# TRICHODERMA SPP.: A BIOCONTROL AGENT FOR SUSTAINABLE MANAGEMENT OF PLANT DISEASES

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#### Abstract

*Trichoderma* spp. are mainly asexual fungi that are present in all types of agricultural soils and also in decaying wood. The antagonistic activity of *Trichoderma* species showed that it is parasitic on many soil-borne and foliage pathogens. The fungus is also a decomposer of cellulosic waste materials. Recent discoveries show that the fungi not only act as biocontrol agents, but also stimulate plant resistance, and plant growth and development resulting in an increase in crop production. The biocontrol activity involving mycoparasitism, antibiotics and competition for nutrients, also induces defence responses or systemic resistance responses in plants. These responses are an important part of *Trichoderma* in biocontrol program. Currently, *Trichoderma* spp., is being used to control plant diseases in sustainable diseases management systems. This paper reviews the published information on *Trichoderma* spp., and its biocontrol activity in sustainable disease management programs.

## Introduction

Plant disease management as well as improvement of yields using traditional methods such as chemical pesticides, herbicides, or fertilizer are not an ecofriendly approach, as they consist of various aromatic groups or methylated and ethylated substances which to a large extent have extreme effects on the environment. Long term using of chemical pesticides contaminate water, cause atmosphere pollution, and some-times leave harmful residues which can lead to development of certain resistant organisms. To overcome these problems researchers look for alternative options such as the use of bicontrol agents (BCA) for disease control either alone or in an integrated approach with other chemicals for ecofriendly and sustainable methods of disease control. Currently, several biocontrol agents have been recognized and are available as bacterial agents for example Pseudomas, Bacillus, and Agrobacterinum, and as fungal agents such as Aspergillus, Gliocladium, Trichoderma, Ampelomyces, Candida, and Coniothyrium (Papavizas, 1985; Koumoutsi et al., 2004; Mavrodi et al., 2002; Atehnkeng et al., 2008; Gilardi et al., 2008). Among these biocontrol agents *Trichoderma* spp. is one of the most versatile biocontrol agents which has long been used for managing plant pathogenic fungi. A previous study found that the disease of seed rot, damping off, root rot of sunflower and mugbean caused by Sclerotium rolfsii was prevented as well as the plant growth was enhanced when plants were treated with the conidial suspensions of Trichoderma spp. (Yaqub & shahzad, 2008). The pathogenic fungal growth of Ganoderma was inhibited by T. harzianum and T. virens (Naher et al., 2012).

*Trichoderma* spp., are typical anaerobic, facultative and cosmopolitan fungi that can be found in large numbers in agricultural soils and in other substrates such as decaying wood (Samuels, 1996; Irina & Christian, 2004). They belong to the subdivision Deuteromycetes, members of which do not have or do not exhibit a determinate sexual state as most strains are adapted to an asexual life cycle (Harman, 2004a). The role of *Trichoderma* spp. is not only to control growth of pathogenic microbes, but there are various other uses for *Trichoderma* such as, i) stimulate colonization of rhizosphores, (ii) stimulates plant growth, root growth, and (iii) enhance plant defence responses (Vinale *et al.*, 2008; Harman, 2004a).

In the early 1930s Trichoderma was introduced as possessing biocontrol ability (Weindling, 1934). Trichoderma is an opportunistic, avirulent plant symbiont fungus which acts as an antagonistic and parasitic fungus against many plant pathogenic fungi and offers protection from phytopathogenic plant diseases. It has been proven in numerous studies that Trichoderma spp. are effective biocontrol agents for managing plant disease, and currently commercial products of Trichoderma are available as biopesticides or soil amendments or as enhancers for plant growth (Papavizas, 1985; Chet 1987; Harman, 2004a; Vinale et al., 2008). Weindling (1932) demonstrated the biocontrol activity of Trichoderma lingnorum (viride) on soil-borne fungal pathogen of Rhizoctonia solani. Later the mycoparsitic action of the same species of Trichoderma on Phytophthora, Pythium, Rhizopus and Sclerotium rolfsii was observed (Well, 1988). Today fungal biocontrol observation and foundation are based on Trichoderma spp. and thus, this fungus has drawn much attention as a biocontrol model (Chet, 1993).

This review paper highlights information on the mechanisms of *Trichoderma* biocontrol activity and its role as a plant health enhancer, its commercial production and applications.

**Biocontrol mechanisms of** *Trichoderma* **spp.:** *Trichoderma* spp. are biocontrol agents effective against fungal phytopathogens. They can act indirectly, by competing for nutrients and space, modifying environmental conditions, or promoting plant growth and plant defensive mechanisms and antibiosis, or directly, by mechanisms such as mycoparasitism (Papavizas, 1985; Howell, 2003; Vinale *et al.*, 2008). The mechanisms can be described as:

**i.** Biocontrol by competition for nutrients and living space: *Trichoderma* spp., are rapidly growing fungi that have persistent conidia and a broad spectrum of substrate utilization. They are very efficient competitors for nutrition and living space (Hjeljord *et al.*, 2000). In addition, *Trichoderma* spp., are naturally resistant to many toxic compounds, including herbicides, fungicides, and phenolic compounds. Therefore, they can grow rapidly and impact pathogens by producing metabolic compounds that impede

spore germination (fungistasis), kill the cells (antibiosis), or modify the rhizosphere, (e.g. by acidifying the soil so that the pathogens cannot grow) (Benitez *et al.*, 2004). Starvation is the most common cause of death for microorganisms, so competition for limited nutrients is especially important in the biocontrol of phytopathogens. Iron uptake is essential for filamentous fungi and under iron starvation; fungi excrete low-molecular weight ferriciron-specific chelators, termed siderophores. *Trichoderma* spp. produce highly efficient siderophores that chelate iron and stop the growth of other fungi (Benitez *et al.*, 2004). Therefore, soil characteristics influence *Trichoderma* as a biocontrol agent.

ii. Biocontrol by mycoparasitism: The direct interaction between Trichoderma and pathogen is called mycoparasitism. As mentioned earlier, Weindling (1932) was the first to recognize that Trichoderma spp., is a biocontrol agent and at the same time he also noticed mycoparasitism of T. lignorum (viride) hypae coiling and killing R. solani (Wells, 1988). Mycoparasitism is a complex mechanism that generally involves the production of a cell wall lytic enzyme. Chet et al., (1998) described that the mycoparasitism process involves four sequential steps: chemotropism and recognition; attachment and coiling; cell wall penetration; and digestion of host cell. Trichoderma strains detect other fungi, grow straight towards them, and sequentially produce hydrolytic cellwall degrading enzymes. Trichoderma attach to the host. and coil hyphae around the host, form appressoria on the host surface, penetrate the host cell, and collapse the host hyphae (Steyaert et al., 2003).

The molecular level induction of mycoparasitism was first reported in 1994 (Carsolio *et al.*, 1999), based on the study of regulation of an endochitinase-encoding gene (ech42). Ech42 was expressed during the mycoparasitic interaction between *T. harzianum* and *Rhizoctonia solani*. Another study showed that in the P1 mutant strain of *T. atroviride*, the expression of exochitinase *nagI* or endochitinase *ech42* gene was needed to induce mycoparasitism in treatments containing purified colloidal chitin from the fungal cell walls (Vinale *et al.*, 2008). Production and regulation of lytic enzymes such as chitinases, glucanases, and proteases by *Trichoderma* spp. also play key roles in the mycoparasitism/biocontrol process (Mukherjee *et al.*, 2008).

**Plant growth enhancement by** *Trichoderma* **spp.:** *Trichoderma* spp. are not only control pathogens, they also enhance plant growth and root development (biofertilizer) and stimulate plant defence mechanisms (Harman, 2004a). Some *Trichoderma* strains have been shown to penetrate the epidermis and establish robust and long-lasting colonization of root surfaces (Harman, 2004a). *Trichoderma* spp. have been shown to improve growth of lettuce, tomato and pepper plants (Vinale *et al.*, 2006). In a study of maize plants, several months after treatment with *Trichoderma harzianum* strain T-22, the plant roots were about twice as long when compared to untreated plants (Harman, 2004a). Cutler (1986, 1989) showed that the secondary metabolites produced by *Trichoderma koningii* (koniningin A) and *Trichoderma harzianum* (6-pentylalpha pryone) act as plant growth regulators. *Trichoderma* spp. also produced gluconic and citric acids, decreased the soil pH, and enhanced the solubilization of phosphates, micronutrients, and mineral components such as iron, magnesium, and manganese (Benitez *et al.*, 2004; Harman *et al.*, 2004b; Vinale *et al.*, 2008).

Induction of plant defence by Trichoderma spp.: It is well documented that Trichoderma spp. induce gene expression of proteins in plants such as chitinase, glucanase, and peroxidase against antagonistic microbes (Yedidia et al., 2003; Hanson et al., 2004; Harman, 2004b). It has also been shown that pre-treatment of plants with Trichoderma spp. increased plant resistance to pathogen attack (Harman, 2004a). Trichoderma spp. are opportunistic invaders, fast growers and large spore producers. They contain cell wall degrading enzymes (e.g., celluloses, chitinases, and glucanases) and produce antibiotics (Vinale et al., 2008). Moreover, the presence of Trichoderma spp. stimulates the induction of the hypersensitive response, systemic acquired resistance (SAR), and induced systemic resistance (ISR) in plants (Benitez et al., 2004; Vinale et al., 2008). For example, tomato plants colonized by T. hamatum actively induced systemic changes in plant physiology and disease resistance (Alfano et al., 2007). In a study of cucumber plants, T. asperellum induced a systemic response of two defence genes encoding phenylalanine and hydroperoxidase lyase and systemic accumulation of phytoalexins against Pseudomonas syringae pv. lachrymans (Yedidia et al., 2003). In oil palm plants the defence gene of chitinase expression was increased in T. harzianum and Ganoderma boninense treated plants compared to G. boninense alone treated plants (Naher et al., 2011). Several studies also showed that Trichoderma spp. may indirectly contribute to systemic resistance (Ahmed et al., 2000; Lo et al., 2000). Harman et al., (2004a) reported that the induction of localized or systemic resistance is an important component for plant disease control by Trichoderma spp. Thus, disease control by root-colonizing Trichoderma spp. involves a complex interaction between the host plant, the pathogen, the biocontrol agent and several environmental factors (Harman, 2004a; Hoitink, et al., 2006; Alfano et al., 2007).

Plant root colonization by Trichoderma spp.: Studies of the early invading fungi Trichoderma spp. showed that root colonization stimulated plant defence responses such as induction of peroxidases, chitinases,  $\beta$ -1, 3 glucanase, phenylalanine, and hydroperoxidase lyase; activated signaling of biosynthetic pathways; and caused accumulation of low-molecular weight phytoalexins (Howell et al., 2000; Yedidia et al., 2003; Harman et al., 2004a;). Yedidia et al., (1999) observed the physical interaction between T. harzianum T-203 and a cucumber plant under the electron microscope and found that the fungus penetrated the root and grew in the epidermis and outer cortex, which stimulated increases of peroxidase and chitinase. Therefore, the interaction appears to be a symbiotic relationship in which Trichoderma lives in the nutritional niche provided by the plant, and the plant was protected from disease.

Production of antibiotics and secondary compounds by Trichoderma spp.: Secondary compounds and antibiotics produced by Trichoderma spp. play a vital role in antagonistic biocontrol activity (Vinale et al., 2008; Ajitha & Lakshmidevi, 2010). Sivasithamparam and Ghisalberti (1998) reported that Trichoderma spp. produced several secondary compounds, including antibacterial and antifungal antibiotics such as polyketides, pyrones, and terpenes. Secondary metabolites, including antibiotics, that are not directly involved in natural growth, development, or reproduction and are chemically different from natural compounds may play important roles in the defence response, symbiosis, metal transport, differentiation, and stimulating or inhibiting spore formation and germination (Demain & Fang, 2000; Vinale et al., 2008). Antibiotics are often associated with biocontrol activity. For example, the production of a pyrone-like antibiotic from T. harzianum exhibited biocontrol activity against Ganumannomyces graminis (Ghisalberti et al., 1990). The peptide antibiotic paracelsin was the first secondary metabolite characterized in Trichoderma spp. (Bruckner & Graf, 1983; Bruckner et al., 1984). Sivasithamparam & Ghisalberti (1991) suggested that secondary metabolites produced by Trichoderma spp., can be grouped into three categories: (i) volatile compounds (e.g., 6-pentyl-alpha-pyrone), (ii) water-soluble compounds (e.g., heptelidic acid), and (iii) peptaibol compounds, which are linear oligopeptides composed of 12-22 amino acids that are rich in alpha-aminoisobutyrate, N-acetylated at the Nterminus and have an amino alcohol group at the C-terminus.

Other uses of Trichoderma spp.: The discovery of cellulase production by Trichoderma reesei, which was isolated by Reese (1976), led to it becoming a very important cellulase or enzyme producer. The cellulase produced by Trichoderma spp. is used mainly for malting, baking, and grain alcohol production (Galante et al., 1998b). The filamentous cellulolytic Trichoderma spp., produce a broad range of cellulases and hemicellulases. The main application of lignocellulosic biomass is the production of biofuels such as ethanol (Lin & Tanaka, 2006: Gimbert et al., 2008), although it is also used in the pulp, paper and textile industries (Galante et al., 1998a). Trichoderma spp. are also used for safe industrial enzyme production (Nevalaines et al., 1994). Macerating enzymes are used to improve the brewing process for fruit juice production and as a feed additive for livestock and pet food (Schaster & Schmoll, 2010). Trichoderma also used for seed germination for example, a study showed that sunflower seeds germination significantly increased in T. viride or T. resei treated plants compared to control plants (Anis et al., 2012). The commercial use of several Trichoderma species for the protection and growth enhancement of a number of crops is ongoing (Lumsden et *al.*, 1993; Samuels, 1996). Currently, the commercially available formulations are RootShield<sup>TM</sup>, BioTrek 22<sup>TM</sup>, T- $22G^{TM}$ , and T-22HB<sup>TM</sup> (Bio-works, USA); Suprevisit<sup>TM</sup> (Borregaard BioPlant, Denmark); Binb<sup>TM</sup> (Bio-Innovation Sweden); Trichopel<sup>TM</sup>, Trichojet<sup>TM</sup>, Trichodowels<sup>TM</sup>, and Trichoseal<sup>TM</sup> (Agimm, New Zealand); Tricco<sup>TM</sup> (Ecosense Labs, India), and Tricho-green (Mycology Lab, Malaysia). Not all of these products are registered as biocontrol agent, but they are marketed as plant growth promoters, plant strengtheners, or soil conditioners.

## Conclusion

In 1930, Weindling first discovered the genus *Trichoderm* spp. as a biocontrol agent and since then numerous studies have demonstrated that *Trichoderma* is an effective bicontrol agent for phytopathogenic microorganisms (Harman, 1996). A biocontrol program is only established when the bicontrol agent can successfully manage the interaction between the host plant and pathogen. The ability of *Trichoderma* to successfully manage this interaction has been well established. The fungi have also been demonstrated to enhance the defence responses in plants. Thus, as an effective biocontrol agent the use of *Trichoderma* will certainly ensure sustainable disease management.

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