REVIEW OF LITERATURE
Sunflower (*Helianthus annuus* L.) is one of the three important edible oilseed crops grown in the world, after soybean (*Glycine max* L.) and groundnut (*Arachis hypogaea* L.). In India, sunflower was introduced in commercial cultivation around 1969 and within a short span it has reached a level of 2.1 M ha with the annual production of 1.3 M t of seed (Damodaram and Hegde, 2002). Presently in India, sunflower is cultivated over an area of 0.72 M ha with a production of 0.50 M t and productivity of 692 kg ha\(^{-1}\) (Table 1).

**Diseases of sunflower**

More than 30 diseases have been reported on sunflower, but only a few of them are of economically important (Gulya *et al.*, 1994). Sunflower is an important oil yielding crop and its protection measures should be taken at an early stage to minimize the loss caused by insects, pests and diseases. Among the insects, pests and diseases, diseases alone cause 10% yield loss in the field itself and some of the important diseases of sunflower are alternaria blight caused by *Alternaria solani*, head rot caused by *Rhizopus* sp., charcoal rot caused by *Macrophomina phaseolina*, sclerotial wilt caused by *Sclerotium rolfsii*, downy mildew caused by *Plasmopara halstedii*, powdery mildew caused by *Erysiphe cichoracearum*, rust caused by *Puccinia helianthi* and mosaic caused by Potyvirus. Among these, downy mildew is an important disease and causes more than 80% yield loss and quality in some areas of the world (Gore, 2008).

**Downy mildew disease of sunflower**

Amongst the sunflower diseases, downy mildew caused by the fungus *Plasmopara halstedii* (Farl.) Berl. & De Toni is important. Wide-spread occurrence of sunflower downy mildew is reported in many sunflower growing temperate, tropical and subtropical countries of the world except New Zealand (Viranyi, 1990). Downy mildew is the major biotic constraint in sunflower production. The typical symptoms appear as early as at the seedling stage. Stunting of the seedlings, yellowing or chlorosis and the downy growth of the zoosporangia on the abaxial side of the leaf are the typical symptoms of sunflower downy mildew. Majority of the seedlings will not survive due to severity of the disease and those which survive become unproductive with no flowers or no seeds. Infected plants serve as source of inoculum spread
through debris and the soil (Delanoe and Hamant, 1972). The primary infection is initiated by oospore germination and zoospores production, which systemically infect young seedlings causing either death or stunting the plant. The spread of infection in a crop depends on different factors such as the initial inoculum level in the soil and climatic conditions (Zimmer and Hoes, 1978). Soil and air moisture and temperature are particularly important during the germination and early growth stage of sunflower for disease development (Sackston, 1981).

The disease is initiated through oospores contaminated seeds or soil which upon favourable conditions germinate during spring in wet soils and produce swimming structures called zoospores, which migrate to roots, encyst and germinate. Conducive environment like cool, water-saturated soil during this period greatly favours infection spread (Kolte, 1985). Plant’s resistance appears in sunflower and the plants become increasingly resistant to infection with age, therefore, systemic infection occurs as early as during one to two weeks after seed germination. Spores are carried long distances through wind during favourable conditions. Spores may also adhere to soil particles and move to neighbouring fields during dust storms. Water running through an infested field may also carry mildew spores into fields which were disease-free earlier.

Occurrence of sunflower downy mildew depends on the growth stage and climatic conditions during the sunflower growing season. If the season is rainy, the number of diseased plants increases in proportion with the number of rainy days. The number of infected sunflower plants may vary from 1% to 100%. Extent of damage depends on infection type, i.e., whether it is primary (systemic) or secondary infection. While the primary or systemic infection causes significant yield reductions, secondary infection has no importance for the production of sunflower (Acimovic, 1998).

According to Tihonov (1975), downy mildew was first discovered on sunflower in the United States in 1883 and in 1892 it was found on *Helianthus tuberosus* in Russia. As the sunflower expanded to other countries, the disease followed it closely, especially after the World War II. The rapid expansion of the disease may be explained by its transfer with infected sunflower seeds. In the former Yugoslavia, it was discovered by Perisic (1949) and described by Nikolic (1952). Due to the seedborne nature of the fungus, the disease has been rapidly distributed throughout the
world by seed trade (Leepik, 1966) and the main cause is attributed to the latent infection of the fungus (Cohen and Sackston, 1974). In India, Ramnath et al. (1981) detected the presence of oospores on sunflower seeds imported from Bulgaria during early eighties. The sunflower downy mildew in India was reported around 1986 in Marathwada region of Maharashtra where sunflower is extensively grown. Later, Mayee and Patil (1986) reported its field occurrence at Oilseeds Research Station, Latur (Maharashtra) India. The disease incidence was also reported from adjoining major sunflower growing states like Andhra Pradesh and Karnataka (Mayee, 1988). During 1995-1996, Shirshikar (1997) conducted a major survey covering six districts of Marathwada region to establish the status of downy mildew in the farmers’ fields. The results revealed that 36.67% of sunflower fields were infected by downy mildew, with disease intensity ranging from 1 to 30%. During 2003-2004, the incidence level was dramatically minimized and this trend was noticed in other sunflower growing areas as well. This indicated that the release of resistant hybrids/varieties through screening at Latur must have contributed to minimizing the disease incidence in farmers’ fields. The sunflower downy mildew screening work initiated at Oilseeds Research Station, Latur has helped in identifying resistant hybrids and minimizing the disease problem in farmers’ fields. Apart from this, the identification of resistant germplasm material and parental lines has helped sunflower breeders to initiate sunflower downy mildew disease resistant breeding programme.

Downy mildew is seed borne in nature and release of resistant hybrids curbs the spread of the disease to new localities. Of the 1,944 sunflower genotypes screened against downy mildew in infected plot, eight hybrids and one population that showed resistance to downy mildew and high yield potential were released for commercial cultivation in India. The release of these resistant hybrids has helped to considerably minimize the disease incidence on farmers’ fields (Shirshikar, 2005a). Disease management strategies include use of resistant hybrids/cultivars, chemical control and cultural practices. Sunflower hybrids resistant to downy mildew are available, but new pathogenic races of the fungus are being formed in nature, making questionable the use of formerly resistant cultivars in a particular area (Molinero-Ruiz et al., 2002).
Management of sunflower downy mildew

Measures of protection against downy mildew include cultivation/cultural practices, chemical measures and the use of resistance breeding. The recommended cultural practices are the use of healthy seeds for planting, seed treatment with fungicides against downy mildew, proper crop rotation, i.e., intervals of 4-5 years between two sunflower crops in the same field, selection of fields for sunflower growing that are at least 500 m away from a field planted in the previous year because of infected harvest remains in that field, removal of volunteer plants, sowing at optimum time and avoiding late planting, and deep ploughing of the field after sunflower harvest.

The most effective chemical measure of downy mildew control is seed treatment with metalaxyl based preparations. This measure protects the sunflower crop at the time of the primary infection, i.e., at early stages of development of sunflower. In addition, various chemicals for post-emergence treatment are available. Patil et al. (1992) evaluated the efficiency of different fungicides against sunflower downy mildew and found that metalaxyl at 6 g kg\(^{-1}\) seed reduced the downy mildew incidence. In addition, ridomil was an effective fungicide in reducing the disease but it was on par with fosetyl-al, while thiophanate methyl and carbendazim were ineffective. Phytoalexin G84 was effective when sprayed on the radicle of germinated seeds after inoculation. Furthermore, use of Apron XL-35 ES (a metalaxyl fungicide) has been recommended for downy mildew disease management (Shirshikar, 2005b).

Metalaxyl seed dressing at 1, 2 or 4 g.a.i. kg\(^{-1}\) to sunflower seeds prevented \textit{P. helianthi} sporulation on seedlings inoculated by dipping the roots into a zoosporangial suspension. Washing seedlings with water before inoculation did not influence results, suggesting a systemic activity of the fungicide in the seedlings. One or two drenches of soil in pots with metalaxyl at concentration of 125, 250 or 500 mg a.i.l\(^{-1}\) reduced symptom severity in \textit{P. helianthi} infected seedlings and allowed plants to resume normal growth. Symptom remission in infected plants was also achieved by fungicide sprays in the field (Melero-Vara \textit{et al.}, 1982). However, over-use of the fungicide has led to the selection of pathogen strains resistant to pesticides. The occurrence of resistant isolates of the pathogen was first reported under greenhouse by Oros and Viranyi (1984). The resistance of the oomycete has been found at doses of metalaxyl lower than 2 g.a.i. kg\(^{-1}\) seed in Hungary, USA and Turkey (Viranyi \textit{et al.}, 1992; Gulya
and at commercial doses of 2 g.a.i. kg\(^{-1}\) seed in France and Spain (Albourie \textit{et al.}, 1998; Molinero-Ruiz \textit{et al.}, 2003). Mefenoxyam was marketed 10 years later than metalaxyl and it contains the biologically active enantiomer of the racemic fungicide metalaxyl (Shetty, 1998). Both fungicides are used worldwide against sunflower downy mildew. Resistance to mefenoxam has also been already reported in natural populations of the oomycetes \textit{Peronospora parasitica} and \textit{Phytophthora capsici} (Parra and Ristaino, 1998, 2001; Lamour and Hausbeck, 2000). Pesticides that are adopted to prevent or cure oomycete plant pathogens either do not exist or are not economically feasible for marginal farmers (Attard \textit{et al.}, 2008). These limitations associated with the routinely used strategies emphasize a search for alternative chemicals for sunflower downy mildew disease management.

Some newly developed sunflower hybrids and varieties included in All India Coordinated Programme were screened against downy mildew disease in the downy mildew infected plot at Latur Station. The main objective of this trial was to establish the downy mildew disease reaction of these hybrids/varieties in view of their further exploitation. Seeds treated with ridomil MZ \textsuperscript{®} 2 g kg\(^{-1}\) of seed and foliar spray \textsuperscript{®} 2.5 ml l\(^{-1}\) twice at 30 and 45 days after planting gave good result.

The efficacy of three commercial formulations of strobilurins \textit{viz.}, trifloxystrobin, kresoxim-methyl and azoxystrobin was evaluated against sunflower downy mildew disease caused by \textit{P. halstedii} under laboratory, greenhouse and field conditions. Complete inhibition of sporangial development, zoospore formation, release and motility were observed with 21 g ml\(^{-1}\) in trifloxystrobin, 51 g ml\(^{-1}\) for azoxystrobin and kresoxim-methyl. Seed treatment with different concentrations of strobilurins enhanced seed germination and seedling vigor of sunflower to varying degrees compared to control. For the three strobilurins seed treatment along with foliar application enhanced the protection of the plants compared to only the treatment of seeds. Foliar spray treatments alone provided an intermediate control of the disease. Trifloxystrobin showed a better effect than kresoxim-methyl and azoxystrobin. Disease curative activity of trifloxystrobin was higher compared to kresoxim-methyl and azoxystrobin (Sudisha \textit{et al.}, 2010).
**Plant growth promoting fungi**

Bringing down the use of fertilizers and chemicals in agricultural production is necessary to maintain ecosystems and to develop sustainable agriculture. The use of both biofertilizer and biocontrol systems can have minimal effect on the environment and such strategies have been widely researched. In soils, numerous microorganisms co-exist in association with plant roots. Some microorganisms live specifically in rhizosphere or on plant root surfaces and these can have many effects on performance of the plant and may also affect the structure of the plant community (Hyakumachi and Kubota, 2004). A unique microflora is particularly present around the plant root surface, where various substances are secreted. Most of the microorganisms distributed around plant root surface have a role in the decomposition of organic matter and some may suppress deleterious microorganisms, which could inhibit plant growth. Some of the root-associated microorganisms can promote plant growth and they have been called “plant growth-promoting fungi” (PGPF; Hyakumachi, 1994). PGPF are known to suppress many plant diseases. Similar effects are also observed in plants treated with mycorrhizal fungi, which have a symbiotic relationship with most plant species. Endophytes can also promote plant growth and these have been considered as potential biological control agents (Hyakumachi and Kubota, 2004).

Soil-borne fungi, such as *Trichoderma* sp., *Rhizoctonia solani* and others, can promote significant plant growth. Most of these PGPF have a high rhizosphere competence as a character. Because the genera found to be PGPF are common soil-borne fungi, there is a possibility that fungi having a similar role of PGPF exist widely in natural ecosystems. Most of these studies were quantified from the relative dry weight of root or above-ground part of treated plant seedlings with PGPF compared to non treated ones over a period as short as 4 weeks. In some cases, significant growth promoting effects of PGPF were observed as increased yield of plants grown in fields over longer period of 14 weeks or more (Shivanna *et al*., 1994). *Piriformospora indica* a newly described axenically cultivable phytopromotional endosymbiont, showed pronounced growth-promotional effects by mobilizing the insoluble phosphates and translocated the phosphorus to the host in an energy-dependent process (Varma *et al*., 2012).
Management of plant diseases using PGPF

The ability of PGPF to manage plant pathogens and diseases is well documented. Most of the PGPF identified have shown a pronounced suppressive effect against soil-borne diseases. One example of this is the suppression by *T. harzianum* of damping-off disease on barley, cucumber, radish and tomato caused by *Pythium ultimum* (Ahmad and Baker, 1987). Non-pathogenic *R. solani* AG4 has been reported to suppress damping-off disease caused by virulent *R. solani* and *R. zeae* by 76-94% on cotton, radish and wheat. The sterile fungi, sterile black fungus, sterile dark fungus and sterile red fungus have been shown to decrease the occurrence of take-all disease of wheat caused by *Gaeumannomyces graminis* var. *tritici* (Speakman and Kruger, 1984; Dewan and Sivasithamparam, 1989; Narita and Suzuki, 1991). The PGPF isolated from zoysiagrass rhizosphere have been shown high suppressive ability against soil-borne diseases caused by *P. aphanidermatum*, *P. irregulare*, *R. solani*, *Sclerotium rolfsii*, *F. oxysporum* f. sp. *melonis*, *F. oxysporum* f. sp. *cucumerinum*, *G. graminis* var. *tritici* and *Cochliobolus sativus* (Hyakumachi, 1994). When cucumber plants inoculated with PGPF isolates from zoysiagrass rhizosphere, disease suppression was observed against the air-borne pathogen, *Colletotrichum orbiculare* (Meera et al., 1994). The results therefore suggested that induced systemic resistance is involved as one of the mechanisms for disease suppression by PGPF. The PGPF isolates from zoysiagrass rhizosphere, *Trichoderma*, *Fusarium*, *Penicillium*, *Phoma* and sterile fungi, all provided significantly protection to air-borne anthracnose caused by *C. orbiculare*, bacterial angular leaf spot caused by *Pseudomonas syringae* pv. *lacryma*, and soil-borne fusarium wilt by *F. oxysporum* f. sp. *cucumeris* (Koike et al., 2001).

*Arabidopsis thaliana* grown in soil amended with barley grain inocula of *Penicillium simplicissimum* GP17-2 or receiving root treatment with its culture filtrate exhibited clear resistance to *P. syringae* pv. *tomato* DC3000 (Pst) (Hossain et al., 2007). Growth of tomato plants was enhanced and also the reduction of the incidence of *Meloidogyne incognita* was mediated due to the application of antagonistic fungi *Paecilomyces lilacinus*, *Pochonia chlamydosporia*, *T. harzianum* and *Gliocladium virens* with or without the PGPR, *Pseudomonas putida* and composted cow manure (CCM) in greenhouse experiments. *P. putida*, *G. intraradices* or CCM caused a significant increase in the growth of plants without nematodes. Use of *P. putida* also
caused a 39% reduction in galling and nematode multiplication followed by CCM (34%) and *G. intraradices* (32%) (Siddiqui and Akthar, 2008).

*Aspergillus niger* and *Penicillium* spp. were isolated from different sources (Rice field, Mangroves and Effluent soil) and were biochemically characterized and screened *in vitro* for their plant growth promoting traits like production of indole acetic acid (IAA), ammonia, HCN and catalase production. All the isolates were able to produce IAA. Production of ammonia was commonly detected in all the isolates. All the test isolates were positive for catalase but none of the isolates produced HCN (Samuel and Muthukkaruppan, 2011). *Fusarium, Penicillium, Phoma* and a sterile fungus from zoysiagrass rhizosphere induced systemic resistance in cucumber plants against *C. orbiculare* resulting in significant reduction of the disease. These PGPF also induced systemic resistance against bacterial angular leaf spot and fusarium wilt by treatment with barley grain inoculum. Roots treated with CFs of these fungal isolates induced lignification at *Colletotrichum* penetration points indicating the presence of an elicitor in the CFs (Koike *et al*., 2001).

The plant growth promoting fungus *F. equiseti* GF183 effectively controlled fusarium wilt of spinach caused by *F. oxysporum* f. sp. *spinaciae* in transplanting systems using paper pots reducing the disease severity which ranged from 43.5 to 91.8%. Double application of *F. equiseti* GF183 increased the protective effects (Horinouchi *et al*., 2010). The number of colony forming units of *F. oxysporum* f. sp. *spinaciae* g$^{-1}$ fresh weight of roots was significantly reduced in plants treated with *F. equiseti*. Root extracts from both *F. equiseti* treated plants and *F. equiseti* and pathogen treated plants significantly inhibited new production of budding cells of *F. oxysporum* f. sp. *spinaciae*.

**Plant growth promotion by Trichoderma**

*Trichoderma* spp. are free living fungi that are common in soil and root ecosystems. They are highly interactive in root, soil and foliar environment. They produce or release a variety of compounds that induce localized or systemic resistance responses in plants. *Trichoderma* strains have long been recognized as biological agents for the control of plant disease and for their ability to increase root growth and development, crop productivity, resistance to abiotic stresses and uptake and use of nutrients (Harman *et al*., 2004). *Trichoderma* is a potent biocontrol agent and used
extensively for post-harvest disease control. It has been used successfully against various pathogenic fungi belonging to various genera *viz.*, *Fusarium*, *Phytophthora* and *Sclerotinia*. *Trichoderma* strains solubilize phosphates and micronutrients (Harman *et al*., 2004; Islam *et al*., 2011). Application of *Trichoderma* strains with plants such as grasses increases the number of deep roots, thereby increasing the plant's ability to resist drought. *Trichoderma* strains are known to induce resistance in plants. Three classes of compounds that are produced by *Trichoderma* which induce resistance in plants are now known. These compounds induce ethylene production, hypersensitive responses and other related reaction in plants (Ranasingh *et al*., 2006).

*Trichoderma* spp. are very widely used to control various crop diseases effectively such as collar rot of elephant foot yam caused by *S. rolfsii*, damping-off of chilli, tomato, brinjal and rhizome rot of ginger, turmeric, and onion caused by species of *Phytophthora*, and *Pythium* respectively and *Fusarium* wilt of banana, cotton, tomato and brinjal caused by *F. oxysporum* (Ranasingh *et al*., 2006; Harel *et al*., 2014).

Species of *Trichoderma* are commonly used in the biocontrol of soil-borne plant pathogens of several economically important plant pathogenic fungi (Howell, 1998). The classical mechanisms of control have included antibiosis, mycoparasitism and competition for nutrients. *Trichoderma* strains inhibit or kill plant pathogenic fungi through production of antifungal, antibiotics and/or hydrolytic enzymes. The ability to promote growth and induce resistance in plants has been described for members of this genus (Monte, 2001; Harman *et al*., 2004).

Earlier work revealed that *Trichoderma* promotes growth responses in radish, pepper, cucumber and tomato (Baker *et al*., 1984; Chang *et al*., 1986). Further studies demonstrated that *Trichoderma* also increases root development and crop yield, the proliferation of secondary roots, seedling fresh weight and foliar area (Harman, 2000). Moreover, *T. harzianum* can solubilize several plant nutrients (Altomore *et al*., 1999), and the colonization of cucumber roots by *T. asperellum* has been shown to enhance the availability of P and Fe to plants, with significant increases in dry weight, shoot length and leaf area (Shoresh *et al*., 2005). Moreover, *Trichoderma* spp. produces auxins that are able to stimulate plant growth and root development (Contreras-Cornejo *et al*., 2009). As indicated above, an auxin-like effect has been observed in etiolated pea stems treated with harzianolide and 6-pentyl-a-pyrone, the major
secondary metabolites produced by different *Trichoderma* strains (Vinale *et al*., 2008). Role of auxin in regulating the growth and development of *Arabidopsis* seedlings in response to inoculation with *T. virens* and *T. atroviride* by developing a plant-fungus interaction system was investigated by Contreras-Cornejo *et al*. (2009). Wild type *Arabidopsis* seedlings inoculated with either *T. virens* or *T. atroviride* showed characteristic auxin related phenotypes including increased biomass production and stimulated lateral root development. When grown under axenic conditions, *T. virens* produced the auxin-related compounds indole-3-acetic acid, indole-3-acetaldehyde and indole-3-ethanol. These results highlighted the important role of auxin signalling for plant growth promotion by *T. virens*.

Maize rhizosphere colonization by *T. virens* also induces higher photosynthetic rates and systemic increases in the uptake of CO$_2$ in leaves (Vargas *et al*., 2009). The beneficial effects of *Trichoderma* on abiotic stress have been well documented (Donoso *et al*., 2008; Bae *et al*., 2009), although the mechanisms controlling multiple plant stress factors are still unknown. Mastouri *et al*. (2010) reported that the treatment of tomato seeds with *T. harzianum* accelerates seed germination, increases seedling vigor and ameliorates water, osmotic, salinity, chilling and heat stresses by inducing physiological protection in plants against oxidative damage.

1-Aminocyclopropane-1-carboxylate (ACC) deaminase activity was evaluated in the biocontrol and plant growth-promoting fungus *T. asperellum* T203. Fungal cultures grown with ACC as the sole nitrogen source showed high enzymatic activity. The enzyme encoding gene (Tas-acdS) was isolated and an average 3.5-fold induction of the gene by 3 mM ACC was detected by real-time PCR. *Escherichia coli* carrying the intron-free cDNA of Tas-acdS cloned into the vector pAlter-EX1 under the control of the tac promoter revealed specific ACC deaminase (ACCD) activity and the ability to promote canola root elongation (Viterbo *et al*., 2010).

Effect of *Trichoderma* along with *Glomus aggregatum* and *Bacillus coagulans* on growth, nutrition and content of secondary metabolites of *Solanum viarum* seedlings was assessed in greenhouse conditions. Triple inoculation of *G. aggregatum* + *B. coagulans* + *T. harzianum* with *S. viarum* in a greenhouse nursery study resulted in maximum plant biomass with P, Fe, Zn, Cu and Mn and secondary metabolites, orthohydroxy phenols, flavonoids, alkaloids, saponins and tannins of *S. viarum* seedlings. Activities of the enzymes, acid phosphatase, alkaline phosphatase and
dehydrogenase was enhanced due to *G. aggregatum + B. coagulans + T. harzianum* treatment (Hemashenpagam and Selvaraj, 2011).

*Aspergillus niger* strain BHUAS01, *Penicillium citrinum* strain BHUPC01 and *T. harzianum* were tested for phosphate solubilizing potential and plant hormones production (indole acetic acid). *Aspergillus niger* showed a maximum amount of soluble phosphate followed by *P. citrinum* and *T. harzianum*. Indole acetic acid production was estimated maximum in *A. niger* followed by *T. harzianum* and *P. citrinum*. Further, *T. harzianum* showed antagonistic effect against *F. oxysporum* and *R. solani*. Co-inoculation of *T. harzianum* and *A. niger* showed significant increase in chickpea growth parameters including shoot length, root length and dry weight of shoot and root (Yadav *et al*., 2011).

Colonization of *A. thaliana* seedlings by *T. atroviride* and its effect on plant growth and development was investigated by Salas-Marina *et al.* (2011). Results suggest that indole acetic acid related indoles produced by *T. atroviride* may have a stimulatory effect on plant growth. In addition, colonization of *Arabidopsis* roots by *T. atroviride* induced systemic protection against foliar pathogens the expression profile of salicylic acid, jasmonic acid/ethylene, oxidative burst and camalexin related genes was assessed in *Arabidopsis*. *Trichoderma atroviride* induced an overlapped expression of related genes of SA and JA/ET pathways and of the gene involved in the synthesis of the antimicrobial phytoalexin, camalexin, both locally and systemically.

Khan *et al.* (2012) investigated plant growth promoting activity of roots inhabiting endophytic fungi and evaluated their role in the survival of host plants under extreme sand dune environment of coastal regions. The results showed that 101 fungal isolates (82.7%) promoted plant height and shoot length of Waito-c rice, while 21 fungal isolates (17.2%) inhibited growth attributes. Root colonization by *P. indica* results in an increase in plant growth, early flowering, higher seed yield, alteration in the secondary metabolites and adaptation to abiotic and biotic stresses. The colonization of roots begins with a biotrophic growth phase, in which living cells are colonized and continues with a cell death dependent phase, in which root cells are actively killed by the fungus. The complexity of sebacinalean symbiosis is further enhanced by the presence of endocellular bacteria which may represent significant determinants for a successful outcome of the symbiosis. *Piriformospora indica* is
shown to have enormous bioprotective potential against plant pathogens and insect pests of agricultural and horticultural crops. Recently, decoding of \textit{P. indica}'s genome has revealed its potential for application as a plant growth promoting mycorrhizal fungus for realizing the targeted improvement in the production of crop plants (Varma \textit{et al.}, 2012).

\textbf{Management of plant diseases using \textit{Trichoderma harzianum}}

\textit{Trichoderma} is a very effective biological mean for plant disease management. Species of \textit{Trichoderma} are commonly used in the biocontrol of soil-borne plant pathogens of several economically important plant-pathogenic fungi (Howell, 1998; Sriram \textit{et al.}, 2013). \textit{Trichoderma} spp. in particular has been reported to control soil-borne plant pathogens such as \textit{R. solani}, \textit{S. rolfsii}, \textit{Pythium} spp. and \textit{Fusarium} spp. (Sivan and Chet, 1986, 1987; Calvet \textit{et al.}, 1990; Samuel, 1996; Lewis and Lumsden, 2001; Prasad \textit{et al.}, 2002). So far, \textit{Trichoderma} spp. are among the most studied fungal biological control agents (BCAs) and commercially marketed as biopesticides, biofertilizers and soil amendments (Harman, 2000; Harman \textit{et al.}, 2004; Lorito \textit{et al.}, 2004).

One of the most studied commercial biocontrol agents is isolate T39 of \textit{T. harzianum} which is regarded as a model to demonstrate control under commercial conditions and mechanisms of biocontrol. The use of this biocontrol agent for the control of \textit{Botrytis cinerea} in vineyards around the world has been described (Elad, 1994; O’Neill \textit{et al.}, 1996). This biocontrol agent has also been used for the control of tomato and cucumber diseases in commercial greenhouses (Elad, 2000). \textit{Trichoderma} spp. isolated from rhizosphere of banana were evaluated under \textit{in vitro} conditions for their antagonistic potential against \textit{F. oxysporum}, the banana fusarium wilt pathogen and it was found that \textit{T. harzianum} isolate Th-10 was most effective in inhibiting the mycelial growth of \textit{Fusarium} under \textit{in vitro}. In two field trials, soil application of \textit{T. harzianum} Th-10 as dried formulation effectively controlled fusarium wilt with an efficacy comparable to that of the fungicide carbendazim (Thangavelu \textit{et al.}, 2004). \textit{Trichoderma harzianum} ITEM 3636 and \textit{T. longibrachiatum} ITEM 3635 were evaluated in a field trial for their efficiency against \textit{F. solani} causing peanut brown root rot in a commercial field with a previous history of the disease. Two seed treatments were evaluated; seeds coated with a conidial suspension using
carboxymethylcellulose (CMC) as sticker and seeds coated with the antagonist fungal biomass on Biodac particles. *Trichoderma harzianum* in both seed treatments was more effective than *T. longibrachiatum* in decreasing the disease, increasing the frequency of healthy plants and boosting yield (Rojo et al., 2007).

*Trichoderma harzianum* isolate was evaluated *in vitro* and *in vivo* as potential BCAs against *Sclerotinia sclerotiorum*. The study showed that *T. harzianum* inhibited the growth and production of mycelia and sclerotia. *Trichoderma harzianum* appeared to exhibit mycoparasitism and protected over 80% of tomato, squash and eggplant seedlings inoculated with *S. sclerotiorum*. The efficacy of *T. harzianum* compared with two commercial products, PlantShield and SoilGard in the control of *S. sclerotiorum* was similar or slightly lower depending on the crop plant (Mansour et al., 2008). T39 of *T. harzianum* is an important BCA that controls the foliar pathogens, *B. cinerea*, *Pseudoperonospora cubensis*, *S. sclerotiorum* and *Sphaerotheca fusca* (syn. *S. fuliginea*) in cucumber under commercial greenhouse conditions. Cells of the BCA applied to the roots and dead cells applied to the leaves of cucumber plants induced control of powdery mildew. The BCA suppressed enzymes of *B. cinerea*, such as pectinases, cutinase, glucanase and chitinase, through the action of protease secreted on plant surfaces. A combination of several modes of action is responsible for biocontrol (Elad et al., 2000).

Three BCAs; *T. hamatum* (TM), *T. harzianum* (TZ) and *Paecilomyces lilacinus* (PL) and two resistance inducers (RIs); Bion [Benzo (1,2,3) thiadiazole-7-carbothioic acid S-methyl ester] (BTH)], salicylic acid (SA) were applied individually or in combination to test their efficacy in controlling cotton root rot disease caused by *F. oxysporum* (FO) and *P. debaryanum* (PD) under greenhouse and field conditions. In greenhouse experiments, all applied treatments protected cotton seedlings against FO root rot. All treatments significantly reduced the disease compared to infected controls. Increase in cell wall fractions (cellulose, hemicelluloses and lignin) resulted from application of both BCAs and RIs in case of PD.

*Pyrenophora tritici-repentis* (Died) Drechs. (anamorph - *Drechslera tritici-repentis* (Died) Shoem. is one of the most important and widespread necrotrophic pathogens of wheat in Argentina. The potential of *T. harzianum*, *T. aureoviride* and *T. koningii* as biocontrol agents of *D. tritici-repentis* was evaluated under *in vitro* and greenhouse conditions. Dual cultures in petri dishes containing potato dextrose agar
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showed that the isolates of *Trichoderma* spp. tested inhibited significantly the mycelial growth of *D. tritici-repentis*. Microscopic examination of cultures of *Trichoderma* spp. and *D. tritici-repentis* in close proximity showed plasmolysis of conidia and hyphae of the pathogen. The results of the greenhouse tests indicated that strains of *Trichoderma* spp. significantly reduced the disease severity on wheat plants compared with untreated plants (Perelloa *et al.*, 2003).

Biological control of botrytis gray mold (*B. cinerea*) of chickpea by foliar, seeds or soil treatments of seven BCA isolated from commercial biopesticides was evaluated by Khan *et al.* (2011). Foliar inoculation with BGM fungus caused disease of greater severity in comparison to soil or seed inoculation and reduced the plant dry weight and yield of chickpea. Soil treatment of *T. harzianum* (PBAT-1 and Biowilt-X) significantly improved the yield of soil inoculated plants. Similarly seed treatment with *T. harzianum* decreased the disease severity and increased the yield by 8-13% over seed inoculated control. *Trichoderma harzianum* PBAT-1, *T. virens* Sanjeevni and *P. fluorescens* Biocomp-X were found to be the most effective biopesticide strains. The ability of various strains of *Trichoderma* spp. to control bunch rot of grape was evaluated. Three strains of *Trichoderma* spp. were evaluated for their biocontrol ability and all provided significant control of *B. cinerea*. Trials in New York in 1992 and in Chile in 1992-1993 indicated that *T. harzianum* could replace some applications of iprodione or vinclozolin with little reduction in efficacy. In New York, in 1993, it was found that applications of *T. harzianum* at bloom and early fruit development followed by a tank-mix application of *T. harzianum* and half rates of iprodione gave extremely effective control of bunch rot. Thus, biological control of bunch rot of grape with *T. harzianum* can be an effective method of management of this disease (Harman *et al.*, 1996).

**Induction of systemic resistance in crop plants by Trichoderma**

Researchers have shown that *Trichoderma* are opportunistic, avirulent plant symbionts, as well as mycoparasites. At least some strains establish robust and long-lasting colonization of root surfaces and penetrate into the epidermis and a few cells below this level. They produce or release a variety of compounds that induce localized or systemic resistance responses and this explains their lack of pathogenicity to plants. These root-microorganism associations cause substantial changes to the
plant proteome and metabolism. Plants are protected from numerous classes of plant pathogen by responses that are similar to systemic acquired resistance (SAR) and rhizobacteria-induced systemic resistance (ISR). Inoculation of an industry standard light sphagnum peat potting mix with *T. hamatum* 382 (T382) significantly reduced the severity of botrytis blight on begonia plants grown in a greenhouse. Spatial separation was maintained in begonias between the biocontrol agent T382 and the pathogen. It was concluded, therefore, that the decrease in disease severity provided by inoculation of the peat mix with T382 most likely was due to systemic resistance induced in begonia against botrytis blight (Horst et al., 2005).

Induction of plant responses in various plants, both mono and dicotyledonous species, showed increased resistance to pathogen attack when pre-treated with species of *Trichoderma* (Harman et al., 2004). The induction of plant resistance by colonization with some *Trichoderma* sp. is similar to that elicited by rhizobacteria, which enhances the systemic resistance but do not involve in the production of pathogenesis-related proteins (PR proteins) (Van Loon et al., 1998; Stacey and Keen, 1999; Harman et al., 2004). Alfano et al. (2007) investigated at a molecular level, the plant genes involved in *T. hamatum* 382 induction of resistance by using a high density oligonucleotide microarray approach. Interestingly, *Trichoderma*-induced genes were associated with biotic or abiotic stresses, as well as RNA, DNA and protein metabolism. During the interaction of *Trichoderma* with the plant, different classes of metabolites may act as elicitors or resistance inducers (Harman et al., 2004; Woo et al., 2006; Woo and Lorito, 2007). These molecules include: (i) proteins with enzymatic activity, such as xylanase (Lotan and Fluhr, 1990); (ii) avirulence-like gene products able to induce reactions in plants (Woo et al., 2004); (iii) low-molecular-weight compounds released from fungal or plant cell walls by the activity of *Trichoderma* enzymes (Harman et al., 2004; Woo et al., 2006; Woo and Lorito, 2007). Some of the low-molecular-weight degradation products released from fungal cell walls were purified and characterized, and found to consist of short oligosaccharides comprised of two types of monomers, with and without an amino acid residue (Woo et al., 2006; Woo and Lorito, 2007). Djonovic et al. (2006) identified a small protein (Sm1) elicitor secreted by *T. virens* and demonstrated its involvement in the activation of plant defense mechanism and the induction of systemic resistance.
Three root-colonizing fungi, binucleate Rhizoctonia (BNR) isolates BNR621 and P9023 and T. hamatum isolate 382 (T382), were studied for suppression of botrytis blight in Geranium by induction of host systemic resistance. Resistance to botrytis blight was observed in geraniums transplanted into potting mix amended with formulations of P9023 and T382 two weeks prior to inoculation with B. cinerea when grown under environments either highly or less conducive to disease development (Olson and Benson, 2007). Colonization of roots by T. virens to induce systemic protection against a foliar pathogen in the monocot maize was studied by Djonovic et al. (2007), who demonstrated the importance of hydrophobin like elicitor Sm1 during maize-fungal interactions using a functional genomics approach. Maize seedlings were inoculated with T. virens Gv29-8 wild type and transformants in which Sm1 was disrupted or constitutively over expressed in a hydroponic system or in soil-grown maize seedlings challenged with the pathogen C. graminicola. It was shown that similar to dicot plants, colonization of maize roots by T. virens induces systemic protection of the leaves inoculated with C. graminicola.

The ability of 28 Trichoderma isolates to promote the growth of tomato seedlings and to induce systemic resistance (ISR) against Xanthomonas euvesicatoria and A. solani was evaluated by Fontenelle et al. (2011). Twelve isolates promoted the increase of plant dry matter mass (DMM) above 100%, showing the great potential of these strains. The plant growth promoting isolates were further evaluated for potential elicitation of ISR. Treatment of the soil with all Trichoderma isolates provided protection in tomato plants from 24.13% to 95.94% against X. euvesicatoria and 30.69% to 95.23% against A. solani.

The effect of Trichoderma spp. on resistance induction in tomato plants against root rot caused by F. oxysporum f. sp. radicis-lycopersici was evaluated in vivo, in growth chamber by bringing separately the pathogen and the antagonist to the same plant root level was investigated by Hibar et al. (2007). Results obtained showed that inspite of the physical separation from FORL, Trichoderma spp. have significantly reduced disease incidence especially when they were applied one week before inoculation with the pathogen. Light photomicrograph of samples from tomato roots treated with Trichoderma spp. showed elaboration of structural barriers in regions situated within striking distance of the pathogen penetration, formation of cell wall
thickenings and occlusion of intercellular spaces by a densely stained material, preventing pathogen invasion and penetration.

*Trichoderma harzianum* RU01 consistently triggered a significant degree of protection against rust in bean under greenhouse conditions. Control efficacy was similar to that of rhizobacterium, *P. aeruginosa* KMPCH, a test strain included in this study, which previously demonstrated induction of systemic resistance in bean. Given the spatial separation of challenging pathogen and biocontrol agent, this effect can be attributed to the induction of systemic resistance by *T. harzianum* RU01 (Abeysinghe, 2009). Efficacy of various isolates of *T. virens* were evaluated under greenhouse condition for efficacy in suppressing incidence of fusarium wilt disease and promoting plant growth in tomato. *Trichoderma virens* (Tv1) increased the plant growth and highly inhibited the mycelial growth of the pathogen under *in vitro* condition. In greenhouse studies, seed treatment plus soil application of talc based formulation of *T. virens* (Tv1) significantly reduced incidence of the diseases (Christopher *et al.*, 2010).

**Biochemical mechanism of ISR induced by Trichoderma**

*Trichoderma* strains inhibit or kill plant-pathogenic fungi through production of antifungal antibiotics and/or hydrolytic enzymes. The ability to promote growth and induce resistance in plants has also been described for members of this genus (Monte, 2001; Harman *et al.*, 2004). The complex process of mycoparasitism consists of several events, including recognition of the host, attack and subsequent penetration and killing. During this process *Trichoderma* secretes CWDEs that hydrolyze the cell wall of the host fungus, subsequently releasing oligomers from the pathogen cell wall (Kubicek *et al.*, 2001; Howell, 2003; Woo *et al.*, 2006). *Trichoderma* secretes hydrolytic enzymes at a constitutive level and detects the presence of another fungus by sensing the molecules released from the host by enzymatic degradation (Harman *et al.*, 2004; Lorito *et al.*, 2006; Woo and Lorito, 2007; Harman *et al.*, 2008). The antifungal arsenal of *Trichoderma* spp. includes a great variety of lytic enzymes (Lorito *et al.*, 1996; 1998), most of which play a great role in biocontrol (Harman and Kubicek, 1998; Baek *et al.*, 1999; Carsolio *et al.*, 1999; Woo *et al.*, 1999; Zeilinger *et al.*, 1999; Kullnig *et al.*, 2000; Kubicek *et al.*, 2001). When tested alone or in combinations, the purified proteins showed antifungal activity towards a broad
spectrum of fungal pathogens (i.e., species of *Rhizoctonia, Fusarium, Alternaria, Ustilago, Venturia* and *Colletotrichum*, as well as fungus like organisms such as *Pythium* and * Phytophthora* which lack chitin in their cell walls) (Tronsmo, 1991). During the process of mycoparasitism, *Trichoderma* spp. produce several cell wall degrading enzymes, including β-1,3 glucanase, which allow them to bore holes in the cell wall of other fungi and extract nutrients for their own growth (Almeida *et al.*, 2007).

The biochemical nature of the interaction between the antagonistic fungus *T. harzianum* strain T203 and cucumber roots was studied during the early stages of root colonization by the fungus. Pathogenesis related (PR) proteins of the plant and enzyme activity of the fungus following the penetration and colonization of the roots by *T. harzianum* were explored up to 72 hpi. Scanning electron microscopy (SEM) revealed typical fungal structures previously associated with mycoparasitic interactions of *T. harzianum* strains during biological control. These included hyphal coiling and appressoria formation. Compared to untreated control, cucumber roots treated with *T. harzianum* T203 exhibited higher activities of chitinase, β-1,3 glucanase, cellulase and peroxidase, up to 72 hpi (Yedidia *et al.*, 2000).

The effect of soil application of biocontrol agents (*Pseudomonas fluorescens, T. viride* and *T. harzianum*) in combination with chitin on induction of phenolics and enzymes in coconut roots infected with *Ganoderma lucidum*, the causal agent of ganoderma disease, was investigated by Karthikeyan *et al.* (2006). Soil application of these biocontrol formulations in combination with chitin induced a significant increase in the activities of peroxidase, polyphenol oxidase, phenylalanine ammonia lyase, chitinase and glucanase in the *G. lucidum* infected palms. Activities of both PAL and PO reached maximum levels within three days while the activity of PPO reached the maximum level of six days after application of a mixture of *P. fluorescens, T. viride* and chitin. Biological control of root-knot nematode by *T. harzianum* BI was investigated in greenhouse and laboratory experiments. Results showed that different concentrations of *T. harzianum* BI decreased nematode infection and other parameters significantly compared to control. Specific activities of resistance related enzymes, namely peroxidase (POX), polyphenol oxidase (PPO) and phenylalanine ammonia lyase (PAL) increased significantly in *T. harzianum* BI inoculated plants (Sahebani and Hadavi, 2008). Expression of various related
enzymes was found involved in the induction of systemic resistance against pathogen infection. *Trichoderma virens* (Tv1) treatment resulted in induction of defence enzyme such as peroxidase (PO), polyphenol oxidase (PPO) and phenylalanine ammonia lyase (PAL) (Christopher *et al*., 2010). Isozyme analysis revealed that unique PO\textsubscript{3} and PPO\textsubscript{2} isozymes were induced in coconut palms treated with *P. fluorescens* + *T. viride* + Chitin. Accumulation of phenolics was recorded three days after treatment and reached maximum levels i.e., nine days after treatment application. Activity of chitinase was significantly increased from the third day after treatment imposition and continued to increase up to 9 to 12 days in all treatments. Chitinase isozyme analysis revealed that a unique Chit3 isoform was induced in coconut roots treated with *P. fluorescens* + *T. viride* + Chitin. The glucanase activity was maximum nine days after treatment application. The mechanisms by which *P. fluorescens* + *T. viride* + Chitin reduced the incidence of ganoderma disease in coconut may be treated to its ability to induce defence mechanisms in coconut palms (Karthikeyan *et al*., 2006).

The evaluation of production and activity of β-1,3 glucanase are important parameters to be analyzed during screening of efficient candidate of *Trichoderma* for use as a biocontrol agent (Almeida *et al*., 2007). Two strains of *T. harzianum* 101645, and 206040, used for biological control of fungal plant pathogens were investigated for the production of serine protease, chitinase and antibiotic activity in relation to entomopathogenicity. Both strains produced serine protease with aMr of 31 kDa and chitinase with aMr of 44 kDa. Enzymes from both strains had similar characteristics and were produced during the growth phase. Both strains also produced peptaibols active against fungi in late growth and stationary phases which differed in their amino-alcohol content (Shakeri and Foster, 2007).

*Trichoderma* proteins involved in root colonization can also act as MAMPs. Swollenin (Brotman *et al*., 2008) stimulates responses in cucumber roots and leaves and affords local protection against fungi and bacteria, and the endopolygalacturonase. ThPG1 (Moran-Diez *et al*., 2009) generates a response in *Arabidopsis* similar to the ISR triggered by PGPR. Orthologues of the SSCP cerato-platanin family – Sm1 from *T. virens* and Epl1 from *T. atroviride* are accumulated in the hyphae during root colonization and act as MAMPs in cotton and maize (Djonovic *et al*., 2006; Seidl *et al*., 2006). The mycotrophic activity of *Trichoderma* chitinases can also release
chitooligosaccharides and indirectly contribute to the induction of this mechanism. Certain secondary metabolites produced by *Trichoderma* exert an antimicrobial effect at high doses but act as MAMPs and as auxin-like compounds at low concentrations (Vinale *et al*., 2008). The protection of tobacco plants against tobacco mosaic virus by *Trichoderma* peptaibols was shown to involve multiple signalling pathways (Luo *et al*., 2010).

The primary target of *T. harzianum* ETS 323 in the biocontrol mechanism was the cell wall of *B. cinerea* as demonstrated by the activities of the cell wall-degrading enzymes (CWDEs), including β-1,3 glucanases, β-1,6 glucanases, chitinases, proteases and xylanases, which were significantly higher in media with deactivated *B. cinerea* mycelia than in other media (Yang *et al*., 2009). The efficiency of *Trichoderma* spp. in inducing acquired systemic disease resistance in tomato was conducted. Systemic induced resistant reaction was evaluated on chitinolytic and β-1,3 glucanolytic activities produced by tomato plant (*Sida* cultivar) including disease severity of bacterial and gray leaf spot. High chitinase activity was detected from *Trichoderma* isolates in descending order, T1, T9, T13, T18 and T18 (Saksirirat *et al*., 2009).

Efficacy of various isolates of *T. virens* were evaluated under greenhouse condition for efficacy in suppressing incidence of fusarium wilt disease and promoting plant growth in tomato. *Trichoderma virens* (Tv1) increased the plant growth and highly inhibited the mycelial growth of the pathogen under *in vitro* condition. In greenhouse studies, seed treatment plus soil application of talc based formulation of *T. virens* (Tv1) significantly reduced incidence of the diseases. Trichokonins, antimicrobial peptaibols isolated from *T. pseudokoningii* SMF2 induced defence responses and systemic resistance in tobacco (*N. tabaccum* var. *samsun* NN) against tobacco mosaic virus infection. Trichokinin treatment increased the production of reactive oxygen species and phenolic compounds in tobacco. Additionally, application of trichokonins significantly increased activities of pathogenesis-related enzymes PAL and POX, and upregulated the expression of several plant defence genes (Luo *et al*., 2010). *Trichoderma* spp. are commonly used in the biocontrol of soil-borne plant pathogens of several economically important plant pathogenic fungi (Howell, 1998).
Recently, the potential of four *T. asperellum* strains to control *P. myriotylum* was assessed by Mbarga *et al.* (2012). The antagonistic and mycoparasitic potential of the *T. asperellum* strains was evaluated *in vitro* through dual culture and interaction tests. All four *T. asperellum* strains were antagonistic to *P. myriotylum*, although differences were found among the strains. The growth of *P. myriotylum* was inhibited by more than 60%. Furthermore, the four *T. asperellum* strains were aggressive mycoparasites of *P. myriotylum*.

Seed treatment with *T. virens* stimulates defence responses, as indicated by the synthesis of terpenoids in cotton roots. Analysis of extracts of cotton roots and hypocotyls grown from *T. virens*-treated seeds showed that terpenoid synthesis and peroxidase activity were increased in the roots of treated plants, but not in the hypocotyls of these plants or in the untreated controls (Howell *et al.*, 2000). Expression analysis of the exo-β-1,3 glucanase from the mycoparasitic fungus *T. asperellum* and the regulation of the gene encoding the extracellular exo-β-1,3 glucanase (tag83) produced by the mycoparasite *T. asperellum* was studied by Marcello *et al.* (2010). Enzyme activity was detected in all carbon sources, but the highest levels were found when starch and purified cell walls from *R. solani* were used. Experiments using RT-PCR showed that exo-β-1,3 glucanase induction in *T. asperellum* occurred at the transcriptional level. It was shown that the expression of tag83 is significantly increased by the presence of *R. solani*.

*Trichoderma harzianum* T39 mediated reduction of downy mildew severity on susceptible grapevines under controlled greenhouse conditions was modulated by the involvement of jasmonic acid and ethylene signals in the defence processes induced by T39, in contrast to the salicylic acid pathway activated by BTH (Perazzolli *et al.*, 2011). The induction dynamics of chitinases and glucanases in 10 strains of *Trichoderma* spp. was assessed in liquid media with different inducers (Gonzalez *et al.*, 2012). The highest values of chitinase activity were obtained in basal and basal media supplemented with chitin; β-1,3 glucanase, on the other hand, exhibited higher levels of activity in basal and basal media supplemented with gelatin. Soil application of *T. harzianum* on the induction of phenolic accumulation content and defence enzymes in tomato plants infected with *F. oxysporum* f.sp. *Lycopersici* (F. *oxysporum*) resulted in enhanced activities of both peroxidase and polyphenol oxidase (Ojha and Chatterjee, 2012).
Molecular mechanisms of ISR by *Trichoderma*

The protein elicitor xylanase Xyn2/Eix isolated from *T. viride* activated a xylanase that elicits ET biosynthesis and hypersensitive response in tobacco leaf tissues (Rotblat *et al.*, 2002). Cellulases from *T. longibrachiatum* activated and heat denatured cellulases elicited melons through the activation of the SA and ET signalling pathways respectively (Martinez *et al.*, 2001). The effecro Cerato-platanins Sm1/Epl1 derived from *T. virens/T. atroviride* elicited hydrophobin-like SSCP orthologues that can induce expression of defence responses in cotton and maize (Djonovic *et al.*, 2006; Seidl *et al.*, 2006). *Trichoderma* induced MAPK (TIPK) gene was identified and its function characterized in cucumber (*Cucumis sativus*). Plants over expressing TIPK were more resistant to pathogenic bacteria attack than control plants, even in the absence of *Trichoderma* pre-inoculation. On the other hand, plants expressing TIPK-AS revealed increased sensitivity to pathogen attack. Moreover, *Trichoderma* pre-inoculation could not protect these AS plants against subsequent pathogen attack. It was suggested that *Trichoderma* exerts its protective effect on plants through activation of the TIPK gene, a MAPK that is involved in signal transduction pathways of defence responses (Shoresh *et al.*, 2006).

Wild type *Arabidopsis* seedlings inoculated with either *T. virens* or *T. atroviride* showed characteristic auxin related phenotypes, including increased biomass production and stimulated lateral root development. When grown under axenic conditions, *T. virens* produced the auxin-related compounds indole-3-acetic acid, indole-3-acetaldehyde and indole-3-ethanol. A comparative analysis of all three indolic compounds provided detailed information about the structure-activity relationship based on their efficacy at modulating root system architecture, activation of auxin-regulated gene expression and rescue of the root hair defective phenotype of the rhd6 auxin response *Arabidopsis* mutant (Contreras-Cornejo *et al.*, 2009).

Tomato - *F. oxysporum* f. sp. *radicis-lycopersici* pathosystem was used to study induced systemic resistance elicited by *T. koningiopsis* (Th003) using the split root model. Gene expression induced by Th003 was evaluated using the tomato TOM1 microarray. Plant treatment with *T. koningiopsis* affected mRNA levels of 45 genes: 41 in roots and four in leaves (Moreno *et al.*, 2009). Colonization of *A. thaliana* seedlings by *T. atroviride* and its effect on plant growth and development was investigated by Salas-Marina *et al.* (2011). Results suggest that indole acetic acid
related indoles (IAA-related indoles) produced by \textit{T. atroviride} may have a stimulatory effect on plant growth. In addition, colonization of \textit{Arabidopsis} roots by \textit{T. atroviride} induced systemic protection against foliar pathogens and the expression profile of salicylic acid, jasmonic acid/ethylene, oxidative burst and camalexin related genes was assessed in \textit{Arabidopsis}. \textit{Trichoderma atroviride} induced an overlapped expression of related genes of SA and JA/ET pathways and of the gene involved in the synthesis of the antimicrobial phytoalexin, camalexin, both locally and systemically.

Six isolates of \textit{Trichoderma} were tested for parasitic and antimicrobial activity against \textit{Phytophthora capsici} and for endophytic and induced resistance capabilities in pepper. Isolates DIS 70a, DIS 219b and DIS 376f were \textit{P. capsici} parasites, while DIS 70a, DIS 259j, DIS 320c and DIS 376f metabolites inhibited \textit{P. capsici}. DIS 259j, DIS 320c and DIS 376f induced defence-related expressed sequence tags (EST) in 32-day-old peppers (\textit{Piper nigrum} L.). DIS 70a, DIS 259j and DIS 376f delayed disease development. Expression of CaLTP-N, encoding a LTP-like protein in pepper, in \textit{N. benthamiana} leaves reduced disease development in response to \textit{P. nicotianae} inoculation, suggesting LTP are functional components of resistance induced by \textit{Trichoderma} sp. (Bae et al., 2011). The proteomic and histochemical changes activated by \textit{T. harzianum} T39 in grapevine were investigated before and after \textit{P. viticola} inoculation. A comprehensive proteomic analysis of T39 induced resistance in grapevine resulted in the identification and quantification of a total of 800 proteins. Most of the proteins directly affected by T39 were found to be involved in signal transduction. T39 induced resistance was associated with rapid accumulation of reactive oxygen species and callose at infection sites, as well as changes in abundance of proteins involved in response to stress and redox balance, indicating an active response to downy mildew (Palmieri et al., 2012).

The expression patterns of the HR4 gene in \textit{Arabidopsis} seedlings interacting with the beneficial fungus \textit{T. atroviride} were investigated. The HR4 gene was differentially regulated in interactions with the beneficial bacterium \textit{P. fluorescens} and the pathogenic bacterium \textit{P. syringae}. When hormone treatments were applied to \textit{A. thaliana} (Col-0), each hormone treatment induced changes in HR4 gene expression. On the other hand, the expression of the RPW8.1 and RPW8.2 genes of \textit{Arabidopsis} ecotype Ms-0 in interaction with \textit{T. atroviride} was assessed. These genes
were interaction responsive; in particular, the RPW8.1 gene showed a very high level of expression in the later stages of interaction. The results indicated that HR4 and RPW8 genes could play a role in the establishment of *Arabidopsis* interactions with beneficial microbes (Saenz-Mata and Jimenez-Bremont, 2012).

The ability of *T. asperellum* SKT-1 to induce systemic resistance by SKT-1 or its cell-free CF was tested using *A. thaliana* Col-0 plants. Both SKT-1 and its CF elicited an induced systemic resistance against the bacterial leaf speck pathogen *P. syringae pv. tomato* DC3000 in Col-0 plants. Involvement of plant hormones in the induced resistance by SKT-1 and CF was assessed using *Arabidopsis* genotypes such as the jasmonic acid (JA)-resistant mutant *jar1*, the ethylene (ET)-resistant mutant *etr1*, the plant impaired in salicylic acid (SA) signalling transgenic NahG and the mutant *npr1* impaired in NPR1 activity. In soil experiments using SKT-1, no significant disease suppression effect was observed in NahG transgenic plants or *npr1* mutant plants. Expression levels of SA-inducible genes such as PR-1, PR-2 and PR-5 increased substantially in the leaves of Col-0 plants (Yoshioka *et al*., 2012).

**Formulation of Trichoderma**

Several commercial formulations of *Trichoderma* spp. mainly based on inert carriers are available for controlling plant diseases (Lewis *et al*., 1991). The most important factor that must be considered when selecting a biocontrol agent for commercial development is the availability of a cost-effective production and stabilization technology that yields an optimally effective form of the antagonist. More studies on the practical aspects of mass production and formulation need to be undertaken to make new biocontrol products stable, effective, safer and more cost-effective (Fravel *et al*., 1999). Major characteristics to market a biofungicide are the following (Agosin and Aguilera, 1998): abundant and cost-effective production of microbial propagules; ability to survive downstream process; stability and adequate shelf life of the final product upon storage, preferably without refrigeration; tolerance to environmental variations in temperature, desiccation, irradiation and relative humidity in order to survive and establish active populations in the soil; and consistent efficacy under varying field conditions at commercially feasible rates.

One of the most important reasons for the limited commercial diffusion of biofungicides is the high cost of production, due to the cost of substrate, low biomass
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productivity and limited economies of scale (Rhodes, 1996). The practical efficacy of a BCA greatly depends on the quality of the inoculants; itself a function of the production and formulation processes (Whipps, 1997). Two methods are commonly used for producing inoculum of BCA’s: liquid and solid fermentation. In submerged fermentation, specific parameters for aeration, temperature and pH control, carbon and nitrogen sources must be developed for each organism. Extracellular chitinase production by the chitinolytic fungus *T. harzianum* using submerged fermentation was studied (Sandhya *et al*., 2004). Supplementation of additional carbon sources showed no further enhancement in chitinase production while supplementation of nitrogen sources such as peptone and tryptone in the fermentation medium showed a marked increase in production. Many BCAs are easily produced in liquid culture in labscale, but when produced in large scale, they do not produce the expected quantity or quality of propagules, essentially for the low oxygen availability in fermenters. Some filamentous fungi need high oxygen transfer for growth and especially for sporulation. The choice of the adequate propagule of the microorganism is important to provide the desired shelf life. Some bacteria are easily dried and can be provided as dry cells or formulated further. Survival stage structures of the organism, such as chlamydospores, microsclerotia, ascospores or endospores are generally preferred. Sporulating gram-positive bacteria, such as *Bacillus* spp. and *Streptomyces* spp., offer endospores resistant to desiccation and heat that can be formulated readily into stable products, such as dry powder. Instead, gram-negative bacteria, such as *Pseudomonas* spp., are generally formulated as frozen cell pellets that must be kept at low temperature until application, which remain a major obstacle for their large scale use (Slininger *et al*., 1996). If the microorganism is produced by liquid fermentation, it is necessary to reduce the volume of liquid or obtain a final dry formulation. The strain Fo47 of *F. oxysporum* has been produced in submerged fermentation, removing the growth medium by filtration and the propagules were mixed with talcum powder used as an inert carrier and then dried at 18-20°C for two days (Durand *et al*., 1989). Drying can also be accomplished by freeze drying, atomization or bed-fluid drying, preserving the inoculum for a long time with high viability (Beudeker *et al*., 1989). Rapid drying can cause cell membrane damage, particularly if heating is used to speed drying.
For this reason, a glycerol enriched medium was developed to produce high levels of desiccation tolerant conidia of *T. harzianum* (Jin *et al*., 1996). A biofungicide based on the K61 strain of *Streptomyces griseoviridis* is produced by fermentation followed by lyophilization. The commercial product contains at least $10^8$ dormant spores g$^{-1}$ and is stable for 12 months at temperatures inferior to 8°C (Tahvonen and Avikainen, 1987). Sabaratnam and Traquair (2002) demonstrated that vegetative propagules from actively growing filaments are acceptable inoculants for *Streptomyces* sp. formulations with better shelf life at 4°C. The final product is easy to apply by mixing it with nutrient solution delivered to plants in soilless cultures or by mixing with potting mixtures. Solid state fermentation mimics the natural environment conditions and habitat for some microorganisms (Kim *et al*., 1985). The moisture needed is found in the solid matrix in an adsorbed or complex form, with moisture ranging between 12 and 80%. Solid fermentation scaling, necessary for use on an industrial scale, raises engineering problems due to the build up of temperature, pH, O$_2$, substrate and moisture gradients. However, solid fermentation possess several biotechnological advantages, e.g., higher fermentation productivity, higher end concentration of products, higher product stability and lower demand on sterility due to the low water activity (Holker *et al*., 2004). Larena and Melgarejo (2002) produced 250 fold more conidia of *Penicillium oxalicum*, antagonist of *F. oxysporum* f. sp. *lycopersici*, in solid than in liquid fermentation. Conidia produced in solid fermentation had a longer shelf life if stored at −20°C.

Moreover, solid state fermentation permits to save the labour and technical difficulties and generally does not need further formulation. This type of formulation is adopted for horticultural usage where it is mixed with potting mixture, but it does not enable application of the inoculant as a suspension in water. The strain Fo47 of *F. oxysporum* can be produced by solid state fermentation either in sterilized peat or in calcinated clay and can be stored at 4°C or at room temperature without loss of density or activity (Olivain *et al*., 1999). Conidia of *T. harzianum* produced in aerial mycelium with solid substrate fermentation persist longer under harsher environmental conditions than those produced under submerged culture conditions and wall thickness of aerial conidia is nearly twice that of submerged ones (Munoz *et al*., 1995). A big advantage was the culture of the fungus under absolute axenic conditions (Holker *et al*., 2004). After 14 days of sterile fermentation including in
situ drying, the cereal grains used as a substrate were covered and filled with fungal spores. When the fermentation process is completed, the conidia are separated from the culture medium using a microscreen machine that permits to have only the conidia of the processed fungus in the end product.

Formulation and methods of application of biological control has often been idealized as a method for controlling plant diseases, as happened for organic farming compared to the traditional cropping techniques. In organic farming, the bottleneck is represented by keeping remunerative yield, lowering the selling price of the product. For biological control, the major difficulty to reach the market and to be competitive with the chemical fungicides is represented by a consistent and reliable effectiveness and by the length of shelf life. Both problems can be faced with a scientific development of formulation of biocontrol agents. Until now, except for rare cases, formulation has been faced with an empirical approach without a methodology.

Obvious advantages of formulation include greater efficacy, increased shelf life, and ease of handling, increased safety, lower production costs and compatibility with agricultural practices. Minuto et al. (1997) have compared different strains of antagonistic *F. oxysporum* and different commercial formulations of these strains to control fusarium wilt of basil, concluding that the efficacy was strongly dependent on the formulation. Formulation is dependent on the type of fermentation used. In case of solid state fermentation providing the carrier for the inoculum, it is not necessary to develop a sophisticated formulation process (Lewis, 1991). The type of formulation desired depends on the intended use. For application to soilless cultures where the easiest way is to apply the inoculant through the drip irrigation system, a liquid formulation would be preferred. A granular material would be more appropriate for combining with potting mix, while a wettable powder would be more appropriate for root dips or sprays. The application of *Conithyrium minitans* follows one of the two ways: either soil application to reduce the sclerotial inoculums potential or spore sprays onto diseased plants or crop debris to sanitize the crop (de Vrije et al., 2001).

Often a biofungicide comprises many ingredients, such as carriers, diluents, bulking additives, membrane stabilizers, growth and contaminant suppressants, buffering systems, binders, dispersants, lubrificants, activators, food sources and coating compounds, added for various purposes (Paau, 1998). These include, keeping viability of antagonists, manipulating bulk for handling and delivery, promoting the
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activity of the BCAs and arresting growth of potential contaminants. More attention should also be devoted to the special requirements of BCAs in terms of application and delivery technology. Delivery must be easy, effective and timely to the appropriate site of action and compatible with the available agricultural equipment. In contrast to chemical pesticides, they are living organisms; they range in size and are more susceptible to the actual conditions (temperature, humidity and pH) than chemical pesticides (Matthews, 2000). In general, most biocontrol agents are applied with the same equipment currently used to apply chemical pesticides (Mathre et al., 1999).

From the present review of literature it is evident that there are no reports on use of *Trichoderma* spp. for induction of resistance in sunflower against downy mildew disease. Similarly, the biochemical and molecular levels of induction against sunflower downy mildew disease has not been done and hence the present study has been undertaken with following objectives.

**Objectives:**

1. Survey and isolation of PGPF from rhizosphere of crop plants.
2. Screening for growth promotion and induction of systemic resistance in sunflower against downy mildew disease using PGPF.
3. Elucidation of the defense enzymes underlying the induced systemic resistance by the PGPF.
5. Development of formulation of promising PGPF in large scale and test its efficacy against sunflower downy mildew.