2 LITERATURE SURVEY:

2.1 STRAINAL VARIATION BASED ON ANALYSIS OF ITS REGION OF RDNA IN FUNGAL ORGANISMS:

The genus *Trichoderma* (*Ascomycetes, Hypocreales*) contains species that are of vast economic importance owing to their ability to act as biological control agents against plant pathogens since 1920s (Harman, 2006) and their production of antibiotics and industrial enzymes (cellulose and hemi-cellulose) (Kubicek and Penttila, 1998; Sivasithamparam and Ghisalberti, 1998). Taxonomy of *Trichoderma* is largely based on morphological characters such as conidial form, size, color and ornamentation, branching pattern with short side branches, short inflated phialides and the formation of sterile or fertile hyphal elongations from conidiophores (Bissett, 1991; Rifai, 1969). *T. harzianum* is an aggregate species, divided into three, four or five sub specific groups, depending on the strains. However, most species descriptions are based on examination of a limited number of strains where the morphological differences are clear but these differences become less clear as more strains are studied. This suggests that there are not enough morphological and cultural characters for reliable species level definition. Identification of *Trichoderma* isolates at the species level has become difficult, due to their morphological similarities. Rifai, 1969, adopted the concept of “species aggregate” and distinguished nine aggregates, some of which comprised two or more morphologically indistinguishable species. An attempt to differentiate phenotypically similar
species, proposed ‘sections’ based on morphology was made (Gams and Bissett, 1998), to accommodate similar forms within the species concept of Rifai. Current studies find that morphological analysis was highly prone to error and roughly 50% of the *Trichoderma* spp. obtained by morphological analysis alone was wrongly identified (Kubicek *et al.*, 2001).

The genus *Trichoderma* lacks a discrete species concept because of variation within and among species groups which Rifai, 1969, defined as species aggregates. Bissett, 1991 proposed ‘sections’ to accommodate morphologically similar forms within the species aggregates of Rifai.

Knowledge concerning the behavior of these fungi as antagonists is essential for their effective use since they can act against target organisms in several ways (Jeffries and Young, 1994) by producing extracellular enzymes (Haran *et al.*, 1996), antifungal antibiotics (Ghisalberti and Rowland, 1993) and also be competitors to pathogens (Simon and Sivasithamparaman, 1989). Of late, molecular techniques are gaining importance in characterization and diagnosis of microbial population. Molecular characterization and identification of biocontrol isolates of *Trichoderma* *spp.* has been reported by several researchers (Hermosa *et al.*, 2000; Venkateswarulu *et al.*, 2008).

*Trichoderma*, isoenzyme analysis (Leuchtmann *et al.*, 1996; Samuels *et al.*, 1994), RAMS (Cortinas *et al.*, 2006; Latiffah *et al.*, 2005) and rDNA sequencing (Druzhinina *et al.*, 2005; Jaklitsch *et al.*, 2005) have been used to distinguish species within specific groups of strains.
In recent years, various molecular techniques have been used in the systematics of phytopathogenic and other (commercially important) fungi to assess intra- and interspecific variation and to determine phylogenetic relationships (Buchko and Klassen, 1990; Chen et al., 1992; Forster et al., 1990; Gaudet et al., 1989; Levy et al., 1991; Meyer et al., 1992; Moody and Tyler, 1990; O'Donnell, 1992; Vaillancourt and Hanau, 1992).

Meyer et al., 1992 used a DNA fingerprinting technique to analyze the nine species aggregates of *Trichoderma* and recognized only five groups. “Universal” fungal primers are aimed at the specific amplification of pan-fungal DNAs (Borneman and Hartin, 2000; White et al., 1990; Zhou, et al., 2000).

Kindermann et al., 1998 attempted first phylogenetic analysis of the genus *Trichoderma*, using sequence analysis of ITS 1 region of rDNA. Nevertheless, the use of phylogenies based on single gene sequences is now normally discredited, especially as regards the use of ITS1 and/ or ITS2, as some fungi and plants have been shown to contain paralogous copies (Lieckfeldt and Seifert, 2000). Taylor et al., 1999 proposed phylogenetic species concepts between five or more gene trees.

Although the morpho-taxonomic characteristics of isolates of three groups fall within the broad range of variation described for the species aggregate *T. harzianum* (Rifai, 1969), the molecular data could suggest their separation into three DNA-based species (Reynolds and Taylor, 1991).
Barnes et al., 2001 studied the ribosomal DNA (ITS1, ITS2 and 5.8s gene) and suggested analysis failed to separate the cypress canker fungi.

Lieckfeldt et al., 2000; Muthumeenakshi et al., 1994 and Ospina et al., 1999 observed the amplified rDNA fragment of approximately 500 to 600 bp ITS-PCR of *Trichoderma*. ITS-PCR has helped to detect polymorphism at ITS region of rDNA among the *Trichoderma* isolates. But based on these results it is not possible to conclude that all the isolates were the same.

Romaine et al., 2001 studied the molecular genetics and pathogenicity trials on bio-control and pathogenic isolates of *T. harzianum*. Both randomly amplified polymorphic DNA and sequence specific PCR analyses discriminated the bio-control isolates from the highly pathogenic *T. harzianum* biotypes 2 and 4 (Th4) on cultivated mushrooms (*Agaricus bisporus*). Phylogenetic analysis based on beta tubulin gene sequence suggested that biological control and pathogenic isolates share a recent common ancestry, but have diverged considerably.

Molecular techniques exploiting the variations in ribosomal DNA or mitochondrial rDNA genes are sensitive, reliable and quicker than conventional methods to identify fungal strains (Arora et al., 1996)

Yli-Mattila et al., 2002 studied the difference in morphology, ITS, IGS, mtSSU and beta-tubulin sequences and PCR hybridization were compared between morphologically similar *Trichoderma* spp.
Phylogenetic trees were inferred from the 5.8s subunit and flanking internal transcribed spacers (ITS1 and ITS2) of r DNA as well as the beta-tubulin gene, separated species in accordance with their morphological features and characteristics. Better resolution in separation of species was achieved from the beta-tubulin data (Crous et al., 1999).

The gene ech42 coding for the endochitinase Ech42 was highly expressed when the fungus is grown on media containing chitin or in dual cultures with a host - direct confrontation assays (Carsolio et al., 1994).

The major destructive activity of opportunistic fungi is thought to be enzymatic disruption of nematode egg shells, larval cuticles and physiological disturbances brought about by biosynthesis of diffusible toxic metabolites (Morgan-Jones and Rodriguez-Kabana, 1985).

Trichoderma spp. has been reported to be a promising bio-control agent of plant-parasitic nematodes. Several reports show that Trichoderma spp. are able to suppress Meloidogyne spp. populations (one of the most economically important group of plant-parasitic nematodes worldwide) and increase crop yields (Rao et al., 1996; Sharon et al., 2001; Spiegel and Chet, 1998; Windham et al., 1993).

Lytic enzymes such as β-1, 3-glucanases, chitinases and proteases are probably responsible for hyphal lysis through the digestion of major cell wall components. The chitinolytic system of T. harzianum comprises six distinct
enzymes, two of which were classified as β-1, 4-N- acetylglucosaminidases and the rest as endochitinases (Haran et al., 1995).

A trypsin like protease produced by PRA1 in T. harzianum has been reported having major nematicidal activity. It was also proved for its nematicidal activity by biochemical methods (Belen et al., 2004).

**Effect of culture filtrates of organisms:**

Effect of fungal filtrates were evaluated for their effect on root penetration, development and reproduction of M. javanica on tomato by root dip treatment (Khan and Saxena, 1997). Three mechanisms of action were thought to be responsible for reduction in nematode infection (i) production of toxic metabolites which reduce hatch and attraction, (ii) degradation of specific root exudates which control nematode behavior and/or (iii) enhanced defense mechanism leading to systemic resistance in plants (Sikora et al., 1993).

Culture filtrates of T. harzianum and P. fluorescens, T. viride were used to study their effect on egg hatch on M. javanica. (Ansari et al., 2002). Culture filtrates from T. virens G1-3 could inhibit the egg hatch and J2 mobility of root-knot nematode – M. incognita (Meyer et al., 2000). Recently, T. asperellum and T. atroviride were reported to show an in vitro parasitic effect in M. javanica eggs and larvae (Sharon et al., 2007). The demonstration of the nematicidal ability of *Trichoderma* species suggests their potential in nematode biocontrol (Harman and Björkman, 1998; Chen et al., 2009).
Bio-control activity of *T. harzianum*:

The ability of *T. harzianum* Rifai to colonize eggs and infect second-stage juveniles (J$_2$) of root-knot nematodes *in vitro* has been demonstrated (Saifullah and Thomas, 1996; Sharon *et al.*, 2001). Extracellular proteolytic activities in *Trichoderma* spp. have long been recognized and they have been attributed to antagonistic and bio-control activities (Bertagnolli *et al.*, 1996; De Marco and Felix, 2002; Elad and Kapat, 1999; Rodriguez Kabana *et al.*, 1978).

This gene is expressed during mycoparasitic interactions in the presence of cell walls or chitin (Cortes *et al.*, 1998; Olmedo-Monfil *et al.*, 2002) and its over expression improves bio-control activities of transformed *T. atroviride* strains against the fungus *Rhizoctonia solani* (Flores *et al.*, 1997). In addition, the protease encoded by *prb1* also appears to attribute virulence against the nematode *Meloidogyne javanica* (Sharon *et al.*, 2001).

Fungal strains grouped in the genus *Trichoderma* possess a wide spectrum of evolutionary responses that range from very effective soil colonization, with high biodegradation potential, to non-strict plant symbiosis by strains colonizing the rhizosphere. In addition, some groups of strains within this conglomerate of biotypes are able to antagonize phytopathogenic fungi by using substrate colonization, antibiosis, and mycoparasitism as the main mechanisms (Hjeljord and Tronsmo, 1998).
This antagonistic potential is the basis for effective use of *Trichoderma* strains as alternatives to the chemical control of a wide set of phytopathogenic fungi and nematodes (Harman and Björkman, 1998).

The antagonistic (antifungal) activity of crude chitinase was found to be located in a molecular weight fraction of the enzyme, which does not possess chitinase activity. Both crude and purified chitinase were able to lyse the cell walls of the intact mycelium. Low molecular weight non enzymatic substances largely determine antagonistic activity of *Bacillus* spp. against micromycetes; whereas the role of chitinase is to utilize chitin, which is ubiquitously present in soil (Melent' et al., 2001). *Trichoderma* spp. has been described as antagonistic agents against various phytopathogenic fungi.

Molecular characterization of lytic isoenzymes of *T. harzianum* was achieved by electrophoretic separation in renaturalizing conditions (Santorum et al., 2001).

The use of light and scanning electron microscopy has demonstrated the penetration of hyphae of *R. solani* by *T. harzianum* (Elad et al., 1983). A complex set of extracellular enzymes was produced by mycoparasitic strains of *Trichoderma* when grown on isolated cell walls of *R. solani* (Ridout et al., 1986). Thus, lytic enzymes such as β-1, 3-glucanases, chitinases and proteases were probably responsible for hyphal lysis through the digestion of major cell wall components. The chitinolytic system of *T. harzianum* comprises six distinct
enzymes, two of which were classified as β-1, 4-N-acetylglucosaminidases and the rest as endochitinases (Haran et al., 1995).

*Trichoderma harzianum* (teleomorph: *Hypocrea lixii*) is active as a hyper parasite and has been extensively tested in field experiments. It has been shown to be an effective bio-control agent, with good in vitro antagonistic abilities against a range of economically important aerial and soil-borne fungal plant pathogens (Gao et al., 2002).

The filamentous fungus *Trichoderma harzianum* is one of the most potent agents for bio-control of plant pathogens. The antagonistic mode of action of the fungus is proposed to be due to the production of antibiotics (Claydon et al., 1987; Schirmbock et al., 1994) and fungal cell wall degrading enzymes such as chitinases, glucanases, and proteases (Chet, 1987; Lorito et al., 1993). Endo chitinase of *T. harzianum* has been shown to be the most effective enzyme when tested alone or in combination with β-1, 3-glucanase. It appears to be more effective than plant and bacterial chitinases against a wider range of target fungi (Lorito et al., 1993; 1994).

Studies of pathogenic fungal β-tubulin have been focused mainly on the interaction with benzimidazole fungicides, as a result of the detection of many benzimidazole-resistant pathogenic isolates in the wild shortly after intensive and exclusive use of these fungicides (Maymon et al., 2006). Mutagenesis studies have identified b-tubulins as primary benzimidazole binding targets (Li et al., 1996). Reduced binding of carbendazim by crude extracts of tubulin has
also been reported for laboratory-induced resistant strains of *Aspergillus nidulans* (Jung et al., 1992).

β-tubulins of biocontrol fungi e.g., *Trichoderma viride* (Goldman et al., 1993) and *Fusarium lateritium* (McMahan et al., 2001) have been cloned and characterized recently in order to explore their interaction with benzimidazole fungicides.

*Trichoderma harzianum* is ubiquitous in most agricultural fields and is a promising antagonist of plant pathogenic fungi and nematodes (Dos Santos et al., 1992; Elad et al., 1982).

Rhizosphere harbours a variety of micro-organisms which may influence the activity of an introduced biocontrol inoculants against root-knot nematode. Recently, Siddiqui and Shaukat, 2003 demonstrated that a nonpathogenic *Fusarium solani* strain Fs5 substantially increased bio-control performance of DAPG-producing *P. fluorescens* strain CHA0 in tomato.

**Disease complex:**

**Introduction to disease complexes in plants:**

Nature does not work with pure cultures alone, but most frequently with associations and so plant pathologists have to study the effect of inoculation of plants with known mixtures of microorganisms on the development of disease (Wallace, 1978).
Under natural conditions a plant is a potential host to various microorganisms and they can influence each other by occupying the same habitat. Different parasites on the same plant interact and result in disease complex. These interactions predispose the plants and make them susceptible to the attack of microorganisms (Sidhu and Webster, 1981).

Plant parasitic nematodes often play a major role in disease interactions. Interaction involving nematodes is important because they contribute substantially to variability in crop growth (Zadoks and Schein, 1979). Nematodes interact with different groups of plant pathogens and root symbionts. It is reported that that infection by one pathogen may alter the host response to subsequent infection by another (Taylor, 1990).

Bacterial wilt caused by *Ralstonia solanacearum* is more severe in resistant cultivars of tomato and eggplant in the presence of *M. incognita*. The combination of the two pathogens suppresses the survival rate of wilt-resistant tomato plants to 33-36%. Both capsicum and eggplant are prone to many soil borne diseases among which the bacterial wilt (*R. solanacearum*) in combination with root-knot nematode (*M. incognita*) takes heavy toll every year all over the world (Naik, 2004).

Ecto and endo-parasitic nematodes can facilitate bacterial invasion by providing wounds in the host root (Poinar and Hansen, 1986).
INTERACTION WITH FUNGI

Nematodes usually assist and enhance the pathogenicity mechanism of fungus towards modifications in host plants. *Meloidogyne incognita* and *Rhizoctonia bataticola* or *Sclerotium rolfsii* when inoculated simultaneously in soil, reduce the germination of seeds in okra, brinjal and tomato (Chhabra and Sharma, 1981; Shukla and Swarup, 1970). The incidence and severity of root rot of brinjal, tomato and okra, caused by the soil-borne fungi such as *R. solani, R. bataticola* and *Phomopsis vexans* was increased in the presence of *M. incognita* (Chahal and Chhabra, 1984; Sharma *et al.*, 1980).

Evidence indicates that interactions between Fusarium and root-knot and cyst nematodes are biological and physiological rather than mechanical in nature (Roy *et al.*, 1989). Root exudates are known to attract the motile stage of fungal pathogens (Zentmyer, 1961). Thus changes induced by a nematode in the root exudates may be the first stage in the synergistic interaction between nematode and fungi (Taylor, 1990).

Plant parasitic nematodes are known to predispose some plants to fungal pathogens (Bergeson, 1972; Mai and Abawi, 1987; Sidhu and Webster, 1977). Nematode cause severe damage in crops every year (CasasFlores and HerreraEstrella, 2007). Migratory nematodes also represent a predisposing factor to infection by certain fungi. Mechanical wounding of the root favors the entry of other pathogens. For example, *Pratylenchus* sp. is reported to interact
synergistically with *Verticillium* and *Belonolaimus* sp. and *Trichodorus* sp. with Fusarium (Faulkner, Bolander, Skotland, and . 1970).

The kinds of effects observed in the nematode-fungus aetiological relationships are: Fungus disease aggravated, host growth suffered, resistance to fungus reduced, fungus suppressed by nematode or nematode suppressed by fungus and susceptibility to fungus increased (Norton, 1978).

**INTERACTION OF NEMATODES WITH ROOT-ROT FUNGI**

Nematodes are important and, at times, vital to the development of root rots caused by fungi. Associations of this type may have greater overall importance than disease complexes involving nematodes with wilt-inducing fungi. Sedentary endoparasitic nematode pathogens (species of *Meloidogyne* and *Heterodera*), migratory endoparasitic nematodes (species of *Pratylenchus* and *Radopholus*), semiendoparasitic nematodes (*Tylenchulus semipenetrans* and *Rotylenchulus reniformis*) and ectoparasitic nematodes (*Tylenchorhynchus brassicae*, *Belonolaimus longicaudatus* and *Macroposthonia xenoplax*) are involved in several root rot disease complexes (with species of *Pythium*, *Phytophthora*, *Rhizoctonia*, *Sclerotium* and *Macrophomina*). It is evident that root-rot complexes involving nematodes and fungi are among the most widespread and important interactions (Parvatha Reddy, 2010).
**NEMATODES FACILITATE ACCESS TO THE ROOT SURFACE:**

The nematodes interact with fungi in disease complex by providing access to the root tissues through the wounds they cause. There are many reports of fungal pathogens growing in and extending the lesions caused by burrowing nematode or root lesion nematodes, and growing along the invasion tracks left by invading juveniles of cyst and root-knot nematodes. For instance, *Cylidrocarpon musae* is only common in the lesions formed in banana roots by *Radopholus similis* (Booth and Stover, 1974). The increased lateral root production following nematode invasion also provides entry sites for fungi.

Application of bio-pesticides reduced incidence of *Phytophthora* foot and root rot and increased the yield by 26% and Cost benefit ratio was 1: 3.8 (Rao *et al.*, 2009).

**GERBERA:**

Gerbera (*Gerbera jamesonii* Bolus ex. Hook f.) belongs to Asteraceae family (Compositae) that produces showy capitula, characterized by its rosette shape of variable colors of high commercial value (Roriz and Cunha 1998, Lorenzi and Souza 2000). It is the largest family of flowering plants, and is one of ten popular cut flowers in the world (Vasudevan and Rao, 2010). According to the global trends in floriculture, it occupies the forth place in cut flowers (Choudhary and Prasad 2000). These plants are grown in polyhouses due to high commercial and economical value for good quality cut flowers.
**Integrated methods of management available in gerbera:**

Application of *Paecilomyces lilacinus* at 0.5 g/kg of soil along with neem cake at 1.0 t/ha efficiently suppressed the nematode population and checked its build up, enhancing the plant growth parameters resulting in better production with increased flower stalk length and flower diameter in carnation. The plants also came to flower early (Johnson, 2000). Combined inoculation of arbuscular mycorrhizal fungus and *P. fluorescens* reduced the populations of root-knot nematodes on Gerbera (Anusuya and Vadivelu, 2002).

Application of *P. lilacinus* and *T. harzianum* at the rate of 0.5 ml per m² (aqueous spore suspension containing $2 \times 10^4$ spores per ml) along with neem cake at 0.5 kg/m² or fenamiphos at 2 g/m² increased plant growth parameters and flower yield of carnation and gerbera. The above treatments also increased root-knot egg parasitization by the parasitic fungi (Nagesh and Parvatha Reddy, 1996).

An experiment carried out for the biological control of root-knot nematode *M. incognita* in carnation (*Dianthus caryophyllus*) showed that, integration of *P. lilacinus* and *P. chlamydosporia*, significantly reduced root galling (Shylaja, 2004).

Combined application of neem cake @ 25 g/m² was found effective for the management of disease complex and increased the flower yield in gerbera cv. Debora (Manoj Kumar *et al.*, 2010).
Integrated management in Gerbera using bioagents and botanicals:

Root galling was reduced in Gerbera by use of combinations of *P. lilacinus/ T. harzianum* + Neem cake (Nagesh and Parvatha Reddy, 1996) and *P. lilacinus + P. chlamydosporia* + Neem cake (Nagesh and Reddy, 2000).

**BIO-MANAGEMENT:**

Interactions between nematodes and fungi:

Interactions between fungal antagonists and nematodes have been known to occur in agricultural soils for many years (Mankau, 1980). Nematode destroying fungi play an important role in regulating nematode population dynamics.

*Trichoderma* spp.:

*Trichoderma* spp. has been described as antagonistic agents against various phytopathogenic fungi and nematodes. This ability makes *Trichoderma* a healthier, safer and environmentally friendlier biological choice, compared with polluting chemical pesticides, usually employed in agriculture. Mycoparasitism is one of the mechanisms involved in antagonistic processes, based on activity of extra cellular lytic enzymes produced by *Trichoderma* and has been proven as a good biological agent.

*Trichoderma* spp. are saprophytic fungi that are highly interactive in root, soil and foliar environments and have been widely described as biocontrol
agents against phytopathogens (Harman, 2006). Some groups of strains belonging to *Trichoderma* spp. are active as hyperparasites and have been extensively tested in field experiments. *Trichoderma* spp. are able to antagonize phytopathogenic fungi by substrate colonization, antibiosis and mycoparasitism as the main mechanisms (Hjeljord and Tronsmo, 1998; Yedidia *et al*., 2001; 2003; Howell, 2003; Harman *et al*., 2004). They also secrete cell wall degrading enzymes (CWDEs) such as chitinases, glucanases and proteases, among others and excrete secondary metabolites active against a number of plant pathogenic fungi *in vitro* and *in vivo* (Chet, 1987; Nelson, 1991; Tronsmo, 1991; Harman and Björkman, 1998; Reithner *et al*., 2011). A principal function in this process has been attributed to chitinolytic and glucanolytic enzymes (Benitez *et al*., 1998; 2004; Lorito *et al*., 1998; Reithner *et al*., 2011). Different biocontrol related serine (subtilisin-like, chymotrypsin/ elastase-like and trypsin-like activities) and aspartic proteases have been detected and/or purified from several *Trichoderma* species (Delgado-Jarana *et al*., 2000; Saurez *et al*., 2004; Williams *et al*., 2003).

Extracellular hydrolytic enzymes, including serine protease, chitinase, lipase and collagenase are believed to play key roles in the infection process of parasitic fungi against plant parasitic nematodes (Yang *et al*., 2007).

*Trichoderma* species have a complex extracellular proteolytic system, in which serine proteases have long been attributed to their antagonistic and biocontrol activities (Kredics *et al*., 2005). However, serine proteases with
nematicidal activity isolated from *Trichoderma* have hardly been reported. The subtilisin-like serine protease PRB1 from *T. atroviride* appeared to participate in virulence against *M. javanica* (Sharon et al., 2001). In a recent study by Chen *et al.*, 2009, *T. pseudokoningii* SMF2 (earlier described as *T. koningii* Song *et al.*, 2006) was shown to have strong nematicidal ability against *M. incognita* and one important virulence factor, a novel nematicidal serine protease SprT, was identified from a crude extract in solid fermentation.

*Trichoderma harzianum:*

*T. harzianum* was found to be a potential bio-agent of root-knot nematodes (Rao *et al.*, 1997; Rao *et al.*, 1998b). *T. harzianum* suppressed *Meloidogyne* spp. by 58.3 - 68.9 per cent in cardamom nursery and increased the number of quality seedlings for transplanting (Eapen and Venugopal, 1995). Colonization of eggs and parasitism of juveniles of *M. javanica* by *T. harzianum*, was documented by Sharon *et al.*, 2001. Plant based formulations of *T. harzianum* for the management of *M. incognita* on egg plant was evaluated by Rao *et al.*, 1998a.

Early in the process of parasitization of *Rhizoctonia solani* by *Trichoderma harzianum*, directed hyphal branching by the mycoparasite appears to be induced as a chemotropic reaction to the presence of the host fungus (Chet and Baker, 1981). Subsequently, the hyphae of *T. harzianum* coil tightly around those of *R. solani*. It has recently been shown that a purified lectin from *Sclerotium rolfsii* induces coiling of hyphae of *T. harzianum* and the formation of
mycoparasitism-related structures (e.g., hooks and appressorium-like bodies) around nylon fibers coated with the lectin, and thus simulates the interaction with the host (Inbar and Chet, 1992). Similar lectins have been identified in *R. solani* and other fungal host species (Jeffries and Young, 1994). Thus, lectins present on the cell wall of the host are suggested to take part in its recognition.

Although the information about the mechanisms of this fungal activity against root-knot nematodes is limited, the ability of *T. harzianum* Rifai to colonize eggs and infect second-stage juveniles (J₂) in vitro has been demonstrated (Saifullah and Thomas, 1996; Sharon *et al*., 2001). Extracellular proteolytic activities in *Trichoderma* species have long been recognized and they have been attributed to antagonistic and biocontrol activities (Bertagnolli *et al*., 1996; De Marco and Felix, 2002; Elad and Kapat, 1999; Rodriguez Kabana *et al*., 1978).

*Pseudomonas spp.*:

*Pseudomonas fluorescens* belongs to Plant Growth Promoting Rhizobacteria (PGPR), the important group of bacteria, which play a major role in the plant growth promotion, induced systemic resistance, biological control of pathogens etc. PGPR are known to enhance plant growth promotion and reduce severity of many nematode diseases. *P. fluorescens* belongs to PGPR, the important group of bacteria, which play a major role in the plant growth promotion, induced systemic resistance, biological control of pathogens etc.
PGPR are known to enhance plant growth promotion and reduce severity of many fungal and nematode diseases (Haas, 2005; Manoj and Rao, 2011a).

Various researchers reported that *P. fluorescens* as a potential bio-control agent against wilt causing incidents (De Leij *et al.*, 1995; Sarniguet *et al.*, 1995). The specific media for the isolation of the bacterial bio-control agent *P. fluorescens* was standardized by King *et al.*, 1954. Isolation of the bacterial biocontrol agent *P. fluorescens* (pf1), and its bio-control nature against most of the diseases was documented by Vidyasekaran *et al.*, 1999. Induction of growth promoting substances from the PGPR (Plant Growth Promoting rhizobacteria) and induction of systemic resistance against pathogenic fungi was reported by Ramamoorthy *et al.*, 2001.

*Pseudomonas fluorescens* is known to control the pathogen by the mechanism called siderophoric effect. It was also found very effective in the control of various soil borne fungi causing root rot in certain vegetables and ornamental crops (Mukhopadhaya, 1987). Shanthi and Sivakumar, 1995 reported the increased plant growth of tomato with *P. fluorescens* strain Pf-1. Bacterial treatment also reduced the level of infestation by the nematode, which was concentration dependent. *P. fluorescens* produce the antibiotics 2,4-diacetylphloroglucinol (DAPG), phenazine-1-carboxylic acid (PCA), phenazine (Phe), antimicrobial metabolites such as pyoluteorin (Plt), hydrogen cyanide (HCN), siderophores pyoverdine (Pvd), salicylic acid (Sal) and pyochelin (Pch) which inhibit a broad spectrum of plant pathogenic fungi, bacteria, nematodes
(Boruah and Kumar, 2002; Raaijmakers et al., 1997; Schmidli-Sacherer et al., 1997) and control a variety of root and seedling diseases. There are many evidences substantiating the importance of DAPG in biological control. The production of DAPG is governed by Phl gene. The population size of DAPG producers in the rhizosphere correlated with the disease suppressiveness of the soil and in situ antibiotic production. The diverse DAPG producing Pseudomonas spp. have been isolated from the rhizosphere of various crop plants and their role in promoting plant growth and inhibiting root diseases are the subjects of ongoing investigations worldwide.

DAPG producing P. fluorescens F113 is proposed as a potential bio-control inoculant for the protection of potato crop against the potato cyst nematode, Globodera rostochiensis (Cronin et al., 1997; Raaijmakers and Weller, 1998).

Application of P. fluorescens at 4 g/plant significantly reduced root galling (40%), egg mass production (60%) and increased root colonization by P. fluorescens and fruit yield in grapevine by 66% over control (Shanthi et al., 1998). Turmeric rhizome treatment with P. fluorescens at 10 g/kg increased plant growth characters (58 and 31% increase in pseudo stem height and number of tillers over control) and decreased nematode population (40%) and gall indices (25%) (Seenivasan et al., 2001).
*P. fluorescens* had induced systemic resistance and inhibited early root penetration of *Heterodera schachtii*, the cyst nematode in sugar beet (Oostendorp and Sikora, 1989).

PGPR are known to enhance plant growth promotion and severity of many fungal diseases (Hoffland *et al*., 1996; Wei *et al*., 1996). These PGPR employ different mechanisms to arrest the fungal growth. Production of lytic enzymes like chitinase and β-1, 3-glucanase by the PGPR strain is considered a major antagonistic property of strains. These lytic enzymes act on chitins and glucans, which are the major constituent of the cell wall of the majority of the fungi except the oomycetes group. Hydrolytic action of these enzymes results in the degradation of the cell wall. Antifungal effects of chitinase produced by bio-control agents like *Trichoderma* spp. and *Pseudomonas* spp. on plant pathogens have been reported by Haran *et al*., 1996 and Krishnamurthy, 1996.

**COMBINATION FORMULATIONS:**

Bio-efficacy of the combinations of *P. fluorescens* + *Bacillus subtilis*, and *P. fluorescens* + *T. viride* for the management of wilt disease in grapevine caused by *F. moniliforme* was reported by Karunakaran *et al*., 2003. Suppression of *Phytophthora capsici* infecting Black Pepper by the combinations of *P. fluorescens* + *T. harzianum* and *P. fluorescens* + *T. viride* was observed by Saju *et al*., 2003. Further there are also reports on the bio-efficacy of the combination of *T. harzianum* + *P. fluorescens* for the management of diseases on the nursery seedlings of Black Pepper (Anandraj *et al*., 2003).
Pseudomonas fluorescens and P. chlamydosporia combination was found to be effective in the management of M. incognita on capsicum (Rao et al., 2004). Further, the successful management of integration of M. incognita on papaya nursery seedlings was achieved by the integration of T. harzianum and Paecilomyces lilacinus (Rao and Naik, 2003).

Pseudomonas fluorescens is proved to be a potential bio-control agent against several root pathogenic fungi and bacteria (Compeau et al., 1988; Rao et al., 2004). It was found to be effective in the control of root-knot nematodes on tomato (Shanthi and Sivakumar, 1995). Siddiqui et al., 1999 reported the effect of combination of Pseudomonas aeruginosa and T. viride in the control of root rot and root knot disease complex in chilli.

Elad et al., 1982 reported on the degradation of pathogenic fungi by T. harzianum. Flores et al., 1997 reported on the improved biocontrol activity of Trichoderma harzianum by over expression of the proteinase encoding gene prb1. Biological control of Phytophthora root rot of capsicum by T. harzianum was reported by Ahmed et al., 1999. Bio-agent was found to be effective in the control of root-knot nematodes on various crops (De Leij and Kerry, 1991; Godoy et al., 1983).