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Review Article

Use of *Trichoderma* in disease management of crop plants caused by soil borne phytopathogenic fungi

Subhash Chandra
Department of Botany, Sri Murli Manohar Town P.G. College, Ballia-277001 (U.P.), India
Email- subhashcbsi1@gmail.com

Abstract

Biological management of soil-borne phytopathogen is a potential alternative to the use of chemical pesticides, which have already been proved to be harmful to the environment. Several species of the genus *Trichoderma* have been isolated and found to be effective biocontrol agents of various soil-borne phytopathogenic fungi under greenhouse and field conditions. Different application approaches have been used including integration of *Trichoderma* with reduced doses of chemical agents. Biochemical and molecular biology studies carried out to explore the mechanisms involved in biological control revealed that *Trichoderma* is a rather specific mycoparasitism, antibiosis and competition and rhizosphere competence. Lectins were found to be involved in the recognition between *Trichoderma* and its host fungi, whereas chitinase is involved in the degradation of the host cell wall. Genetic engineering techniques were employed in order to increase the effectiveness, stability and biocontrol efficacy of *Trichoderma* spp. as well as other biocontrol agents used as disease management of economically important crop plants caused by soil-borne phytopathogenic fungi viz., *Pythium*, *Fusarium*, *Rhizoctonia*, *Phytopthora*, *Sclerotinia*, *Sclerotium* and *Macrophomina*, resulting in heavy losses.

Keywords- *Trichoderma*, Disease management, Crop plants and Phytopathogenic fungi

Introduction

Trichoderma is a very effective biological mean for plant disease management especially the soil-borne. Fungal species belonging to the genus *Trichoderma* are worldwide in occurrence and easily isolated from soil, decaying wood and other forms of plant organic matter. The potential of *Trichoderma* species as biocontrol agents of plant diseases was first recognized in the early 1930's (Weindling, 1932). The species of genus

Trichoderma have been reported as most potential biocontrol agents (BCAs) against a wide range of soil-borne phytopathogen (Lewis and Papavizas, 1991; Haran *et al.*, 1996a; Haran *et al.*, 1996b; Elad, 2000; Joshi *et al*., 2010; Hermosa *et al.*, 2012; Keswani *et al*., 2015) due to their ability to successfully antagonize other fungi. Establishment of the *Trichoderma* and other biocontrol agents in the soil ecosystem has greatly affected by numerous biotic (nature of the target organism and of the host plant, presence of predators, parasites or antagonistic microorganisms among the resident microflora) and abiotic (nature of the soil or substrate, humidity, availability of nutrients, temperature, radiations, salinity and pH) factors (Dandurand and Knudsen, 1993; Eastburn and Butler, 1988a, b; Hubbard *et al*., 1983; Knudsen and Bin, 1990). One of the most interesting aspects of the science of biological control is the study of the mechanisms employed by biocontrol agents to affect disease control. Past research indicates that the mechanisms are many and varied, even within the genus *Trichoderma*. In order to make the most effective use of biocontrol agents for diseases management of economically important, cereals, pulses, oils and horticultural crop plants.

The application or delivery of the biocontrol agents into the soil is done in the following manner:

(a) Apply suspension of agent propagules to soil directly or in a substrate.
(b) Substrate or some other medium reading the antagonists is directly mixed with soil. Several soil-borne diseases have been controlled by this method, e.g. wheat bran colonized by *Trichoderma viride* when incorporated in a strawberry nursery; it decreased the disease severity of damping-off of seedlings caused by *Rhizoctonia solani*, thereby increasing the yield (Elad *et al.*, 1981).
(c) Synthetic substrates such as sodium alginate can be used to form pellets in which biocontrol agent's spores are incorporated. This is an effective method of the delivery of *Trichoderma* spp.
(d) For foliar pathogens, biocontrol agents are applied to the aerial surfaces of the plants.
(e) In other cases, biocontrol agents are applied directly to bulbs, seeds, potato tubers, pieces or harvested fruits, before sowing in the fields.

Methods of disease management

Seed treatment

Mix 6-10 g of *Trichoderma* formulation (conidial suspension) per litre of cow dung slurry for treatment of 1 kg of seed before sowing, particularly for cereals, pulses, vegetables and oil seeds.

Nursery treatment

Drench nursery beds with @ 5 g *Trichoderma* formulation (conidial suspension) per litre of water before sowing.

Cutting and seedling root dip

Mixed 10 g of *Trichoderma* formulation (conidial suspension) per litre of water and dip the cutting and seedlings for 10 minutes before planting.

Soil treatment

Mixed 1 kg of *Trichoderma* formulation (conidial suspension) in 100 kg of farmyard manure and cover it for 7 days with polythene. Turn the mixture in every 3-4 days interval and then broadcast in the field.

Virulent stage

Fungi often become attenuated (lose virulence or antagonistic characteristics) when maintained on artificial media. Cultural conditions must be identified which retain virulence without increasing production costs. At present little progress has been made in this area partly because the underlying mechanisms for attenuation have not been elucidated.

***Trichoderma* formulations**

For any crop protection agent, an efficient formulation is essential to translate laboratory activity into adequate field performance. Little progress has been made in this area despite the fact that formulation will improve the field efficacy of fungal BCAs and expand their market opportunities beyond high value, niche markets. New, more effective formulation components must be found (eg. UV protectants, humectants, carriers, virulence-enhancing factors and stickers). Several important commercial formulations of *Trichoderma* are available in the National and International market in the name of Rot stop, Primastop, Soil Gard (Glio Gard), Soil Gard 12G, Root Shield, Rootgard, Supresivit, Trichopel, Trichoteam, Bio-Trek, Trichoseal, Trichojet, T-22, T-22HB, Binab-T, Trichodex, Trichomax Agrigold, Trigold, Ecosom-TH, Ecosom-TV, Asha TV, Yash Derma, and Trichoderma 2000 (Burges, 1998; Butt and Copping, 2000; Butt *et al*., 1999; 2001).

Improved targeting

Cost- effective control of pests, weed and diseases demands efficient targeting of the BCAs. Recently, it has been shown that honey bee-mediated delivery of the insect pathogen, *Metarhizium anisopliae* and increased pollen beetle control (*Meligethes* spp.) in oil seed rape (Butt *et al*., 1998). The bees were more efficient than conventional sprayers in delivering the inoculum to the pest infested flowers. There is also evidence that the use of systems that attract insects to a trap that contains a fungal entamopathogen where they are contaminated allowing them to take the BCA to other members of the species is also showing promise particularly for communal insects. "Push-pull" pest control strategies entail insect pests being driven out of the cash crop with the application of feeding deterrents and being drawn into a trap crop where they are controlled by inundation with pathogens. To encourage pests into the trap crop, lures such as favoured plant varieties (*i e.* those more attractive than the crop) and chemical attractants (sex hormones and gustatory stimulants) would be used. Feeding attractants incorporated into formulations may be useful to encourage insects to feed on BCAs. As yet, very few in expensive but effective lures and deterrents have been developed for commercial use.

Biocontrol mechanisms of *Trichoderma* spp.

Antagonistic microorganisms, such as *Trichoderma* species, reduce growth, survival or infections caused by soil-borne phytopathogenic fungi by different mechanisms like mycoparasitism, antibiosis and competition and rhizosphere competence.

(A) Mycoparasitism

One of the mechanism involved in the antagonistic activity of *Trichoderma* spp. against a range of economically important pathogens is the mycoparasitism (Dennis and Webster, 1971b), where the production of fungal cell wall-degrading enzymes by *Trichoderma* spp. is believed to play a role. It was shown that extracellular lytic enzymes, β-1, 3-glucanases, chitinases and proteases excreted by *Trichoderma* spp. were involved in cell wall degradation of plant pathogens. *Trichoderma* directly attacks the plant pathogen by excreting lytic enzymes such as β-1, 3-glucanases, chitinases and proteases (Haran *et al.*, 1996 a; Hjeljord and Tronsmo, 1998). Because the skeleton of fungal cell wall contains chitin, glucan and protein, enzymes that hydrolyze these components have to be present in a successful antagonist in order to play a significant role in cell wall lysis of the pathogen (Lorito *et al.*, 1994; Carsolio *et al.*, 1999). β-1, 3-glucanases play in nutritional role in saprophytes and mycoparasites (Sivan and Chet, 1989). Several chitinolytic enzymes have been reported in *T. harzianum* (De la Cruz et al., 1992). These include endochitinases, exochitinases and 1, 4- β-N-acetylglucosaminidases. Enzymatic degradation of chitin is generally involved in many biological processes, such as autolysis (Vessey and Pegg, 1973), morphogenesis and nutrition (Griffin, 1994), and in addition to

Table1. Use of different *Trichoderma* species in disease management of crop plants caused by soil-borne phytopathogenic fungi

***Trichoderma* species**	**Crop plant diseases**	**Phytopathogenic fungi**
T. harzianum	Southern stem blight of soybean	*Sclerotium rolfsii*
T. koningii	White rot disease of onion roots	*Sclerotium cepivorum*

T. harzianum	Fusarium wilt of tomato	*Fusarium oxysporum f. sp. lycopersici*
T. harzianum	Fusarium wilt & corm rot of gladiolus	*Fusarium oxysporum f. sp. gladioli*
T. viride	Pigeon pea wilt	*Fusarium udum*
T. lignorum	Damping-off of bean	*Rhizoctonia solani*
T. viride	Cotton seedling disease	*Rhizopus oryzae*
T. virens	Brown blotch disease of cowpea	*Colletotrichum truncatum*
T. harzianum	Rotting of common vegetables	*Sclerotium rolfsii*
T. harzianum	Sunflower head rot	*Sclerotinia sclerotiorum*
T. harzianum	Collar rot of lentil	*Sclerotium rolfsii*
T. harzianum	Root rot of blackgram	*Macrophomina phaseolina*

mycoparasitism plays also a role in relationships between fungi and other organisms such as plant-fungus interactions (Mauch *et al*., 1988). In addition to chitin and glucans, fungal cell wall contains proteins. Thus, the production of proteases also plays an important role in lysis of cell wall of fungal pathogen during mycoparasitism (Flores *et al.*, 1997).

(B) Antibiosis

This mechanism involves the secretion of both volatile and non volatile anti-microbial metabolites, which are portable in nature and thereby suppressing or killing the fungal pathogen (antibiosis) around the surrounding area (Corley *et al*., 1994; Horvath *et al*., 1995). Dennis and Webster (1971a) found that many isolates of *Trichoderma* spp. produce non volatile antibiotics, which were active against a range of pathogen. Biological activity of antagonistic fungi may partially be associated with production of antibiotic (Estebarian *et al.,* 2000; Faull *et al*., 1994). The production of antibiotics; Trichodermin (Godtfredsen and Vangedal, 1965), ergokonin (Kumeda *et al*., 1994), viridin (Chet *et al*., 1977; Grove *et al*., 1996) and viridin fungin A, B and C (Harris *et al*., 1993) by *Trichoderma* spp. have been reported. The principal functions of these antibiotics are cell membrane disruption, inhibition of metabolic activity and stimulation of plant defense system.

(C) Competition and Rhizosphere Competence

If mycoparasitism and antibiosis are not the principal mechanisms in the biocontrol process, what is? One mechanism that has against adherents in recent years is that of competition through rhizosphere competence. Rhizosphere competence is important because a biocontrol agent cannot compete for space and nutrients if it is unable to grow in the rhizosphere. *Trichoderma* species, either added to the soil or applied as seed treatments, grow readily along with the developing root system of treated plant (Harman, 2000). Although competition through rhizosphere competence may not be among the principal mechanisms that driven biological control, it is certainly a valuable adjunct to those that do. One concept that is associated with competition and rhizosphere competence, the replacement of endogenous fungi on the root surface (Harman, 2001), can be difficult to demonstrate. *Trichoderma* species are often able to suppress the growth of endogenous fungi on an agar medium and therefore make their presence.

Conclusion

Fungal plant diseases are a major concern in agriculture food production worldwide. Soil-borne phytopathogenic fungi viz., *Pythium*, *Fusarium*, *Rhizoctonia*, *Phytopthora*, *Sclerotinia*, *Sclerotium* and *Macrophomina* attack most of the economically important crop plants resulting in heavy losses. So, there is a pressing need to manage fungal diseases to ensure a steady and constant food supply to ever increasing world population. The development of such a global system for sustainable food production is one of the greatest challenges faced by humans. Conventional practice to overcome this problem has been the use of chemical fungicides which have adverse environment effects causing health hazards to humans and other non-targeting living beneficial organisms.

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