojha, 2008). Different species of *Trichoderma* like *T. harzianum*, *T. aureo-viride*, *T. hamatum*, *T. koningii* differ significantly in their ability to infect and to produce antibiotics (Desai and Schlosser, 1999). *T. viride* and othe species of *Trichoderma* are capable of producing some antibiotics in the culture
filtrate which are fungistatic in nature (Ooka et al., 1996; Jackson et al., 1991; Mondal et al., 1995). The culture filtrates of antagonists like *T. reesei*, *T. pseudokoningii* contain some kinds of antibiotics or enzymes (Di Pietro et al., 1993). Kim et al. (1990) discovered glucose oxidase as the antifungal principle from *T. flavus*. Hydrolytic enzymes such as glucanase, chitinase, cellulose, xylanase, α- and β-galactosidase, N-acetyl glucosaminidase and protease are known to be produced by *Trichoderma* upon infection (Aziz et al., 1993; Dutta and Chatterjee, 2004).

Most *Trichoderma* strains produce volatile and non-volatile toxic metabolites that impede colonization by antagonistic microorganisms; among these metabolites the production of harzianic acid, alamethicine, tricholin, peptaibols, antibiotics, 6-pentyl-α-pyrone, massoilactone, viridian, glioviridin, glisopenins, haptilidic acid and others have been described by Vey et al. (2001). *Trichoderma* spp. produce trichodermin which completely controlled *Fusarium* rots in wheat (Krivoshchekova and Mischenk, 1990). A new antifungal compound (6-substituted 2H-Pyran-2-one named viridipyronone) has been isolated from the culture of a strain of *T. viride* showing in vitro activity towards *Sclerotium rolfsii* (Kucuk and Kivanc, 2003).

Isolates of *Trichoderma* checked the growth of many fungal pathogens including *Fusarium oxysporum*, *F. udum* and *F. sporotrichioides* (Bandyopadhyay et al., 2001; Gromovykh, 2002; Osuinde et al., 2002; Shah et al., 2005; Wani, 2005). Bhat et al. (2003) reported that biocontrol agents *T. viride* and *T. harzianum* resulted in reduction of chickpea wilt caused by *F. oxysporum* f.sp.*ciceri* and thereby caused improvement in plant growth. *Trichoderma* species viz. *T. harzianum*, *T. viride* and *T. hamatum* effectively inhibited the growth and sporulation of *F. oxysporum* f.sp.*cumini* in vitro through production of volatile and non-volatile antibiotics (Vyas and Mathur, 2002).

The combination of hydrolytic enzymes and antibiotics results in a higher level of antagonism than that obtained by either mechanism alone (Howell, 1998; Monte, 2001). Sequential roles of antibiosis and hydrolytic enzymes during fungal interaction have also been described when combinations of antibiotics and several kinds of hydrolytic enzymes were applied to propagules of *F. oxysporum*, synergism occurred, but it was lower when the enzymes were added after the antibiotics, indicating that cell wall degradation was needed to establish the interaction (Howell, 1998). Petaibols, produced by many *Trichoderma* strains, are a class of linear peptides that generally have strong antimicrobial activity against gram-positive bacteria and fungi and act
synergistically with cell wall degrading enzymes to inhibit the growth of fungal pathogens and elicit plant resistance to pathogens (Wiest et al., 2002).

**Mycoparasitic action of the antagonists in terms of their degree of hydrolytic enzyme production**

Mycoparasitism, one of the main mechanisms involved in the antagonistic activity of *Trichoderma* strains, depends on the secretion of complex mixtures of hydrolytic enzymes able to degrade the host cell wall (Marco and Felix, 2002; Sanz et al., 2005). Lytic enzymes of mycoparasitic fungi, *Trichoderma* spp. capable of suppressing several fungal phytopathogens that originate in air and soil, are reviewed (Elad et al., 1982b; Srinivasan et al., 1992; Delgado-Jarana et al., 2000; Witkowska and Maj, 2002; De Marco et al., 2003; Markovich and Konovona, 2003). Interstrain and interspecific variations exist among the *Trichoderma* isolates with regard to their ability to produce both laminarinase and chitinase (Srinivasan, 1993; Bruce et al., 1995; Viterbo et al., 2002; Chakraborty, 2005). Characterization of laminarinase from *Trichoderma* sp. was performed by Sharma and Nakas (1987). It was reported that lectins were shown to be involved in the recognition of *Trichoderma* spp. and their host fungus, while chitinase involved in the degradation of host cell wall (Chet and Inbar, 1994). Markovich and Kononova (2003) observed *in vitro* ability of *Trichoderma* species to degrade cell walls and inhibit spore germination or germ tube elongation in various pathopathogenic fungi.

The chitinolytic system of *Trichoderma* comprises many enzymes and the list of its components is rapidly being updated as new enzymes and genes are reported. Chitinase are divided into 1, 4-β-acetylglucosaminidases, endochitinases and exochitinases (Benitez et al., 2004). Ulhoa and Peberdy (1991) purified the extracellular chitinase produced by *T. harzianum*. *T. harzianum* Rifai TM transformants overexpressing chit 36 chitinase inhibited *F. oxysporum* more strongly than the wild type. Moreover, culture filtrates inhibited the germination of *Botrytis cinerea* almost completely (Viterbo et al., 2001). It has been shown that β-1, 3-glucanases inhibit spore germination or the growth of pathogen in synergistic cooperation with chitinases (Benitez et al., 1998; El-Katatny et al., 2001). Proteases involved in the degradation of heterologously produced
proteins have been characterized and alkaline protease pbr 1 from *T. harzianum* IMI 20604 has been demonstrated to play an important role in biological control (Delgado-Jarana et al., 2000; Benitez et al., 1998). Nakkeeran et al. (2005) reported the efficacy of chitinase and β-1, 3-glucanases of *T. viride* in the management of cotton rot. The strain of *T. hamatum* C-1 was able to produce extracellular lytic enzymes such as β-1, 6-glucanases (Maj et al., 2002).

Many β-1, 3-glucanases have been isolated, but only a few genes have been cloned, e.g. bgn 13.1 and lam 1.3 from *T. harzianum* (Benitez et al., 1998). BGN 13.1 has been reported to inhibit the growth of *Botrytis cinerea*, *Rhizoctonia solani* and *Phytophthora citophthora* (Benitez et al., 2004). Transformant T28, which is the highest BGN 13.1 glucanase activity under both repressing and including conditions, showed the highest inhibition of pathogen (Benitez et al., 2004). Crude enzyme preparation of *Trichoderma* species with lytic enzyme activities greatly reduced the germinated conidia of *Fusarium* spp. and aberrant morphology of conidia was observed with germ tube lysis (Shoulkamy et al., 2006).

Production of hydrolytic enzymes appeared to be higher by *Trichoderma* spp. when the growth medium was supplemented with cell wall of some other fungi which are not being parasitized by *Trichoderma* (Sivan and Chet, 1989). From this observation it is extrapolated that the cell wall of host fungi certainly provides some biochemical stimulus for enzyme induction (Sivan and Chet, 1989). Extracellular enzymes corresponding to the main chemical constituents of the fungal cell wall i.e. chitin, glucans, proteins, have been detected when *T. harzianum* is grown on *Rhizoctonia solani* mycelia or cell wall as the sole carbon source (Ridout et al., 1998; Geremia et al., 1993). Shoulkamy et al. (2006) also reported that *Trichoderma* species produced chitinase and β-1, 3 glucanase in liquid culture containing cell wall of *Fusarium* spp. as sole carbon source.

Production of lytic enzymes and the factors which influence their production are therefore, the aspects which will determine the potential of any *Trichoderma* isolate selected for the biological control of plant pathogenic fungi (Bruce et al., 1995; Witkowska and Maj, 2002; Karasuda et al., 2003). Markovich and Kononova (2003) reported the cloning of the expression of genes coding for certain lytic enzymes of *Trichoderma* spp. and *Trichoderma* transformants produce higher levels of one lytic enzyme and thereby exhibit a more pronounced ability to suppress phytopathogenic fungi.
Siderophore mediated competition between the antagonists and the pathogen

The history of iron nutrition of microorganisms dates back to evolutionary period when anaerobic life was come into existence and it was assumed that such form of life was probably using ferrous ions. In due course, in the present context of aerobic environment, majority of microbes have opted to use ferric form instead of ferrous form for their nutrition (Chincholkar et al., 2006).

Iron is an essential nutrient for nearly all organisms because it serves as an obligate component of many indispensable enzymes and other proteins (Askwith et al., 1996). Iron is needed in relatively small amount but is essential as a donor and acceptor of electrons in cellular processes including cytochrome system in aerobic respiration (Deacon, 2006). The optimum requirement of iron for the normal growth and metabolism of soil microorganisms ranges from $10^{-8}$ to $10^{-6}$ M, but Fe (III) in aerobic aqueous environment is limited to an equilibrium concentration of $10^{-7}$ M which is much below than its optimum value. Iron normally occurs in the ferric ($Fe^{3+}$) form, insolubilized as ferric oxides or hydroxides at a pH above 5.5. Microbial pathogens have collectively evolved a diverse repertoire of highly effective iron acquisition mechanisms, some of which appear to be specially designed to defeat the iron withholding system or to target iron-rich niches of the host (Patne, 1993; Howard, 1999; Ratledge and Dover, 2000). A more commonly used strategy for microorganisms to acquire iron is the secretion of high affinity iron chelators called siderophores (Stintzi et al., 2000; Brem et al., 2001). Siderophores (Greek: sideros=iron, phores=bearers) are low molecular weight iron chelating compounds produced by microorganisms under iron stress condition (Neilands, 1981). Thus stress condition of iron is a decisive factor for the biosynthesis of siderophores (Dutta et al., 2006). No systems analogous to siderophores have been found for any other metal ion and thereby making iron unique in requiring such specific ligands (Yeole et al., 2001). Reports are available that large number of fungi and bacteria produce siderophores under iron-limiting conditions in the soil (Hider, 1984; Jellison et al., 1991; Anke et al., 1991; Bakkza et al., 2003; Rane et al., 2005; Chincholkar et al., 2006). Siderophores chelate a ferric ion ($Fe^{3+}$) then they reabsorbed through a specific membrane protein and $Fe^{3+}$ is reduced to $Fe^{2+}$ within the cell, causing its release because the siderophore has a lower affinity for $Fe^{2+}$ than for $Fe^{3+}$ and finally the siderophore is exported again to capture a further ferric ion (Deacon, 2006).
As siderophore produced by a microorganism can bind with iron in a high specificity and affinity thus making the iron unavailable for other microorganisms and thereby limiting the growth of the other microbes, the strategy may certainly be involved in the biological control of plant diseases. Competition for iron by siderophore production has long been recognised as an important antagonistic trait of many biocontrol agents against plant pathogens (Neilands, 1984; Leong, 1986; Bossier et al., 1988; Barash, 1990; Dube, 1995; de Boer et al., 2003).

Siderophores according to their chemical nature may be grouped into three classes like hydroxamate type, catecholate or phenolate type and carboxylate type (Mayer and Abdallah, 1978; Philipson and Lilinas, 1982; Jalal and van der Dick, 1990). A common feature of eukaryotic organisms, especially fungi, is to produce hydroxamate type of siderophores while prokaryotes produce both hydroxamate and catecholate types (Rane et al., 2005). Machuca et al. (2007) reported the production of hydroxamate and catecholate type iron-chelating compounds by ectomycorrhizal fungi. The structure, functions and applications of fungal siderophores were reviewed by Neilands (1995) and Renshaw et al. (2002). Fungal siderophores are classified as (i) ferrichromes, (ii) coprogens, (iii) rhodotorulic acid (iv) fusarimines and rhizoferrins (Chincholkar et al., 2006). Carran et al. (2001) reported a class of siderophores called ‘heterobactins’ containing hydroxamate and catecholate donor groups together. Methods of siderophore analysis have evolved quite fast leading to add accuracy and precision (Chincholkar et al., 2000).

Growth promotion of crop plants followed by higher yield by using siderophore producing microorganisms has been reported by different workers (Kloeper et al., 1980; Teintze et al., 1981; Yeole and Dube, 1997).

Transport of siderophores is an energy-dependent process and is stereoselective, depending on to transporting iron, siderophores have other functions and effects including enhancing pathogenecity, acting as intracellular iron storage compound and suppressing growth of the other microorganisms. Siderophores can complex other metals apart from iron, in particular the actinides (Renshaw et al., 2002).

Some *Trichoderma* spp. produce highly efficient siderophores that chelate iron and stop the growth of other fungi (Chet and Inbar, 1994). Anke et al. (1991) recorded hydroxamate type of
siderophore type of siderophore production by different species of *Trichoderma*. *Trichoderma* spp. are also capable of producing phenolate type siderophores and production of such metabolites by *Trichoderma* may contribute to the biological control of different fungi (Srinivasan et al., 1992). Therefore, siderophore producing microorganisms may promisingly be used in biological control of plant pathogens. Pseudobactin-siderophore producing pseudomonads can suppress germination of the chlamydospires of *Fusarium oxysporum* on low-iron media, whereas mutant pseudomonads, deficient in siderophore production are ineffective (Deacon, 2006). Manwar (2001) had successfully employed siderphoregenic biocontrol microbes in control of ground nut root pathogen *Fusarium oxysporum*.

**Efficacy of botanicals against the pathogen**

Botanicals are in general more compatible with environmental components than the chemical pesticides owing primarily to their susceptibility to degradation by heat, light and microorganisms (Kumar, 2006). Plant based pesticides which are relatively cheaper, safe and non-hazardous can be used successfully against plant pathogenic fungi (Ramachandra and Kalappananavar, 2006b). Approximately 20% of the plants found in the world have been submitted to pharmacological or biological tests (Suffredini et al., 2004). The extracts of higher plants can be very good source of antibiotics (Fridous et al., 1990) against various fungal and bacterial pathogens. Studies to determine the fungistatic and fungicidal nature of plant extracts have been carried out by various workers (Sobti et al., 1995; Gomathi et al., 2000; Bhat et al., 2001; Sharma and Bhora, 2003; Bhattacharjee et al., 2005; Haseeb and Kumar, 2007).

A plant extract may exhibit wide range of fungitoxicity or may show selective toxicity (Tripathi, 1986). Crude aqueous extract of various plants have exhibited some levels of antifungal activity (Awuah, 1989; Hussiani and Deeni, 1991). Such extracts contain active compounds that are biodegradable and are selective in their toxicity (Agabenin and Marley, 2006). Effectiveness of plant extracts depends on the nature and amount of the active principle contained in them. A survey of antifungal compounds from higher plants was carried out by Grayer and Harbone (1994).
Evaluation of botanicals against *Fusarium oxysporum* has been carried out by many workers (Bowers and Locke, 1997; Bansal and Gupta, 2000; Bowers and Locke, 2000; Verma and Dohroo, 2003; Agabenin and Marley, 2006).

Bulb extract of *Allium sativum* showed high inhibitory activity towards the growth of different harmful fungi have been reported by many workers (Augusti, 1996; Bianchi et al., 1997; Shivapuri and Gupta, 2001; Kapadiya et al., 2001). Ark and Thompson (1959) attributed to the antimicrobial properties of garlic extracts to volatile components and Murthy and Amonkar (1974) attributed them more specifically to diallyl-disulphide. Cell free extracts of garlic contain some antifungal metabolites or active principles of which allicin (diallyl thiosulphinate) and ajoene in volatile forms contained in the extract (Singh et al., 1992; Tariq and Magee, 1990). The principle volatiles from garlic extracts have been identified as 2-propenyl disulphide and methyl allyl disulphide although additional sulphides are present at low concentrations (Freeman and Whenham, 1975; Esler and Coley-Smith, 1983). At fungistatic or fungicidal levels, allicin disrupts cell metabolism by oxidizing or binding to sulphhydryl (thiol) groups of essential aminoacids, proteins and enzymes (Barone and Tansley, 1977). Tariq and Magee (1990) observed destruction of microconidia of *F. oxysporum* f.sp. *lycopersici* in the presence of garlic volatiles may have been due to biochemical and structural changes in the immature walls of young spores, caused by the disruption of precursors and enzymes involved in the synthesis of wall components, resulting in changes in wall permeability, disruption of the cytoplasm and ultimate lysis of the spores.

Leaf extracts of *Azadirachta indica* (Neem) effectively inhibited the mycelial growth of different plant pathogenic fungi (Sarvamangala et al., 1993; Biswas et al., 1995; Kamalakannan et al., 2001; Bhattacharya and Pramanik, 1998; Natarajan et al., 2003, Ramachandran and Kalappanavar, 2006b). Neem preparation was significantly efficient to control *Fusarium* spp. (Singh and Prasad, 1993; Dutta and Chatterjee, 2000; Bansal and Gupta, 2000; Sagar et al., 2002; Chandel and Tomar, 2007).

Effectiveness of leaf extracts of *Pongamia pinnata*, *Calotropis giganta* L., *Azadirachta indica* L. against *Fusarium pallidoroseum* and *Fusarium oxysporum* was reported by Gupta et al. (1996). The inhibitory effects of essential oils extracts from 10 Indian plants were evaluated against *Fusarium chlamydosporum*. The plants used for extraction of essential oils were *Eucalyptus* spp.,
Ocimum basilicum, Prosopis cineraria, Derris indica and Pongamia pinnata. The paper disc method and the serial dilution techniques were followed to test the susceptibility of the Fusarium species and the results were compared with miconazole. The essential oils extracted from eucalyptus markedly inhibited fungal growth (Rai et al., 1999). Farrukh Aqil et al. (2003) obtained essential oils of pepper mint (Mentha sp.), clove (Syzygium aromaticum) and eucalyptus (Eucalyptus globulus) and evaluated for their antifungal activity against soil borne fungi, including Aspergillus niger, Alternaria alternata and Fusarium chlamydosporum by agar well diffusion method. Maximum antifungal activity was detected in essential oil of clove oil followed by those of peppermint and eucalyptus. Fusarium chlamydosporum was found to be most susceptible to essential oils in liquid as well as solid media.

Out of twelve botanicals evaluated in vitro, P. hysterophorus leaf extract was found to be most effective in inhibiting the mycelial growth against F. chlamydosporum and R. bataticola. E. globulus leaf extract at 10 per cent was found to be effective in inhibiting the growth of S. rolfsii. The least per cent inhibition was recorded in Cassia occidentalis leaf extract at five per cent against F. chlamydosporum and Prosopis juliflora leaf extract at five per cent against R. bataticola and S. rolfsii (Ramaprasad, 2005).

Haseeb and Kumar (2007) reported that neem seed powder and neem cake were moderately effective against F. oxysporum and can be exploited under field conditions for further experimentation to manage fusarial wilt of brinjal. Chemical basis of the antifungal activity of Azadirachta indica has been attributed by the presence of oil in the plant parts (Singh and Dwivedi, 1990). Commercial formulations of neem viz. neemazal and nimbicidine decreased incidence of wilt disease of gladiolus caused by Fusarium oxysporum f. sp. gladioli (Chandel and Tomar, 2007). Neem products like neem cake and nimin were effective in reducing the disease of club root pathogens Plasmodiphora brassicae under field conditions (Bhattacharya and Pramanik, 1998). Rawat (1995), Meshram (1995) and Sharma (1998) concluded that the antifungal activity of neem extracts might be due to the presence of active chemicals like azadirachtin, nimbidin, nimbinin, nimbolidin, nivasin etc. contained in the extract. Azadirachtin from neem acted as a chitin inhibitor and cause lysis of cell walls of resting pathogenic spores present in sick soil and stimulation of fungal antagonist in soil may have an indirect effect (Bhattacharya and Pramanik, 1998).
Ocimum sanctum (tulsi) extract exhibited strong toxicity inhibiting complete mycelia growth of Penicillium italicum (Tripathy and Dubey, 2003). Antifungal activity of leaves and seeds of O.sanctum have been reported by a number of investigators (Ahmad and Prasad, 1995; Shivpuri et al., 1997; Daya Ram, 1997; Bansal and Gupta, 2000; Singh and Majumdar, 2001; Shivpuri and Gupta, 2001; Ramachandra and Kalappanavar, 2006b). Petroleum ether extract of O.sanctum leaf exhibited strong toxicity inhibiting complete mycelial growth of Penicillium italicum (Tripathi and Dubey, 2003).

Vitex trifolia leaf extract showed fungistatic activity against Rhizoctonia solani, the causal organism of sheath blight of rice (Ansari, 1995). The leaf extract of Vitex negundo contains p-hydroxybenzoic acid and 3, 4-dihydroxy-benzoic acid and these benzoic acids have been reported to have artificial immunising properties against Dutch elm disease causing fungus, Ceratocystis ulani (Harsh, 1998). Singh et al., (2007) studied the influence of Vitex negundo extract on suppression of fusarial wilt and growth of Dalbergia sissoo seedlings. However, Ramachandra and Kalappanavar (2006b) reported that V.negundo extract was not very much effective against Exserohilum hawaiensis, the causal agent of leaf blight of dicoccum wheat.

Rhizome of Zingiber officinale (Ginger) exhibited significantly lower disease severity against Alternaria alternata fruit rot of pomegranate (Singh and Majumdar, 2001). Extract of rhizome of Z.officinale control powdery mildew of pea (Singh et al., 1991) and powdery mildew of mulberry (Biswas et al., 1995). Verma and Dohroo (2003) reported inhibition of mycelial growth of F.oxysporum by ginger rhizome extract. Singh et al. (1983) isolated and characterised the fungitoxic principle from Z.officinale.

Fungitoxic activity of leaf extract of Calotropis procera was studied by different authors (Bansal and Gupta, 2000; Shivpuri and Gupta, 2001; Patni et al., 2005). Mycelial growth and spore germination of Fusarium oxysporum was inhibited by the leaf extract of C.procera.

Antimicrobial activity of crude extracts of Polyalthia longifolia was reported by Annapurna et al. (2006). Extract of P. longifolia leaf inhibited the sclerotial production of Sclerotinia sclerotiorum (Shivpuri and Gupta, 2001). Bark extract of P.longifolia was quite effective against leaf spot and leaf rust diseases of mulberry (Biswas et al., 1995). Patni et al. (2005) reported the fungitoxic activity of leaf extract of P. longifolia against Alternaria blight of mustard. Similar inhibitory response was also recorded by Sobti et al. (1995) against Aspergillus spp. and Macrophomina phaseolina. Several compounds have been isolated from different parts of the plant. Aporphine
and azofluorene type alkaloids have been isolated from the leaves of *P. longifolia* (Jossang et al., 1982; Wu et al., 1990). Proanthocyanidine trimer (Wu, 1989) and clerodane diterpenoids, sistosterols (Phadnis et al., 1988; Annapurna et al., 2006) have also been isolated from this plant. The methanol extract of stem parts of *P. longifolia* showed significant phytotoxic activity (Annapurna et al., 2006).

**Evaluation of fungicides and antibiotics against the pathogen**

The use of chemical compounds toxic to the pathogens is the most common means of controlling plant diseases in the field, in the greenhouse and also in storage. Chemicals were used to control plant diseases long before their causal agencies were known (Manners, 1982). In order to minimize the crop loss and to attain higher yield, chemical use of fungicide has become an essential input in modern agriculture (Rama Krishna Parama et al., 1997). Despite the beneficial impact of these fungicides in improving and sterilizing agricultural productivity, by the control of pest and diseases, a major portion of these agrochemicals tends to affect the soil biological activity in different ways (Nagaraja et al., 1997).

Kopacki and Wagner (2006) studied the effect of fungicides like mancozeb, carbendazim, captan, topsin M on mycelial growth of *Fusarium avenaceum* pathogenic to chrysanthemum and found that Carbendazim was most effect while mancozeb and captan were least effective. Bharath et al. (2005) reported that plants sprayed with Bavistin showed least incidence of wilt diseases; hence it proved its efficacy against *Fusarium* species. Carbendazim (Bavistin) and Benomyl have been reported to offer significant control of Bekanae disease of rice caused by *F. moniliforme* (Ou, 1985; Titone et al., 2004; Bagga and Sharma, 2006). Benomyl, carbendazim and thiophanates have been developed for the tomato diseases caused by *Fusarium oxysporum* and their effects on systemic pathogens like *Verticillium*, *Fusarium* and *Ceratocystis* were promising under controlled condition (Delp, 1987). Urban and Filipowicz (2004) evaluated the efficiency of fungicides to *F. oxysporum* isolates obtained from tomato. Thiram and Bavistin were found to control the collar rot and dry root rot diseases effectively (Jackson, 1975a). Thiram and Bavistin showed the most suitable fungicidal activity in inhibiting the growth of *F. oxysporum in vitro* and these fungicides reduced the incidence of wilt when used as seed treatment or soil drenching (Singh and Jha, 2003). Wilt of gladiolus caused by *F. oxysporum f. sp. gladioli* had been successfully managed by the use of carbendazim, quintal, mancozeb, methyl thiophanate (Baynate) and thiram (Chandel and Tomar, 2007). Das et al. (2004) reported
that carbendazim plus captan as corm dip plus its application (3 times) as a soil treatment effectively reduced severity of *Fusarium* yellows of gladiolus caused by *Fusarium oxysporum* f. sp. *gladioli* and the treatment also increased corm spouting, and reduced the post harvest rotting of corms.

Carbendazim (Bavistin) was recorded as the highly effective fungicide inhibiting growth of several plant pathogens like *Colletotrichum acutatum* (Sattar et al., 2003), *Myrothecium roridum* (Tomar and Shastry, 2006), *Exoserohilum hawaiiensis* (Ramachandra and Kalappanavar, 2006), *Pyricularia penniseti* (Clara et al., 2007). Several fungicides were known which interfere at specific sites of biosynthetic processes in fungi, like respiration, membrane structure or nuclear function (Dekker, 1987). Howard and Aist (1977, 1980) studied the various effects of carbendazim on hyphal tips cells of *Fusarium acuminatum* by light and electron microscopy and a variety of effects ascribed to disappearance of microtubules. According to Arinze and Yubedee (2000), benlate, calixin and captan inhibited the activity of cellulose in *Fusarium moniliforme*.

Antibiotics are substances produced by one microorganism and toxic to another microorganism. Antibiotics used for plant disease control are generally absorbed and translocated systemically by the plant to a limited extent. Antibiotics may control plant diseases by acting on the pathogen or the host (Agrios, 1997). The important antibiotics for the control of plant diseases are streptomycin, Tetracyclines, Griseofulvin, Cycloheximide and Aureofungin (Mehrotra and Aggarwal, 2003). Misato (1977) discussed in detail the development of agricultural antibiotics. Of the nine fungicides and three nitrogenous compounds tested *in vitro*, the fungicides bavistin (carbendazim) and difolatan (Captafol) effectively checked the growth of *Fusarium oxysporum* f. sp. *lycopersici*, whereas PCNB (*Quintozene*) stimulated the growth (Dwivedi and Pathak, 1981). Eleven fungicides were assayed *in vitro* and in the field against *Piper beetle* decline due to *Fusarium solani*. Bavistin (Carbendazim) was found to be the most effective chemical under prevailing soil conditions followed by captan and blitox (Hiremath et al., 1987).

Dilip (1989) reported that, *Fusarium oxysporum* f. sp. *nicotianae* causing wilt of tobacco completely inhibited the growth by carbendazim at 1000 ppm concentration which was followed by ziram and captafol substantially.

The laboratory assay of fungicides against *Fusarium oxysporum* f. sp. *cubense* showed cent percent inhibition with bavistin, dithane M-45, and emisan at all concentrations tested (Sowmya,
1993). Among the six concentrations Carbendazim, captan, copperoxy chloride at 1000 ppm gave total inhibition of the fungal growth. Rawal et al. (2003) reported that systemic fungicides viz., Carbendazim, tridemifon and kitazin (at 10, 25, 50 and 75 ai/ha) were found effective in inhibiting the growth of *Fusarium solani* which caused *Fusarium* rot of sponge gourd fruits.

**Soil solarization in reducing disease incidence**

Increased social and legislative pressure to restrict the use of chemicals has created the impetus to evaluate alternative approaches for management of soil borne diseases (Chattopadhyay and Kalpana Sastry, 2001).

Soil solarization involves the use of transparent polyethylene mulch to trap solar energy for heating the soil to control pests in the soil (Katan, 1981). Mulching with transparent polyethylene mulch (25 µm) resulted in average maximum temperature of 49.7°C at 8 cm soil depth, which was 13.5°C higher over non solarised beds (Raj et al., 1997).

Increased soil temperature at different durations easily controlled several plant pathogenic fungal populations have been reported by many workers (Patel, 2001; Khulbe and Chambe, 2002; Mathur et al., 2002; Raj and Bharadwaz, 2000; Hazarika, 2004). Thermal inactivation could be the reason for suppression of these pathogens by solarization. Moreover, the moist heat generated with available soil moisture might have resulted in pasteurization of soil (Krishna Rao and Krishnappa, 1995). Soil solarization for 20 days was found effective to bring down the soil population of *Fusarium* spp. to zero level upto 15 cm soil depth (Raj et al., 1997). A study of tomato in Florida by Kokalis-Burelle et al. (2002) showed that solarization treatments significantly reduced the colony forming units (on agar) of *Fusarium* compared to control plots. Soil covered with transparent polyethylene for 40 days (during the summer) showed incidence of soil-borne diseases in different crops ranging from 9 to 6%, compared to unsolarized plots that had 10-21% disease incidence. The length of time needed to reduce *Fusarium* disease depends on the initial inoculums density, the climate and the susceptibility of the cultivar. Solarization
can be effective in green house soils raised temperature by 9° C and reduced *Fusarium* population density in the soil by 91 to 98%.

Soil solarization has been reported to control *Fusarium* wilt of cotton (Katan et al., 1980) and *Verticillium* wilt of tomato (Morgan et al., 1991). Katan et al. (1983) reported a long-term effect of soil solarization against *Fusarium oxysporum* f. sp. *vasinfectum* in cotton trials for over three years. Tjamos and Paplomatas (1988) found that soil solarization technique reduce the incidence of *Verticillium* wilt of globe artichokes for three successive cropping seasons. Soil solarization for 60 days with transparent polyethylene mulch (25 µm) kill the propagules of *Fusarium* wilt of tomato (Raj and Kapoor, 1993) and watermelon (Martyn and Hertz, 1986). The initial population of *Fusarium oxysporum* propagules was reduced from $6.56 \times 10^3$ to $1.44 \times 10^3$ cfu/g soil by solarization (Krishna Rao and Krishnappa, 1995).

In addition to direct cumulative heat damage, accumulation of volatiles, interaction of variety of physical, chemical and biological mechanisms taking place under tarps might contribute to control of the pathogen (Stapleton and DeVay, 1982; Sztejnberg et al., 1987). Solarized soil samples showed a significant increase in nitrogen and potassium contents at 10 to 30 cm depths (Sharma and Sharma, 2002). Increased K and N contents had probably helped in controlling the pathogen, since these macroelements have been reported to act directly on the pathogen by reducing its penetration, multiplication and establishment in the host or indirectly by increasing resistance of the host against pathogen (Patil, 1981). Mohanan et al. (2004) concluded that solarization promoted 26.92% increase in germination over non-solarized check. Tomato seedlings grown in solarized nursery beds had better vigour in terms of shoot and root length than seedlings grown in non solarised beds (Raj et al., 1997; Ojha, 2008). A significant increase in yield has been reported in brinjal (Katan et al., 1976) and tomato (Bourbos and Skoudridakis, 1996) after solarization. The amount of yield increases as a result of disease reduction and the damage caused to the crop by the disease (Katan, 1981; Chattopadhyay and Kalpana Sastry, 1999c).

Although soil solarization reduced the population of fungal flora in general, but tolerant fungi such as *Trichoderma* survived the temperature (Abu-Blan and Abu-Ghabrie, 1994; Kalpana Sastry and Chattopadhyay, 1999). Kodama et al. (1980) reported similar effects for another thermophilic fungal group Talaromyces. Soil solarization for six weeks in irrigated-ploughed
treatment is reported to reduce soil population of the pathogen and raised the level of beneficial microflora (Chattopadhyay and Kalpana Sastry, 1999) Katan et al. (1983) has suggested the disease suppressiveness in solarised soils may result from a shift in microbial populations in favour of heat-resistant antagonists. This factor and the possibility of chemical breakdown products from green manure residues may provide an even wider variety of additional interactions leading ultimately to the control of many soil borne pathogens.

**Integrated disease management (IDM) strategies**

Chemical control measures alone are not economical and ecofriendly because of their residual toxic effect and wide spectrum activity. The continuous use of potentially hazardous chemicals is posing an increase threat to environment (Zewain et al., 2005). As biocontrol agents offer disease management alternatives with different mechanisms of action than commercial pesticides, trends in research include the increase use of biorational screening processes to identify microorganisms with potential for biocontrol, increased testing under semicommercial or commercial production conditions, increased emphasis on combining biocontrol strains with each other and with other control methods, integrating biocontrol into an overall system (Fravel, 2005).

Fungicidal seed treatment at the time of sowing does not persist for whole cropping season and may kill non target organisms. Single treatment of fungicide or bioagent can not provide a remedy for disease control. The possibility of introducing a variety of combined treatments with solarization may provide for an even wide spectrum of disease suppression (Gade et al., 2007). Integration of biological and chemical control methods against soil borne diseases has been reported by Elad et al. (1980). Management of soil borne pathogens shall be effective and economically attained following integrated way of disease management of *Fusarium oxysporum* responsible for wilt of different plants have been reported by Chattopadhyay and Sen (1996), Mishra et al. (2000), Reid et al. (2002), Raju and Raoof (2003), Landa et al. (2004), Kapoor et al. (2006), and Singh et al. (2007). Integrated disease management (IDM) through implementation of soil solarization and biological control agent could offer a promising strategy (Abdul Wahid et al., 2001). Solarization has shown to be enhanced in combination with other management regimes, such as amending soil with plant growth-promoting rhizobacteria (Kokalis-Burelle et al., 2002) or in combination with the application of organisms antagonistic towards *Fusarium*
The practice of soil solarization can thus be effectively utilized as a component in integrated management of plant diseases (Chattopadhyay and Kalpana Sastry, 2001).

Integration of soil solarization and *Trichoderma* sp. presented significant control of wilt of tomato caused by *F. oxyporum* f. sp. *lycopersici* (Abdul Wahid et al., 2001) and crown and root rot of tomato caused by *F. oxysporum* f. sp. *radicis-lycopersici* (Sivan and Chet, 1993). Katan (1981) has suggested that addition of suitable organic residues to the soil may enhance the benefits of soil solarization. Beneficial effect of soil solarization and its integration with bioagents and fungicides on incidence of damping off was reported by Khulbe and Chambe (2002).

Successful biological control of pests and diseases in different crops can be achieved through the integrated way of disease management by applying antagonists along with compatible form of fungicides (Papavizas et al., 1982; Elad et al., 1986; Mukhopadhyay, 1994; Khalko et al., 2006). Integration of biocontrol agents with fungicides gave significantly higher disease control in several crops including tomato than that obtained by the biocontrol agents or fungicide alone (Upadhyay and Mukhopadhyay, 1986; Sawant and Mukhopadhyay, 1990; Mukhopadhyay and Kaur, 1990). The integration of *T. harzianum* as bio-control agent along with fungicides for effective management of soil-borne diseases is the present day need (Sharma et al., 2001). Application of *T. harzianum* and *T. viride* in combination with different fungicides were effective against a number of fungal pathogens including *Fusarium* sp. (Kraft and Papavizas, 1983; Vozenikova et al., 1992; Kaur and Mukhopadhyay, 1992; Elad et al., 1993; Muthamilan and Jeyarajan, 1996; Bora et al., 1999; Dubey, 2002; Suruliranjan and Kandhari, 2005; Omar et al., 2006).

Since biocontrol agents have to be applied in soil it becomes imperative to ascertain its tolerance to agrochemicals used in crop production technology (Sharma and Mishra, 1995). *T. harzianum* and *T. viride* were very much sensitive to the fungicide carbendazim but less sensitive to captan at lower concentrations (Sharma et al., 2001; Khalko et al., 2006). Different workers have reported chlorothalonil, methyl benzimidazole carbamate (MBC), Captan and Captafol as tolerant for *T. harzianum* even at higher concentrations (Abd-El Moity et al., 1982; Papavizas et
Attempts have been made to develop heat tolerant strains of the bioagents against carbendazim (Yang and Zhao, 1996).


**Glomus fasciculatum in defense responses to fusarial wilt of Coleus forskohlii**

*Coleus forskohlii* (Wild.) Briq. is a potentially important medicinal plant of the future, being immensely valued for its pharmacoproperties that have been discovered only recently. Its tuberous roots are found to be rich source of forskolin, which is being developed as a remedy for hypertension, glaucoma, asthma, congestive heart failures, and certain types of cancers. The plant suffers tremendously from a wilt pathogen, *Fusarium oxysporum*, which results in tremendous economic loss to our country during its cultivation. Plants have defence mechanisms against pathogen infection by inducing systemic resistance in response to localized pretreatment with biological control agents, thus making them resistant to subsequent pathogen infection (Mohammadi and Kazemi, 2002; Pozo et al., 2002). It is well known that plant-and microorganism symbiosis is a defence mechanism against pathogen infection (Guenoune et al., 2001; Koike et al., 2001; Pozo et al., 2002; Jung et al., 2004). Certain plant-growth-promoting microorganisms (PGPM) could enhance defensive activity and stimulate plant resistance against soil-borne pathogens (Kilic-Ekici and Yuen, 2004).

Among the PGPM, vesicular arbuscular mycorrhizal (VAM) fungi are involved in the most universally intimate and important symbiosis in terrestrial ecosystems which has been shown to assist plants in overcoming biotic and abiotic stresses (Guenoune et al., 2001; Pozo et al., 2002; Zheng et al., 2004). In compatible or incompatible interactions between plants and microorganisms, the salicylic-dependent pathway, jasmonic acid-dependent pathway (Agrios, 1997), and shikimic acid pathway (Haslan, 1983) are involved in plant defence, of which the shikimic acid pathway has received much more attention due to the secondary metabolites such
as lignin and phenolic phytoalexins that are responsible for adding mechanical rigidity and strength to cell walls and for providing barriers to infection of pathogens. In these protection processes, peroxidase (POD), polyphenol oxidase (PPO), and phenylalanine ammonia-lyase (PAL) are directly involved in lignin biosynthesis metabolites (Haslan, 1983). The present study was carried out to assess the effect of an VAM fungus (Glomus fasciculatum) in induction of systemic resistance via production of phenolic compounds, defence enzymes (PPO and PAL), and PR protein (POD) in wilt disease against Fusarium oxysporum and the potential of Glomus fasciculatum as a biological control agent against the disease (Khatun and Chatterjee, 2011).

**Host defense enzymes involved in pathogenesis**

**Total phenol**

Biochemical resistance depends upon some pre existing or induced substances synthesized by plants in response to fungal infection (Das et al., 2003). Synthesis of aromatic substances is a major defence mechanism of plants. These include phenols, phenolic acids, flavonoids, tannins and lignins (Mahadevan, 1991). Dubeler et al. (1997) reported that synthesis of phenolics in plants is altered due to wounding and infection by pathogens. Mohan and Mahadevan (2001) recorded that phenol inhibits the growth of fungal pathogen and its effect is proportional to its concentration. Higher accumulation of phenolic compounds in *Glomus fasciculatum* pre-inoculated Coleus plants being challenged with *F. oxysporum* has been recorded (Khatun and Chatterjee, 2011).

**Polyphenol oxidase and Peroxidase**

The redox enzyme phenol oxidase has been isolated in a latent state from several plant species (Mayer, 1987) and enhancement of phenol oxidase activity under in vitro condition has been achieved by a variety of treatments (Soderhall and Soderhall, 1987). Soderhall et al. (1985) isolated phenol oxidase from carrot cells in a complete inactive form as a soluble prophenol oxidase. The increased amount of total phenols in plant tissues followed by increased activity of phenol oxidizing enzymes like polyphenol oxidase and peroxidase is quite common in many host-pathogen interactions. These enzymes oxidize phenols to quinines for better protection of the host tissues against the invading pathogens. Quinones are more reactive and have more antimicrobial activity than the phenols already exist in plants and thus quinines help in the
resistance mechanism of the host plants (Karhikeyan and Bhaskaran, 1992; Steffens, 2002). It is reported that biochemicals and their oxidation products are implicated in disease resistance (Sukand and Kulkarni, 2006).

Toxic metabolites of the fungus may activate phenol oxidizing enzymes which play a vital role in the tissue browning by their ability to oxidise phenols to quinines which are inhospitable to the invading pathogen (Ramadoss, 1991). Batsa (2004) observed that the activity of phenol oxidase enzymes is increased in infected tissues and that the oxidized phenols i.e. quinines are more reactive and more toxic to microorganisms compared to their non-oxidized form. Increased activity of polyphenol oxidase and peroxidase in response to infection by the pathogen has been reported by many workers (Jennings et al., 1969; Vidyasekaran, 1988). Peroxidase and polyphenol oxidase which are reported to be increased as a result of infection by fungal pathogens are considered to play an active role in contributing to disease resistance in certain host-pathogen interactions following infections (Schipper, 1975).

Polyphenol oxidase activity has been reported to be increased in the infected plants under pathogenic conditions in case of tomato infected by *Fusarium oxysporum* f. sp. *lycopersici* (Retig and Chet, 1974) in mango infected by *Fusarium moniliforme* var. *subglutinans* (Chattopadhyay and Nandi, 1976b), in *Alternaria* leaf blight of *Brassica* sp. (Gupta et al., 1992) in *Cajanus cajan* infected by *Fusarium udum* (Chakraborty and Sengupta, 2001); in *Saraca asoca* infected by *Colletotrichum gloeosporoides* (Ojha et al., 2005); in brinjal infected by *Fusarium solani* (Chakraborty, 2005); in blackspot disease of rose infected by *Alternaria lunata* (Khatun et al., 2008; Khatun et al., 2009); in die-back disease of *Mimusops elengi* infected by *Curvularia lunata* (Khatun et al., 2011e) and in *Coleus* infected by *Fusarium oxysporum* (Khatun and Chatterjee, 2011).Mohammadi and Kazemi (2002), Yoruk and Marshall (2003) are of the opinion that polyphenol oxidase enzyme is capable of oxidizing phenols in the resistant varieties rapidly as compared to the susceptible ones.

It was evident from the study of Reimers and Leach (1991) that high level of phenol synthesis, rapid lignifications and localized necrotization contribute resistance in plants against pathogen. Shriraiishi et al. (1995) established the fact that activation of phenyl propanoid pathway is a common response to pathogen infection in plants. Das et al. (2003) has pointed out that peroxidase enzyme is one of the key enzyme of this pathway and peroxidase catalyses conversion of cinnamyl alcohol to lignin by oxidative polymerization (Lamport, 1986).
Peroxidase enzyme is known to strikingly correlate to the host resistance. The level of peroxidase was enhanced immediately after inoculation with pathogen (Utriani, 1978; Dutta and Chatterjee, 2000; Das et al., 2003; Chakraborty and Chatterjee, 2007a; Khadun et al., 2009). Increased peroxidase activity in disease plants has been reported by Khadun and Chatterjee (2011) in wilt of Coleus caused by *F. oxysporum*; Khadun et al. (2011e) in die-back of *M. elengi* infected by *Curvularia lunata* and Ojha (2008) in tomato wilt caused by *F. oxysporum*. Increased activity of cell wall bound peroxidases has been elicited in tomato plants due to pathogen infection (Mohan et al., 1993).

Banik and Chatterjee (1998) reported that increased activity of peroxidase enzyme is responsible for contributing a physiological status to the host tissues that becomes inhospitable for the pathogen. Changes in the activity of phenol-oxidizing enzymes including peroxidase may also play a role in regulation of metabolic pathways in disease or injured tissues (Mehrotra and Aggarwal, 2003). Polyphenol oxidase and peroxidase might function as an alternate electron transport chain and serve as terminal oxidases in infected plant tissues (Jennings et al., 1969). Pradeep and Jambhale (2002) from their studies concluded that phenolic compounds and related oxidative enzymes are mostly considered as one of the important biochemical parameters for disease resistance, differentiating resistant and susceptible genotypes and as such would be helpful in discriminating the genotypes biochemically in addition to their cytological status.

Peroxidase is considered as one of important PR-proteins (Van Loon, 1997) and plant expresses enzyme activity during host-pathogen interaction (Young et al., 1995; Salkia et al., 2004). Peroxidases are involved in the defense of plant against pathogens either by their direct participation in the cell wall reinforcement and by their role as antioxidants in oxidative stresses generated during plant-pathogen interaction (Hammerschmidt et al., 1982; Ramanathan et al., 2001; Salkia et al., 2004). It has also been implicated in phenol oxidation, IAA oxidation (Beffa et al., 1990), lignifications (Grisebach, 1981) and plant defense (Hammerschmidt et al., 1982). Peroxidase (POD) is an oxido-reductive enzyme that participates in the cell wall polysaccharides processes such as oxidation of phenols, suberization and lignification of host plant cells during the defense reaction against pathogenic agents (Ray et al., 1998). The resistance is associated with the induction of peroxidase in host tissues (Breusegem et al., 2001; Lin and Kao, 2001).

**Phenylalanine ammonia-lyase**
Phenylalanine ammonia-lyase (PAL) is the entry point enzyme in the phenyl propanoid biosynthesis pathway whose presence has been demonstrated in pathogen-infected plant (Chen et al., 1993). PAL activity increases in C. forskohlii with infection with G. fasciculatum/ and F. oxysporum infection has been reported (Khatun and Chatterjee, 2011).

**Vesicular-arbuscular mycorrhizae (VAM) on host resistance and yield**

Roots support the growth of a complex of microbes that in turn have profound effect on growth and survival of plants. Of the different microbes interacting with roots, symbiotic mycorrhizal fungi are the most ubiquitous which actively help the plants in absorbing nutrients under natural conditions. These fungi use some of the root exudates and modify root physiology thereby altering the microbial equilibrium on the root surface (Bali and Mukherji, 1991; Bansal and Mukherji, 1994; Mc Allister et al., 1995). Concomitant colonization and infection of roots by mycorrhizal fungi, root pathogens and other microbes inevitably interfere with the activities of each other (Linderman, 1985). These interactions are of great importance, if maintained or enhanced could result in biological control of plant pathogens (Mukherji et al., 2003).

The mycorrhizal fungus is a specialized member of rhizosphere microorganisms (Game and Navale, 2006). Vesicular arbuscular mycorrhizal (VAM) fungi refer to the symbiotic association formed by the fungi of family Endogonaceae and roots of higher plants (Joshi, 2003). Arbuscular mycorrhizal (AM) fungi are known to colonize a number of tropical plants including vegetables (Reddy et al., 2006).

Mycorrhizal fungi offer an environmentally sound biological alternative to chemical fertilizers and pesticide for maintaining plant quality and productivity in agriculture, horticulture and forestry (Wood, 1992). The mycorrhizal association improves the uptake of macronutrients, particularly nitrogen and immobile phosphorus and micronutrients like zinc and copper (Mukherji and Dixon, 1992; Sonawane et al., 1997; Abdul Khaliq et al., 2001; Liu et al., 2002; Ratti et al., 2002; Game and Navale, 2006). Mycorrhizal plants are known to have altered nutritional status, increased photosynthetic rates, altered levels of growth regulating substances and altered patterns of root exudation due to changes in membrane permeability. These physiological changes along with the physical and chemical presence of external hyphae of the
mycorrhizal fungi significantly alter the chemical, physical and microbiological composition of the rhizosphere soil (Meyer and Linderman, 1986; Linderman, 1991).

To evaluate the influence of VAM fungi on disease incidence and development, different variables i.e. pathogen, symbiotic fungus and environmental conditions are to be considered simultaneously (Joshi, 2003). The interaction between VAM fungi and plant pathogens has been studied in details by several workers. Root pathogens are major limiting factors for plant production. Since VAM fungi are formed in the roots of plants, maximum attention has been paid towards mycorrhizae-disease incidence interactions in relation to soil and root borne pathogens. Host plants previously inoculated with the VAM fungi exhibited increased resistance to several soil borne root pathogens (Davis and Menge, 1981; Jalali and Jalai, 1991; Barea and Jeffries, 1996; Bethlenfalvay and Linderman, 1992; Trotta et al., 1996; Quilambo, 2003; Reddy et al., 2006). VAM fungi have been reported to protect roots from certain root infecting fungi (Schonbeck, 1979; Caron et al., 1986a; Bali and Mukherji, 1991; Hwang et al., 1992; Chakraborty, 2005; Ojha, 2008). VAM fungi are able to increase nutritional status of the host plant thereby improving resistance against the pathogens (Davis and Menge, 1980). Kulshreshtha and Khan (2002) reported that root colonization by *Glomus caledonium* showed greater amount of chlorophyll, seed protein in *Vigna mungo* than uninoculated plants. AM association are known to help in the growth of various crops including horticultural plants like carrot, tomato etc. (Sasal, 1991). Gill et al. (2002) studied the effect of inoculation of four arbuscular mycorrhizal fungi on growth of chickpea and reported that *G. fasciculatum* inoculated plants showed higher plant height, root length and fresh and dry weight compared to other three AM fungi. Similar results were observed in lentil (Singh and Singh, 1986), in *Sandersonia aurantiaca* (Matsubara and Sukurai, 2000) and in *Vetiveria zizanoides* (Ratti et al., 2002) due to inoculation of *G. fasciculatum*. Mycorrhizal inoculation with *G. fasciculatum* not only reduced the tomato damping-off disease incidence but also significantly increased the host plant height, total biomass, dry matter and fruit yield (Reddy et al., 2006). Akhtar and Siddiqui (2007) reported significant increase in plant growth, pod number, chlorophyll, nitrogen, phosphorus and potassium contents and reduced root-rot index in chickpea following combined inoculation of plants with *G. intraradices* and *Psuedomonas putida*. Arbuscular mycorrhizal fungi also increase the root diameter of *Lycopersicon esculentum* and are linked to an increase of the apex size with larger meristem and quicent centre (Fusconi et al., 1999).
When both the pathogen and VAM are free living in the soil before penetration, pathogens often have competitive advantage because of their saprophytic ability, many of them are less strictly biotrophic than the mycorrhizal fungi. But situation is different when mycorrhizal fungi are already established, the mycorrhizal fungus does not have to compete for substrate and may prevent penetration by pathogens (Mukherji, 2003). Caron et al., (1986a) studied the effect of inoculation sequence of *Glomus intraradices* and *Fusarium* f. sp. *radicis lycopersici* on the growth of tomato. Mycorrhizal root with *G. intraradices* exhibited reduction in root necrosis due to *Fusarium* infection independent of the sequence of inoculation of the two fungi. The effect being more pronounced when *G. intraradices* was inoculated four weeks prior to *F. oxysporum*. VAM fungi also reduce number of propagules of pathogenic fungi. Inoculation of *G. intraradices* increased plant dry mass and saved loss due to inoculation by *F. oxysporum* even when VAM fungus was inoculated later. Mycorrhization in guava with *G. mosseae* may be an effective management tool in the wilt disease caused by *F. oxysporum* steadily declined in the various treatments (Srivastava et al., 2001).

Linderman (1992), Ozgonen et al. (2001), Akkopru and Demir (2005) used arbuscular mycorrhizal fungi as biocontrol agents against *Fusarium* wilt of tomato. Seedlings of alfalfa inoculated with *Glomus* spp. show lower incidence of wilt caused by *F. oxysporum* f. sp. *medicago* than non mycorrhizal ones (Hwang et al., 1992). There is a significant increase in phosphorus contents and dry weight of roots of *F. oxysporum* infected tomato plants when inoculated with *G. intraradices* (Akkopru and Demir, 2005). The synergistic effect in the reduction of bacterial wilt incidence of tomato was observed when *G. mosseae* was combined with antagonists (Kumar and Sood, 2002). Kavita et al. (2003) observed suppression of damping-off in chilli following dual inoculation of native arbuscular mycorrhizal fungi and *Azospirillum*.

*Acacia nilotica* plants show enhanced growth response when inoculated with *G. mosseae* and potential biocontrol agent *Trichoderma harzianum* (Rani et al., 1999). It has been reported that dual application of *G. fasciculatum* and *T. harzianum* caused fewer plant death than single application (Hazarika and Phookan, 2003). Clavet et al. (1988) found a positive stimulation on growth of the mycelium of *G. mosseae* by two isolates of *T. aureoviride* that were able to antagonise *Fusarium* species. *Trichoderma* in combination with mycorrhizal fungi improve plant growth and vigour to supresss root diseases (Azcon-Aguilar and Barea, 1992) and *Trichoderma*
spp. also stimulate ectomycorrhizae formation (Davey, 1971). Combined inoculation of *Glomus macrocarpus* and *T. harzianum* has been found most effective in suppressing *Sclerotium rolfsii* in chilli (Sreenivasa, 1994). Green house experiments conducted by McAllister et al. (1994) showed that when *Trichoderma koningi* and *Fusarium solani* were inoculated simultaneously with two different host plants, maize and lettuce and were subsequently challenged with arbuscular mycorrhizal fungus, *G. mosseae, F. solani* did not exert any adverse effect on the colonization of *G. mosseae* in the root system and it also reduced population of *F. solani* significantly in the rhizosphere soil. Singh et al. (2007) studied the influence of interaction of different biocontrol agents on suppression of *Fusarium* wilt of *Dalbergia sissoo* and reported that there was least mortality of *Fusarium* infected seedlings in presence of *Trichoderma* spp. and *Glomus* spp.

Arbuscular mycorrhizal fungi may limit fungal root diseases by strengthening morphological traits of plants with some physiological and microbial modifications in the mycorrhizosphere, competition for space and nutrients, changes in root-system, altering the chemical composition of plant tissues and activation of plant defense mechanisms (Azcon-Aguilar and Barea, 1996; Linderman, 2000; Barea et al., 2002; Demir and Akkopru, 2005). Mycorrhizal fungi colonize the root system rapidly, restrict pathogenic infection. Mycorrhizal fungi stimulate host roots to produce and accumulate sufficient concentrations of different metabolites and impart resistance to the host tissue against the invasion by pathogens and mycorrhizal fungi also known to produce antifungal and antibacterial antibiotics (Mukherji et al., 2003).

Disease inhibition by arbuscular mycorrhizal fungi was linked to their ameliorative effects for plant nutrients especially for phosphorus content (Caron et al., 1986b). Variation in phosphorus nutrition is influencing host defense-related gene expression (Lambais and Mehdy, 1998). Schoenbeck (1979) postulated that the VAM fungi indirectly induce changes in host tissues. They stimulate lignifications or development of callosities or lignitubers in the host cells and thus create a physical barrier to check penetration of the pathogen. The increase in amino acids (Baltruschat and Schoenbeck, 1972b), sugars (Safir, 1968), phenolic compounds (Krishna and Bhagyaraj, 1983) and enzyme activity (Dehne et al., 1978) in the roots of mycorrhizal plants has been observed and suggested as the possible reasons for the checking of disease development on
mycorrhizal plants. Occurrence of phytoalexins and phenolic compounds in mycorrhizal interactions and their potential role in biological control was observed by Morandi (1996).

Root tissues colonized first by VAM fungi physically exclude a pathogen competing for the same infection site. Bennett et al. (2006) recognized that mycorrhizal fungi could affect plant enemies by improving plant nutrition, modifying plant tolerance, or modifying plant defences and in addition mycorrhizal fungi could directly interfere with pathogen, herbivory and parasitism by occupying root space. High chitinase activity of the mycorrhizal tissue inhibits the growth of the pathogen competing for the same infection site and also inhibits the growth of the pathogens in the host system (Mukherjee, 2003).