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TRICHODERMA AS POTENTIAL BIOCONTROL AGENT, ITS EXPLOITATION IN AGRICULTURE: A REVIEW

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ABSTRACT

The novel technologies in all areas of agriculture have improved agricultural production, but some modern practices affect the environment. The recent challenge faced by advanced farming is to achieve higher yields in an environment-friendly manner. Thus, there is an immediate need to find eco-friendly solutions. Among various types of species being used as biocontrol agents, *Trichoderma* is widely used as biocontrol agent against different kinds of plant pathogens. *Trichoderma* spp. are asexual fungi that are present in all types of agricultural soils and also in decaying wood. The hostile activity of *Trichoderma* species showed that it is parasitic on many soil-borne and foliar plant pathogens. Recent studies showed that this fungus not only acts as biocontrol agent but also stimulates plant resistance, plant growth and development resulting in an increase in crop production. The antagonistic activity involves mycoparasitism, antibiotics, competition for nutrients and also induces systemic resistance in plants. Currently, *Trichoderma* spp. are being used to control plant diseases in sustainable disease management system. This paper reviews the already published information on *Trichoderma* as biocontrol agent, its biocontrol activity and its commercial production and application in plant disease management programs.

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INTRODUCTION

The term 'biocontrol or biological control' was introduced for the first time in 1914 by Tubeuf and Smith in 1919 with special concern to plant pathogens and insects respectively. Biocontrol refers to the reduction in plant pest population by naturally occurring organisms that are part of integrated disease management. Biocontrol agent of plant pathogens known as antagonist inspires development and research work in many fields to meet the needs of rising human population by managing the pest. These antagonistic microorganisms belong to various groups of fungi and bacteria while plant pests include plant pathogens, weeds, and insects.

A diversity of biocontrol agents (BCAs) or bio-fungicides are present in the ecosystem and there is need to isolate for bringing into play because BCAs have a low cost of production, long lasting effect on the growth of pathogen and no effect on human health. Virtually, all plant pathogens of plant diseases are subjected to biological control or natural microorganisms having antagonistic effect that are already present in soil and environment. Only between 1-10% microbes show the ability to inhibit pathogen growth *in vitro* when fungal and bacterial isolates tested for biocontrol activities. Some of these have the ability to suppress plant pathogen under *in vivo* favorable conditions while few have broad-spectrum

activities against miscellaneous pathogens texa. Some important microbes belonging to different genera that are currently being marketed worldwide for promoting trend of organic farming among farmers by reducing the use of chemical pesticides include *Agrobacterium, Ampelomyces candida*, *Bacillus*, *Coniothyrium*, *Pseudomonas* (Haas and Défago, 2005), *Streptomyces* and *Trichoderma*. These BCAs interact with plant and pathogens that suppress the pathogen growth by direct and indirect mechanisms involved in the working of antagonist include hyperparasitism, competition, induction of host resistance, production of antibiotics (Phenazines, 2,4-diacetyle phloroglucinol), lytic enzymes (Chitinases, proteases, glucanases), non regulatory waste products (Hydrogen cyanide, ammonia, carbon dioxide) and physical/chemical interference (blockage of soil pores) that leads to biocontrol of plant parasitic pathogens (Pal and Gardener, 2006).

***Trichoderma* as biological control agent:** In the early 1930s, *Trichoderma* was firstly reported as biocontrol agent (Weindling, 1932) and species of genus *Trichoderma* are free-living and cosmopolitan fungi in soils, decaying organic and vegetable matter (Harman et al., 2004a). *Trichoderma* species are successful antagonists having biocontrol abilities against economically important plant parasitic soil-borne pathogens and present abundantly in almost all type of soils (Kushwaha and Verma, 2014; Olabiyi and Ruocco, 2013; Shahid et al., 2014). Biocontrol antagonists played important role in the management of plant diseases and parasitic microorganisms (Alwathnani and Perveen, 2012; Hajieghrari et al., 2008; Zhang et al., 2013). *Trichoderma* attacked other plant pathogenic fungi and promotes plant and root growth. It uses different mechanisms for the control of plant pathogenic pathogens including antibiosis, mycoparasitism, the induced resistance of host cell and competition for nutrient and space. Species of *Trichoderma* can control and antagonize broad range of economically important postharvest phytopathogenic fungal pathogens and plant-pathogenic fungi as well as also control bacteria and viruses (Harman, 2006; Yedidia et al., 2003). Significant information on nutrition of *Trichoderma* are available in literature but very little is well-known about specific carbon and nitrogen nutrients on mass production of *Trichoderma* antagonists (Rajput et al., 2014). Generally, it is considered aggressive competitor that rapidly colonizes the pathogen especially soil-borne pathogens such as *Fusarium* spp., *Phytophthora* spp. and *Rhizoctonia* etc.

***Trichoderma* biology:** Mycoflora belonging to genus *Trichoderma* usually cosmopolitan shows a high level of genetic diversity and frequently found in varying habitats (Grinyer et al., 2004; Samuels, 2006; Zhang et al., 2007). *Trichoderma* species have been easily isolated from natural soil, decaying plant organic matter and wood and is classified as imperfect fungi belonging to order Hypocreales of Ascomycota (Howell, 2003; Küçük and Kivanç, 2003). *Trichoderma* multiplies and grows very fast in different nutrient sources such as Malt Agar (MA), Czapek Dox Agar (CDA) as well as Potato Dextrose Agar (PDA) and produces conidia/spores of various shades characterized by green color (Chaverri et al., 2003; Rey et al., 2001) and some species produce thick walled chlamydospores (Lu et al., 2004). The salient feature of this genus is the ability to parasitize other pathogenic fungal mycoflora specially associated with root rot and wilt diseases (Santoro et al., 2014; Verma et al., 2007). *Trichoderma* species have been reported as endophytic fungi while generally found in all types of soils such as agricultural soil, orchard soil as well as forest soil as opportunistic plant symbionts (Chaverri et al., 2011) and usually considered successful competitor of plant pathogens (Kim et al., 2012; Woo et al., 2006).

Morphological characteristics: Morphological based identification of *Trichoderma* species is a primary method of identification that is not a precise method to differentiate diversity between species (Zhang et al., 2005). *Trichoderma* species are fast growing under the optimum range between 25-30 ºC (Latifian et al., 2007). *Trichoderma* used a variety of compounds such as carbon and nitrogen sources as a growth medium for its sporulation (Gao et al., 2007; Seyis and Aksoz, 2005) and sporulates of *Trichoderma* abundantly produce powder masses characterized by green conidia (Chaudhari et al., 2011) which is diagnostic tool that is also found in related and unrelated genera such as *Myrothecium*, *Clonostachys* and *Aspergillus* as well as *Penicillium* respectively (Alvindia and Hirooka, 2011). Conidiophore is not well defined but mostly branched contains unicellular conidia and phialides at the tip of branched hyphal system that cannot be seen on one week old media (Lu et al., 2004). Generally, conidia shape is ellipsoidal to oblong and some *Trichoderma* species have globose to subglobose with the length/width ratio 1.4 and 1-3 respectively (Bissett et al., 2003; Jaklitsch et al., 2006) while few species have smooth conidia

(Samuels et al., 2002). Conidia color morphology varies from species to species but typically green or may be gray, white and yellow (Jaklitsch et al., 2006).

***Trichoderma* ecology:** *Trichoderma* is usually widely distributed and ubiquitous in almost all types of soils (Olabiyi and Ruocco, 2013; Röhrich et al., 2013; Singh et al., 2014) and found on decaying bark and plant root surface when damaged by phytopathogens (Brotman et al., 2013; Samolski et al., 2011). Species of this genus are fast growing saprophytes that can be detected by coconut smell due to volatile compound such as 6-pentyl-2pyrone and competitors for nutrient as well as space (Tsai et al., 2008). They comprise up to 3.1% and 15% of total fungal propagules from forest and pasture soil respectively (Hagn et al., 2003). Climatic conditions affect distribution of *Trichoderma* species. For instance, *T. harzianum* favors warm climate while *T. viride* and *T. polysporum* characteristic of cool temperature (Sarhy-Bagnon et al., 2000). Similarly, *T. citrinoviride* has been reported in South East Asia but not found in India (Zhang et al., 2005). In general, *Trichoderma* is more ubiquitous in acidic soil condition (Carreras-Villasenor et al., 2012) while *T. pseudookoningi* and *T. hamatum* show more tolerant behavior with excessive moisture as compared to other species (Kumar, 2007).

Effect of environment on *Trichoderma*: The environmental and nutritional parameters play an important role in enhancing mycelial growth and biomass production of *Trichoderma* species and growth and multiplication of biocontrol agents varies with the substrates (Romero-Arenas et al., 2012). The maximum mass production of *Trichoderma* for commercialization product against soil-borne phytopathogen mainly relies on its physiological and environmental factors such as temperature, pH, light, nitrogen and carbon sources (Jayaswal et al., 2003).

Species of *Trichoderma* spp. are reported to be more sensitive to light and nutrient media (Steyaert et al., 2010) and *Trichoderma* conidial response not restricted to light. Light affects many pathways of *Trichoderma* including oxidative stress, development, vegetative growth, sulfur and carbon mechanism and reproduction that involves in the signaling process. Today, BLR-1 and BLR-2 are known to be photoreceptor-orthologs and light regulatory protein ENVOY that regulates expression of cellulase gene established connection between nutrient signaling and light response in *Trichoderma* (Schmoll et al., 2009). Sun emits radiation of various wavelengths that initiates photochemical reactions and wavelength response of photo-conidiation lies within blue or UV spectrum that describes the fungus belonging to blue-light fungi. *Trichoderma* spp. light regulator proteins BLR-1 and BLR-2 are the key regulators of this response (Casas-Flores et al., 2004). *Trichoderma* can sporulate and grow on a variety of artificial nutrient media but not all of them. Aslam et al. (2010) compared cellulase activity of *Trichoderma* spp. and different fermented media with carbon sources for the production of cellulase. *Trichoderma* exhibited maximum mycelial growth on glucose culture but no production of cellulase enzyme was observed. Macroscopic characteristic like mycelial growth and development rate of *Trichoderma* spp. on yeast complete medium (YCM) and potato dextrose agar (PDA) adjusted with sodium hydroxide (SQ) and industrial chemical products (PQIND) at approximately 7, 9 and 11 pH and resulted that development rate of *Trichoderma viride* strain CP-50 and *Pleurotus ostreatus* strain CP-T4 at pH 11.2 were 0.41 mm/day and 6.10 mm/day respectively. *T. viride* development and growth rate was negative in an alkaline medium (Romero-Arenas et al., 2012). Compared mycelial growth, biomass yield and conidia production of *T. harzianum, T. longibrachiatum* and *T. viride* checked on different nutrient media including PDA, Waksman Agar, Agar Agar, Corn Meal Agar, Czepak's Agar and tested their efficacy by dual culture technique against seed borne pathogens such as *Alternaria alternata, Botryodiplodia theobromae, Fusarium moniliforme, F. oxysporum, F. solani* and *Rhizoctonia solani*. Maximum mycelial growth inhibition of these pathogenic fungi was noted by *T. harzianum* and PDA medium was the best for biomass and spore production of *Trichoderma* species (Mustafa et al., 2009). All microorganisms growth including *Trichoderma* species is influenced by environmental parameters that affect antagonistic potential of biocontrol agents. Among the environmental parameters pH is the most important factor that affects mycelial growth of *Trichoderma*. Onilude et al. (2013) described effect of cultural and environmental parameter such as carbon (Mannitol, wheat and rice bran), nitrogen (peptone, $NaNO_3$ and NH_4SO_4) sources and pH as well as temperature respectively on sporulation and growth of *T. viride* under batch and fed-batch culture. Mycelial growth was good at the temperature range of 30-37ºC while sporulation was favored by 30-45ºC. Moreover, maximum growth and sporulation of *T. viride* was observed at 4.5-6.0 pH. The influence of pH on biomass and mycelial growth

demonstrated that acidic pH is the most important key factor for biomass production of all *Trichoderma* species (Singh et al., 2014).

da Silva et al. (2012) evaluated temperature and pH effects on Trichoderma spp. like *T. harzianum*, *T. polysporum, T. koningii and T. viride for chitosanase production under solid state fermentation. T. viride, T. harzianum and T. polysporum, T. koningii showed optimum chitosanase activity at 5.0 and 5.5 pH respectively with maximum chitosanase with enzymes production about* 1.4 IU/gds from *T. polysporum* followed by *T. viride* (~1.2 IU/gds) and *T. harzianum* (1.06 IU/gds). Temperature between ranges of 40-50 ºC did not affect the activity of the enzyme.

Inam-Ul-Haq et al. (2009) scrutinized *Fusarium oxysporum* survival on chickpea plants under the three parameters such as moisture, temperature and *Trichoderma* spp. when *Trichoderma* spore suspension incubated on chick pea branches at temperature 25 and 35ºC in sandy clay loam soil for the period of 4 months with different water potential -0.03 MPa, -0.3 MPa and less than -50 MPa then resulted that maximum *F. oxysporum* growth reduced in moist soil as compared to others water potentials. The survival of *F. oxysporum* was 100% and completely eradicated in air-dry and moist soil respectively after 6 months but up to 10-12% killed by antagonist at 35 ºC on branches chickpea plant.

Efficacy of *Trichoderma* spp. against pathogenic microflora: Species of *Trichoderma* are reported as ubiquitous and cosmopolitan soil-borne Ascomycetes (Singh et al., 2014) that reproduce asexually which are present in almost all types of soil habitats and other environment such as manure, decaying plant tissues, and wood. It produces as discrete colonies with various shades of green or white conidia and colony color from the reverse side of Petri plate often uncolored, yellow, amber, buff, and yellow green. Some species can be identified from their aroma which include *T. atroviride*, *T. lignorum* and *T. fertile* produces odor of coconut and mold respectively (Gams and Bissett, 2002). Conidia become mature after initial production of conidia within 7 to 14 days thus branching pattern and conidiophore morphology is critical for identification. *Trichoderma* used as biological control agent and have the ability to compete with other parasitic and saprophytic pathogens (Table 1) specially soil-borne fungi due to metabolic activities and competitive nature that make them decomposer of herbaceous and woody material (Elad, 2000) in which sexual perfect stage (*Teleomorph* genus *Hypocrea*) has frequently found (Harman et al., 2004b). *Trichoderma* readily obtained by Multiple Tube Dilution Technique (MTDT) due to its formation of chlamydospores and colonization of organic substrates (Khandelwal et al., 2012).

Importance of Trichoderma: The perspective character of *Trichoderma* species as biological control of fungal plant pathogens was first introduced in the early 1930s and later on research indicates that it can control effectively foliar, seed and specially soil-borne pathogenic fungi belong to various genera (El-Mohamedy and Alla, 2013; Gveroska and Ziberoski, 2012).

Trichoderma species are opportunistic, avirulent, and plant symbionts that can compete as well as survive in the complex ecosystem (Harman et al., 2004b). Although, these are capable of successful root colonizer and their number increases when abundant healthy roots are present in the ecosystem (Brotman et al., 2008) and protect the roots and plants from pathogens as well as diseases (Howell, 2003). They increase plant resistant ability against drought conditions and promote the growth of a plant by phosphate, micro-nutrients, and solubilization (Kumar, 2013). Some species of *Trichoderma* are efficient producer of extracellular enzymes that degrade complex compounds of polysaccharides and also used commercially (Samanta et al., 2012). *Trichoderma* species are environmental friendly (Singh et al., 2008) and an alternative to synthetic chemicals (Gupta and Dikshit, 2010) that developed symbiotic relationship with plants rather than parasitic relationship reduced chances of behavioral changes in human caused by the use of synthetic chemicals (Brimner and Boland, 2003).

Plant growth enhancement by *Trichoderma* specie: *Trichoderma* spp. not only controlled pathogens, they also enhance plant growth and root development (biofertilizer) and stimulate plant defense mechanisms (Harman et al., 2004b). Some *Trichoderma* strains have been shown to penetrate the epidermis and establish robust and long-lasting colonization of root surfaces. *Trichoderma* spp. has been shown to improve growth of lettuce, tomato, and pepper plants (Vinale et al., 2008). In a study of maize plants, several months after treatment with *T. harzianum* strain T-22, the plant roots were about twice as long when compared to untreated plants. *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 (Vinale et al., 2008).

Table 1: Efficacy of *Trichoderma* species against soil-borne fungal pathogens.

Trichoderma strains	Pathogen(s)	Plant/ Crop	Disease	Efficacy (Inhibition)	Experiment	Reference
T. harzianum TH-H-3 *T. virens* TV-K-3	*Rhizoctonia solani*	Tomato	Wilt	5 %	Pot Exp.	Kumar (2013)
T. harzianum *T. viride*	*Fusarium solani*	Tomato	Root rot	70-72%	*In vitro*	Haggag and El-Gamal, 2012
T. harzianum *T. viride*	*R. solani*	Tomato	Damping off	51% 39%	*In vitro*	Haggag and El-Gamal (2012)
T. harzianum Mutants	*R. solani*	Tomato	Damping off	40% in greenhouse 100% in field	Greenhouse and field	Montealegre et al. (2010)
T. viride (Tv-R)	*M. phaseolina*	Chickpea	Dry root rot	62%	laboratory conditions	Manjunatha et al. (2013)
T. harzianum *T. viride* *T. virens*	*R. bataticola*	Mungbean	Dry root rot	87%	Pot and field conditions	Dubey et al. (2009)
T. harzianum T22	*Pythium ultimum*	Tomato	Wilt	74%	*In vitro*	Mastouri et al. (2010)
T. harzianum T-22	*F. verticillioides*	Maize	Ear and kernel rot	65% reduce size of necrotic area		Ferrigo et al. (2014)
T. harzianum *T. viride*	*F. oxysporum* f. sp. *Ciceris*	Chickpea	Chickpea wilt	44-60%	Field exp.	Dubey et al. (2007)
T. harzianum	*F. oxysporum* f. sp. *Radicis cucumerinum* *Botrytis cinerea*	Cucumber *Arabidopsis thaliana*	Stem and root rot	12-79%	Pots experiments	Alizadeh et al. (2013)
T. viride	*F. oxysporum* f. sp. *adzuki*	Soybean	Root rot	-	*In vitro*	John et al. (2010)

	Pythium arrhenomanes		Damping off			
Trichoderma spp.	*Phytophthora cactorum*	Strawberry	Leather rot	88% in 2001 97.6% in 2002 99.0% in 2003	Field Exp.	Porras et al. (2007)
T. harzianum *T. viride* *T. virens* T. *koningii* *T. pseudokoningii*	*Alternaria tenuissima*	Sorrel	Leaf spot	67-76% 78-80% 72-77% 77-80% 72-80%	*In vitro*	Ambuse et al. (2012)
	M. phaseolina		Charcoal rot	72%		
T. harzianum 1	*F. solani*	Cotton	Wilt and boll rot	71%	*In vitro*	Asran-Amal et al. (2010)
	R. solani		Boll rot and leaf spot	58%		
T. atrovirde *T. Longibrachiaum* *T. virens* *T. hazianum*.	*F. sambucinum*	Potato	Potato dry rot	*T. longibrachiatum* showed the strongest inhibition	*In vitro*	Ru and Di (2012)
T. harzianum	*A. alternate*	Tobacco	Brown spot	Diffusible metabolites more effective than volatile diffusible	*In vitro*	Gveroska and Ziberoski (2012)
	B. cinerea,		Grey mould	35-44% on fruit and 43-64% on stem		
T. harzianum T39	*Pseuperonospora cubensis*, *Sclerotinia sclerotiorum*	Cucumber	Downy mildew white mould	48-78% 64 on fruit and 30-35% on stem,	Greenhouse	Elad, 2000
	Sphaerotheca fusca		Powdery mildew	45-71 %		

T. hazianum.	*R. solani*	Bean	Root rot	Reduced disease severity and protect seedlings from pre-emergence damping-off	Greenhouse conditions	Júnior et al. (2007)
T. hazianum.	*F. oxysporum* f. sp. *lycopersici*	Tomato	Tomato wilt	44.4%	*In vitro* and pot conditions	Alwathnani and Perveen (2012)
T. hazianum	*Botryodiplodia theobromae* *F. solani*	Yam	Root rot	53-84% 59-87%	*In vitro*	Okigbo and Emeka (2010)
T. hazianum BHU-51 *T. hazianum* BHU-105	*S. Sclerotiorum*	Brinjal	Damping-off	38-44%	Field Exp.	Singh and Singh (2012)
T. hazianum	*F. solani* *F. oxysporum* *R. solani* *Pythium spp.* *Sclerotium rolfsii*	Green bean	root rot	58-100% 56-100% 46--100% 42--100% 40-100%	*In vitro* and greenhouse	El-Mohamedy and Alla (2013)
T. harzianum (Th azad) *T. viride* (01 PP)	*F. oxysporum udum*	Pigeon Pea	Wilt	58% 60%	*In vitro*	Shahid et al. (2014)
T. hazianum *T. viride*	*F. oxysporum*	Banana	Wilt	1.4cm inhibition zone 1.0cm inhibition zone	*In vitro*	Thangavelu et al. (2004)
T. hamatum	*F. oxysporum*	Lentil	Vascular wilt	33%	Glasshouse	El-Hassan et al. (2013)
T. hazianum	*F. oxysporum* *R. solani*	Chickpea	Root rot and wilt	Significantly inhibit growth of phytopathogens	*In vitro*	Verma et al. (2014)

T. virens GL3 and GL21	*Pythium ultimum* and *R. solani*	Cucumber	Damping-off	Most effective in greenhouse Most consistent & effective in growth chamber	Field and greenhouse	Roberts et al. (2005)
T. hazianum ITEM 3636 *T. longibrachiatum* ITEM 3635	*F. solani*	Peanut	Brown root	*T. harzianum* effective than *T. longibrachiatum* in decreasing mean disease severity index and boosting yield	Field exp.	Rojo et al. (2007)
T. harzianum Th908	*F. oxysporum* Fo2797	Tomato	Wilt	15-35%	*In vitro*	Marzano et al. (2013)
T. harzianum	*F. udum*	Pigeon pea	Wilt	Soil application of *T. harzianum* more effective than seed treatment	Field exp.	Prasad et al. (2002)
T. citrinoviride	*S. sclerotiorum*	Soybean	Stem Rot (White Mold)	96%	*In vitro*	Thakkar and Saraf (2014)
	M. pheseolina		Charcoal rot			
T. atroviride *T. koningiopsis* *T. asperellum* *T. spirale* *T. brevicompactum* *T. longibrachiatum*	*S. sclerotiorum*	-	-	70% germination promoted by *T. asperellum* Th034, *T. atroviride* Th002 and *T. harzianum* Th203	*In vitro*	Smith et al. (2013)
T. harzianum	*F. solani*			33% by volatile metabolites and showed highly efficient antagonism		
	R. solani			41% by volatile metabolites and showed highly efficient antagonism		

	S. sclerotiorum			66% by volatile metabolites and showed highly efficient antagonism		
	F. solani	-	-	32% by volatile metabolites and showed highly efficient antagonism		
	R. solani			33% by volatile metabolites and showed highly antagonism		
T. ghanense						
	S. sclerotiorum			33% by volatile metabolites and showed efficient antagonism		
	F. solani			71% by volatile metabolites and showed highly efficient antagonism		
T. asperellum	*R. solani*			66% by volatile metabolites and showed highly efficient antagonism	*In vitro*	Qualhato et al. (2013)
	S. sclerotiorum			17% by volatile metabolites and showed highly efficient antagonism		
	F. solani			28% by volatile metabolites and showed efficient antagonism		
T. tomentosum	*R. solani*			52% by volatile metabolites and showed highly efficient antagonism		
	S. sclerotiorum			0% by volatile metabolites and showed efficient antagonism		
T. harzianum	*S. delphinii*	Cotton				

T. atroviride			Seed rot and seedling rot	*T. harzianum* exhibited highest disease suppression	*In vitro* and greenhouse	Mukherjee et al. (2013)
T. hazianum *T. asperellum*	*R. solani*	Sugar beet	Damping-off	1.88-3.04% increase in healthy seedlings as compared to control	*In vitro* and Greenhouse	Kakvan et al. (2013)
T. viride *T. harzianum* *T. aurepviride*	*F. moniliforme*	Maize	Stalk rot	*T. harzianum* showed maximum antagonism and seed germination	*In vitro* and *In vivo*	Harleen and Chander (2011)
T. viride	*Colletotrichum capsici*	Chilli	Fruit rot	58%	*In vitro*	Sangeetha et al. (2011)
T. harzianum	*F. oxysporum* f. sp. *lycopersici*	Tomato	Wilt	5-7.5%	*In vitro*	Sriram et al. (2010)
	Phytophthora capsici	Chilli	Damping-off	50%		
T. harzianum	*A. porri*	Onion	Purple blotch	73%	*In vitro* under greenhouse	Abo-Elyousr et al. (2014)
T. longibrachiatum				70%		
T. viride	*R. solani*	Tomato	Root rot and wilt	80%	*In vitro*	Hafez et al. (2013)
	F. oxysporum			77%		
	F. verticilloid			67%		
	A. alternata			80%		
	Mucor racemosus			40%		
T. harzianum *T. pseudokoningii*	*A. porri*	Onion	Onion blotch	73-75% inhibition of mycelial growth 71-73% inhibition of mycelial growth	*In vitro*	Imtiaj and Lee (2008)
T. virens				75% inhibition of mycelial growth		
T. viride	*Fusarium sp.*	-	-	40-45%	*In vitro*	

	Curvularia sp.			38-50%		
	Aspergillus niger			41%		Reena et al. (2012)
	Rhizopus			41%		
	Aspergillus flavus			45%		
	Aspergillus fumigates			39-50%		
T. viride				73%		
T. harzianum				71%		
T. longibrachiatum	*S. rolfsii*	Sugarbeet	Damping-off	53%	*In vitro*	Paramasivan et al. (2013)
T. reesei				52%		
T. koningii				55%		
T. harzianum						
T. harzianum T100	*F. oxysporum* and *F. proliferatum*	-	-	Antagonistic activity of *Trichoderma* spp. was more on *F. proliferatum* than on *F. oxysporum*.	*In vitro*	Ghanbarzadeh et al. (2014)
T. viride						
T. hamatum						
T. harzianum	*F. solani* f. sp. Melongena and *F. oxysporum* f. sp. *lycopersici*	Brinjal and Tomato	Wilt	*T. Longibrachiaum* showed 100% inhibition than others	*In vitro*	Enespa and Dwivedi (2014)
T. atroviride						
T. Longibrachiaum						
T. harzianum T-39	*Plasmopara viticola*	Grapevine cultivars	Downy mildew (D.M)	Reduced D.M symptoms, but degree of efficacy differed among cultivars	Field experiment	Banani et al. (2013)

Plant root colonization by *Trichoderma* spp.: Studies of the early invading fungi *Trichoderma* spp. showed that root colonization stimulated plant defense responses such as induction of peroxidases, chitinases, β-1, 3 glucanase, phenylalanine, and hydroperoxidase lyase; activated signaling of biosynthetic pathways; and caused accumulation of low-molecular weight phytoalexins (Yedidia et al., 2003).

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.

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. 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 although it is also used in the pulp, paper and textile industries. *Trichoderma* is also used for safe industrial enzyme production. Macerating enzymes are used to improve the brewing process for fruit juice production and as a feed additive for livestock and pet food. *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. The commercial use of several *Trichoderma* species for the protection and growth enhancement of a number of crops is ongoing (Samuels, 2006). Currently, the commercially available formulations are RootShield TM, BioTrek 22 TM, T- 22G TM, and T-22 HBTM (Bio-works, USA); Suprevisit TM (Borregaard BioPlant, Denmark); Binab TM (Bio-Innovation Sweden); Trichopel TM, Trichojet TM, Trichodowels TM, and Trichoseal TM (Agimm, New Zealand); Trieco TM (Ecosense Labs, India), and Tricho-green (Mycology Lab, Malaysia). Not all of these products are registered as a biocontrol agent, but they are marketed as plant growth promoters, plant strengtheners, or soil conditioners.

Interaction of *Trichoderma* with other microorganisms and plants: Fungi from the genus *Trichoderma*, due to their colonization of different environments, are forced to compete for nutrients and space with many other organisms. The mechanisms facilitating colonization of different ecological niches are well-developed and highly diverse in *Trichoderma* spp. (Vinale et al., 2008).

Hyperparasitism is connected with the direct contact of an antagonist with a pathogen and is composed of such stages as pathogen recognition, attack, gradual penetration of the pathogen cells and death (Vinale et al., 2008). In this process, a considerable role is played by CWDE (Cell Wall Degrading Enzymes) lytic enzymes, synthesized by *Trichoderma* species that facilitate hydrolytic degradation of pathogen cell walls, composed of chitin and glucan polysaccharides. *Trichoderma* species are also capable of producing cell wall degrading enzymes such as cellulase, xylanase, pectinase, glucanase, lipase, amylase, arabinase, and protease as well as many volatile metabolites, such as 6-n-pentyl-2H--pyran-2-one (6-PAP). Chitinases are the most important lytic enzymes playing a key role in the degradation of cell walls of other plant pathogenic fungi. Other enzymes determining the capacity of *Trichoderma* fungi for hyperparasitism, mainly in relation to fungus-like organisms, i.e. *Phytophthora* sp. and *Pythium* sp. are *β*-1, 3- and *β*-1, 6-glucanases. Cellulases form yet another group of enzymes produced by the *Trichoderma* species. These enzymes are capable of hydrolyzing lignocellulose biomass and comprise three types of enzymes which act synergistically.

When considering the interactions of *Trichoderma* fungi with plants, it was found that these fungi have an advantageous effect on plants. Stimulation of plant growth and yield takes place thanks to this interaction and the advantageous effects are seen in the production of vitamins, the increased availability of biogenic elements (nitrogen, phosphorus), the mobilization of nutrients from the soil and organic matter, and the enhanced intensity of mineral uptake and transport. Furthermore, *Trichoderma* fungi are capable of producing zeaxanthin and gibberellin, i.e. compounds accelerating seed germination. Many *Trichoderma* strains produce acids, e.g. gluconic, citric, and coumaric acids, causing the release of phosphorus ions and microelements, which subsequently become available to plants (Harman et al., 2004a).

Application of *Trichoderma* in biological plant protection: *Trichoderma* fungi are microorganisms that have been most frequently tested and applied in biological plant protection. The use of *Trichoderma* fungi may cause a considerable limitation in the use of chemical fungicides in agriculture (Akhtar et al., 2012). It is estimated that 90% of all the antagonistic fungi used in plant protection belong to the genus *Trichoderma*. Recent studies showed the potential of *Trichoderma* spp. to control stem canker of brassicas, severity greatly depending on avirulence genes in fungi *vs.* resistance genes in plants. The species of *Trichoderma* significantly differed in their hyperparasitic effects towards pathogen.

Prospects for the application of *Trichoderma*: Increased ecological awareness of whole societies and growing interest in alternative sources of energy make it possible to use fungi from the genus *Trichoderma* in the production of the so-called second generation biofuels (Schuster and Schmoll, 2010). The development of an adequately high efficiency of this process to ensure its economic viability poses a serious challenge for

researchers. Fungi from the genus *Trichoderma* may also be applied in modern plant cultivation technologies, in which considerable emphasis is placed on the environmental impact.

***Trichoderma* as a pathogen of humans:** Apart from the beneficial species used for human needs, the genus *Trichoderma* also comprises species which are highly dangerous to human health (Druzhinina et al., 2008). The pathogenic species include *Hypocrea orientalis*, genetically close but clonal *Trichoderma citrinoviride* Bis- set as well as *T. harzianum*, and *T. longibrachiatum,* with the prevalence of the first two closely related species. They constitute a lethal hazard for individuals with reduced resistance, including patients with leukemia, HIV- positive or having transplants (Kredics et al., 2003). Infections caused by *Trichoderma* are typically diagnosed late and are difficult to treat, as these fungi exhibit a low sensitivity to commonly applied antifungal drugs (Kratzer et al., 2006) and combined treatment is frequently necessary (Alanio et al., 2008).

Genomes of *Trichoderma*: Contemporary techniques allow one to sequence and compare whole genomes of different organisms, including fungi. In recent years three species of *Trichoderma* (*Hypocrea*); *T. reesei* (*H. jecorina*), *T. atroviride* (*H. atroviri- dis*), and *T. virens* (*H. virens*) have been sequenced, and the results are publically available. The smallest genome size (34 Mb) was found in the weakly mycoparasitic *T. reesei*. The largest genome (38.8 Mb) was one of the highly parasitic *T. virens* (Mukherjee and Kenerley, 2010). The genome of *Trichoderma* was of intermediate size (36.1 Mb). Similarly to some fungi, such as *Neurospora crassa* (Irelan et al., 1994) or *L. maculans* (Fudal et al., 2009), the genomes of *Trichoderma* contain fragmented transposable elements, called Repeat Induced Point (RIP) mutations. However, the comparative genomics have also revealed great differences between the genomes of *Trichoderma* and even closely related fungi, such as *Giberella zeae*. These are differences in respect to the decreased number of repetitive DNA sequences and numerous unique genes or gene families.

Studies on the expression of some genes produced by *Trichoderma* have proved difficult, as their activity may be connected solely with defense against other microbes or multicellular organisms (Osbourn, 2010). Brakhage and Schroeckh (2011) suggested some strategies to activate silent gene clusters by cultivating fungi in conditions that simulate competition and allow the usual biosynthetic pathways to be initiated. A detailed metabolomic-genomic study is suggested for elucidating the roles of the numerous gene products of *Trichoderma* (Mukherjee et al., 2012).

Tools for genetic manipulation of *Trichoderma*: Due to industrial application of *Trichoderma*, the genetic tool kit for this fungus is the most extensive of the genus, although also research with other species is not limited by technical obstacles and most tools can also be used for all species with slight modifications. Transformation of many species is possible, and different approaches such as Agrobacterium-mediated transformation (Zeilinger, 2004), or biolistic transformation were developed. The range of selectable marker cassettes, which includes hygromycin and benomyl resistance (Schuster et al., 2007), the *Aspergillus nidulans* and S gene, which enables growth on acetamide as sole nitrogen source as well as the auxotrophic markers, pyr4, arg2, and hxk1 allows for construction of multiple mutants, which is now facilitated by the availability of a *Trichoderma* spp. strain with perturbed non homologous end-joining pathway (Guangtao et al., 2009; Guangtao et al., 2010). Sequential deletions despite a limited number of selection markers became possible by the use of a blaster cassette comprising direct repeats for homologous recombination and excision of the marker gene (Hartl and Seiboth, 2005). Besides knockout strategies for functional analysis of genes, also expression of antisense constructs for knockdown (Rocha-Ramírez et al., 2002; Schmoll et al., 2009) was reported for *Trichoderma*, and RNAi has been shown to function in *Trichoderma* (Brody and Maiyuran, 2009). Last but not least, the recent discovery of a sexual cycle in *Trichoderma* spp. (Seidl et al., 2009) further boosts the versatility of this fungus for research and industry.

Mechanisms of *Trichoderma*: The most important and fascinating feature of *Trichoderma* is the study of mechanisms varying for management of phytopathogens and plant diseases in which pathogen antagonized by biocontrol agent results from different types of interaction between organisms (Pal and Gardener, 2006). The followings are direct and indirect biocontrol mechanisms to control plant pathogens (Figure 1).

Direct Antagonism

Direct antagonism results in the physical contact of biocontrol agent with the pathogen. It includes;

Mycoparasitism: Mycoparasitisim or hyperparasitism is

a complex process (Harman et al., 2004b) which involves the parasitic interaction of two or more fungi in which one parasitize mycelia of other (Druzhinina et al., 2011) and species of *Trichoderma* parasitize a wide range of mycoparaites especially soil-borne pathogens (Hajieghrari et al., 2008). In this process, firstly *Trichoderma* species sense the pathogen and come into contact with host involves morphological changes such as coiling and appressorium formation which developed hole on the surface of host or target pathogen (Omann and Zeilinger, 2010). Secondly, *Trichoderma* species recognize signals from host fungus that activate penetration of *Trichoderma* hyphae into the lumen of target parasitized fungus (Kubicek and Druzhinina, 2013). Thirdly, active multiplication takes place inside the hyphae of target fungi. *Trichoderma* and pathogen attachment mediated by binding of carbohydrates and lectin that are present in *Trichoderma* cell wall and target pathogen respectively.

Interestingly, *Trichoderma* extracellular enzymes are produced during the penetration process that inhibited the hyphael growth of the target pathogen. It is believed that a variety of chitinolytic enzymes and *ß*-1,3-glucanases are key enzymes in mycoparasitism (Kamala and Devi, 2012) of *Trichoderma* species that has shown great potential against phytopathogens. In the recent studies, more than 1100 strains of *Trichoderma* have been found to be mycoparasite from molecularly defined 75 species (Druzhinina et al., 2011).

Mixed- path Antagonism

Antibiosis and secondary metabolites: *Trichoderma* species cause decay of phytopathogenic fungi without any physical contact between microorganisms by producing the antimicrobial compounds. This process generally called as "antibiosis" and term secondary metabolites is a group of heterogeneous chemically divergent natural compounds might be associated with survival functions such as symbiosis, differentiation, and competition against organisms etc. for the producing organism (Wu et al., 2014). Antibiotics are natural products having the ability to inhibit target pathogen growth correlated with biocontrol activity. Antibiosis occurs during the interaction process of *Trichoderma* strains with pathogenic microbes involving antibiotics or low molecular diffusible compounds that retard the growth of pathogen (Reino et al., 2008). Most of the *Trichoderma* species produced volatile and non-volatile metabolic compounds including tricholin, massoilactone, heptelidic acid, gliovirin, 6-penthyl-α-pyrone, harzianic acid, glisoprenins, peptaibols, alamethicins, and others have been studied (Qualhato et al., 2013) which are toxic to target pathogen. The synergetic effect of antibiotics and hydrolytic enzymes achieve maximum level of antagonism rather than alone mechanism (Monte, 2001). *T. harzianum* and *T. virens* are the most effective biocontrol agents with respect to antibiotics that produce gliovirin and pyrone respectively.

Indirect Antagonism

Competition: Malnourishment is the foundation of death for every living organism. Competition is a phenomenon in which *Trichoderma* species and pathogen compete for limited nutrient and space availability. *Trichoderma* is generally considered as an aggressive competitor against soil-borne fungal pathogens that grow very fast towards pathogen and rapidly colonize it (Cuervo-Parra et al., 2014). Nutrients competition linked to soil rhizosphere and competition for infection sites appear inside or on roots of the plant. In most of the filamentous fungi, iron uptake is necessary for viability and most fungi produced low molecular ferric iron specific chelators called as siderophores under iron starvation that mobilize environmental iron (Eisendle et al., 2004). Species of *Trichoderma* have more ability to mobilize soil nutrients and their take up as compared to other microbes. Among all the mechanisms, nutrient competition is the most important (Verma et al., 2007) that prevent infection from the pathogen.

Induced systemic host resistance: Induction of host resistance in the plant is a complex mechanism. Generally, there are three pathways to induce resistance in the host plant. Two of these involve direct assembly of pathogenesis-related (PR) proteins that can be induced by the mechanism of other organisms. In one pathway PR proteins production results by an attack of pathogenic microbe while in other pathway PR proteins production as a result of necrosis or wound i.e. herbivory by insects. In the third type pathway of resistant induced by root associated with non-pathogenic bacteria such as rhizobacteria referred to as Rhizobacteria-Induced Systemic Resistance (RISR).

Pathogen-related induced pathway and herbivory pathway depends upon salicylic acid and jasmonic acid that are signaling molecules produced by plant respectively and their exogenous application induces a similar response as are produced in naturally produced molecules (Bostock et al., 2001). The pathway linked with

jasmonate referred to as induced systemic resistance that is relatively different in the process initiated by rhizobacteria. Both pathways (Jasmonate- and salicylate-induced pathways) characterized by cascade of pathogenesis-related (PR) proteins production that include antifungal enzymes including chitinases, thaumatins, glucanases and oxidative enzymes. The triggering molecules produced in *Trichoderma* response are still unknown (Druzhinina et al., 2011) that results in direct accumulation of phytoalexins or PR proteins referred to as Systemic Acquired Resistance (SAR).

The third kind of resistant induced by nonpathogenic rhizobacteria associated with roots of the plant described as rhizobacteria-induced systemic resistance (RISR) that is phenotypically similar to salicylic and jasmonate-induced resistant and functionally different results reduction of plant disease by systemic resistant. However, the Induced systemic resistance elicited by some important *Trichoderma* spp. mentioned in Table 2.

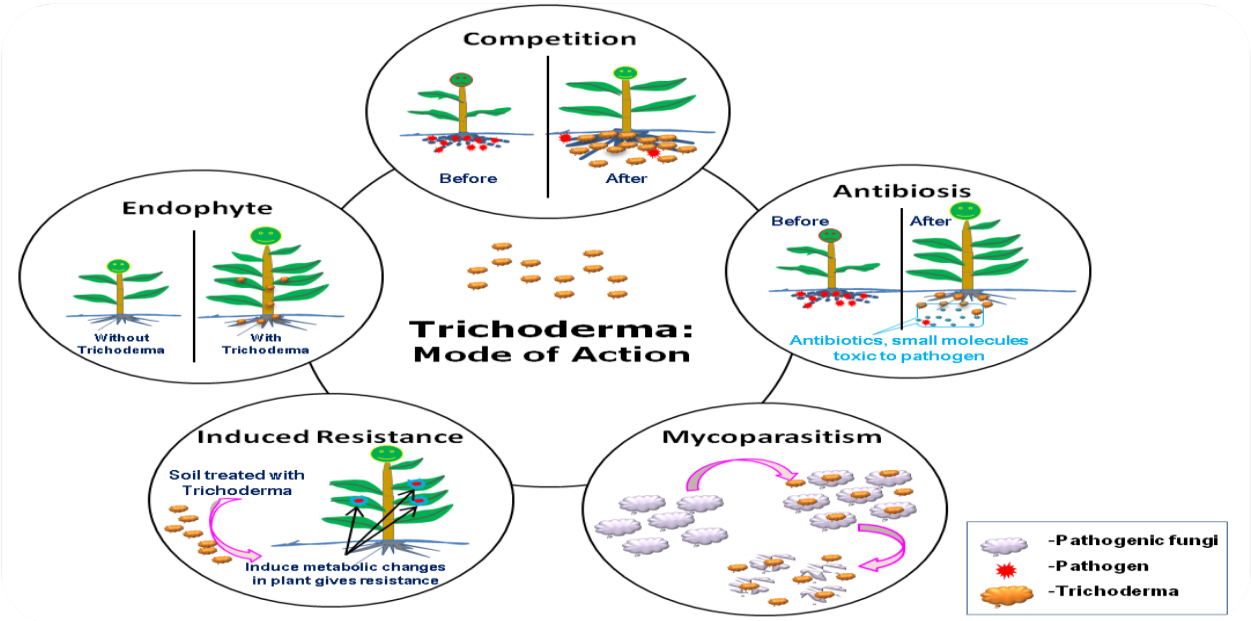

Figure 1. Model depicting mode of action of Trichoderma spp. against pathogen and plant growth improvement.

Table 2. Induced systemic resistance elicited by some important *Trichoderma* spp.

Species	Plant species	Pathogens	Outcome	References
T. virens	Cotton	*Rhizoctonia solani*	Protected plant by inducing terpenoid phytoalexins toxic to fungi	Kumar et al. (2009)
T. harzianum	Pepper	Phytophthora capsici	Improved production of the phytoalexins capsidiol toxic to pathogen	Ahamed and Vermette (2009)
T. virens	Tomato	*Pseudomonas syringe*	Secreted proteins-Sm1 and Ep11 both induced systemic acquired resistance	Salas-Marina et al. (2015)
T. asperellum	Cucumber	*Pseudomonas syringe*	Modulated the expression of proteins related to jasmonic acid/ethylene signaling	Shoresh et al. (2005)

Endophytic activity: Endophytic activity of many microorganisms (growth inside plant tissue without any harm) may useful to host plant by stimulating of plant growth, a postponement to the beginning of drought stress and the obstruction to pathogens. Endosymbiotic species are capable of establishing colonies in plant roots and triggers the expression of many plant genes affecting stress responses. Recently, there are reports showing *Trichoderma* isolates acting as endophytic plant symbionts in some woody plants (Chaverri et al., 2011).

Interestingly, strains forming an association with roots are altering the gene expression pattern in shoots. These changes are the key points in altering plant physiology and this can be exploited in the improvement of many important traits like uptake of nitrogen fertilizer, abiotic/ biotic stress resistance, and photosynthetic efficiency leading to higher yields (Harman et al., 2012). Phylogenetic analysis classifies all known endophytic species as a separate taxon with the exception of *T. koningiopsis, T. stilbohypoxyli* and *T. stromaticum* within their clades at terminal position suggesting endophytism is not an old trait but recently evolved in *Trichoderma* species (Druzhinina et al., 2011).

Efficacy of *Trichoderma* spp. against soil-borne mycoflora: Biological control of phytopathogens is a prospective non-chemical way for disease control. *Trichoderma* species found to be most effective biocontrol under greenhouse and field conditions against many plant diseases caused by soil-borne pathogens (Srinivasa and Devi, 2014) and their efficacy highly depend upon physiological and environmental parameters such as temperature (Colussi et al., 2012), pH (Romero-Arenas et al., 2012), growth medium (Blaya et al., 2013), light (Schmoll et al., 2009), carbon and nitrogen sources (Onilude et al., 2013). However, hyphal establishment, growth and biocontrol potential of *Trichoderma* greatly depend upon the biotic component interaction of various agricultural soil and all climatic zones (Bae and Knudsen, 2005) and *Trichoderma* isolates reported as successful biocontrol that retard growth of soil fungi (Keswani et al., 2014).

***Trichoderma* as a protector of plant health:** The beneficial action of *Trichoderma* spp. is not limited to fighting pathogens; they have also been shown to be opportunistic plant symbionts, enhancing systemic resistance of plants (Shoresh et al., 2010), a response which is improved by ceratoplatanin family proteins (Djonović et al., 2006). Perception of the signals transmitted by *Trichoderma* in the plant requires the function of a MAPK and also in the fungus itself, a MAPK signaling is crucial for full induction of systemic response in the plant (Viterbo et al., 2005). By colonizing plant roots, which is significantly enhanced by swollenin (Brotman et al., 2008) or invading them, they are also carried through soil and occupy new niches. This interaction with plants as well as their rhizosphere competence leads to enhanced root proliferation, better growth, and protection of the plants against toxic chemicals, against which *Trichoderma* spp. themselves show a remarkable resistance. Hence, these fungi are promising agents that can be applied for remediation of polluted soil and water by treatment of appropriate plants with spores (Harman et al., 2004a).

Food Industry: With their long history of safe industrial scale enzyme production, *Trichoderma* spp. has also been extensively applied for production of food additives and related products (Blumenthal, 2004). Currently, various *Trichoderma* enzymes are applied to improve the brewing process (β-glucanases), as macerating enzymes in fruit juice production (pectinases, cellulases, hemicellulases), as a feed additive in livestock farming (xylanases) and for pet food. Cellulases are mainly applied in baking, malting, and grain alcohol production. However, not only enzymes but also metabolites of *Trichoderma* spp. are used as additives. One of the first products isolated from *T. viride* was a chemical with characteristic coconut-like aroma, a 6-pentyl-α-pyrone with antibiotic properties, the production of which was constantly improved to reach concentrations of more than 7 g/L in extractive fermentation cultures in *T. atroviride* nowadays (Oda et al., 2009). An interesting idea is the application of cell wall-degrading enzymes, for example of *T. harzianum*, as food preservatives because of their antifungal effect, but so far this suggestion has not found broad application. With a similar aim, *T. harzianum* mutanase can be used in toothpaste to prevent accumulation of mutan in dental plaque (Wiater et al., 2005).

Commercialization of *Trichoderma* products: Commercialization of *Trichoderma* or biocontrol agents depends upon the screening process of biocontrol microorganism and its efficacy against pathogenic mycoflora. The first species of *Trichoderma* (*Trichoderma harzianum*) registered with EPA in 1989 for control of plant pathogens and diseases (Fravel, 2005).

Commercialization of biocontrol products is a multi step process and includes (Table 3):

a) Isolation of microorganisms
b) Evaluation of antagonists in lab and field conditions
c) Selecting best isolate in field conditions
d) Mass production
e) Formulation
f) Delivery
g) Compatibility
h) Registration and release

Methods of application *Trichoderma* species

Seed treatment: Seed treatment is also known as seed priming used for multiple purposes on many crops to provide inexpensive cover against wilting and rotting of planted seeds by soil-borne fungi such as *Rhizoctonia*, *Sclerotinia,* and *Macrophomina* species. For this purpose, mix 10 grams *Trichoderma* formulation for 1 kg of seed to per liter of cow dung slurry before sowing especially for pulses and cereal crops. *Trichoderma* multiply, reproduce and move towards the root of germinating seed where it fixes nitrogen and increases nutrients, various toxic metals and metabolites uptake and (Harman, 2006) root colonization by *Trichoderma* species promote root growth and increases resistant to abiotic stresses. *Trichoderma* seed treatment enhance chances of germination, vigor index and defense mechanism of the plant (Harman et al., 2004b).

Table 3. *Trichoderma* based commercial products against various diseases.

Commercial Product/ Trade name	*Trichoderma* species	Target disease	Company/ Manufacturer or distributor
Anti-Fungus	*Trichoderma* spp.	Root rot	Grondoontsmettingen De Ceuster, Belgium
Binab	*Trichoderma* spp.	Root rot and wilt	Binab, Sweden
Biofungus, Superesivit	*Trichoderma* spp.	Root rot and wilt	Bioplant, Denmark
Root Pro	*T. harzianum*	Root rot	Efal Agri, Israel
Root Shield, Plant Shield, T-22 Planter box	*T. harzianum* T-22	Root rot	Bioworks, Geneva, USA
T-22B, T-22G	*T. harzianum T-22*	Root rot	TGT Inc. New York, USA
T35	*T. harzianum*	Wilt	Makhteshim-Agan Chemical Israel
ECO T/T22	*T. harzianum*	Root rot	PHP Ltd., South-Africa
GlioGard and SoilGard	*T. virens*	Root rot	Grace-Sierra Co. Maryland
Harzian 20, Harzian 10	*T. harzianum*	Wilt	Natural Plant Protection, Noguerres, France
Biospark Trichoderma	*T. parceramosum T. pseudokoningii*	Wilt	Biospark Corporation, Phillipines
F-stop	*T. harzianum*	Root rot	Eastman Kodal Co. TGT Inc., New York, USA
Soil Gard	*T. virens* GL-21	Root rot	Certis, USA
Tricho-X	*T. viride*	Root rot	Excel Industries Ltd., ,India
Trichodex	*T. harzianum*	Grey mold	Makhteshim Chemical Works, Israel
Trichopel	*Trichoderma* spp.	Root rot	Agrimm Technologies, New Zealand
Bip T	*T. viride*	Wilt	Poland
Trieco	*T. viride*	Root rot, wilt	Ecosense Laboratories, India
Pant biocontrol agent-1	*T. harzianum*	Root rot, wilt	Deptt. of Plant Pathology, GB plant University of Agriculture & Technology, Panatnagar, Uttarakhand
Plant helper	*T. atroviride*	Root rot	Ampac, California

Biobus 1.00WP	*T. viride*	Root rot	Nam Bac, Vietnam
Biocon	*T. viride*	Root rot, wilt	Tocklai Experimental Station Tea Research Association, India
TRICO-DHCT	*Trichoderma* spp.	Sheath blight	Can Tho University, Vietnam
Defense SF	*T. viride*	Wilt	Wockhardt Life Science Ltd., India
NLU-Tri	*T. virens*	Wilt	Ho Chi Minh University of Agriculture and Forestry, Vietnam
Ecoderma	*T. viride+ T. harzianum*	Root rot and wilt	Morgo Biocontrol Pvt. Ltd., India
Bio – Humaxin Sen Vàng 6SC	*Trichoderma* spp.	Cottony rot	An Hung Tuong, Vietnam
Trichogourd	*T. viride*	Damping off	Anu Biotech international Ltd. India
Promot PlusWP and Promot PlusDD	*Trichoderma* spp. *T. koningii* *T. harzianum*	Damping off, Root rot and wilt	Tan Quy
Vi-DK	*Trichoderma* spp.	Root rot and wilt	Pesticide Corp.
Fulhumaxin 5.15SC	*Trichoderma* spp.	Root rot	An Hung Tuong, Vietnam
Trichotech	*Trichoderma* spp.	Wilt	Finlay International Kenya Ltd. Dudutech, Laboratory
Ecofit	*T. viride*	Root rot	Hoechest and Schering Agro. Evo. Ltd., India
Funginil	*T. viride*	Root rot	Crop Helath Bioproduct Research Centre, India
Trichopel Trichodowe Trichoject	*T. harzianum +T. viride*	Wilt	Agrimm, Technologies Ltd., New Zealand
Binap- T&W	*T. harzianum+ T. polysporum*	Wilt	Bio Innovation AB, Toreboda, Sweden
Antagon-TV	*T. viride*	Seed and soil-borne diseases	-
Dfence-SF	*T. viride*	Seed and soil-borne diseases	-

Nursery treatment: Young seedlings are more susceptible to disease that leads to the development of the diseased plant. Moist soil during the germination period increases the chances of infection by root rot and wilt fungi. Nursery beds amendment with the application of *Trichoderma* spore suspension or product facilitate management of soil-borne pathogens and protect the crop results significant increase in the yield under field condition (Reglinski and Dick, 2005). Before sowing of the crop, drench nursery bed treated with @ 5kg *Trichoderma* formulation per liter of water (Ranasinghe et al., 2005). Effect of *T. harzianum* on artificially inoculated *Phaeomoniella chlamydospora* on grapevine under greenhouse and nursery trial has been reported that *T. harzianum* level of the necrotic area which was higher in old inoculating nurseries and also increase tolerance in plants against stress condition.

Cutting and seedling root dip: Dipping of plant cuttings and seedling roots in *Trichoderma* suspension is another way of *Trichoderma* application aims to protect seedling and cuttings from pathogen infection. It is done by dipping of cutting and seedling roots before planting for 10 minutes in a mixture of 10 g *Trichoderma* formulation per liter of water.

Soil application: *Trichoderma* is an actively growing fungus and can be applied in the soil and nursery as drench as well as granular form. Soil can also be treated with 1 kg *Trichoderma* formulation mix with 100 kg FYM

and cover it with polythene for 7 days. Turn the position of the mixture after 4-5 days interval and apply in the field.

Application recommendation and precautions

Recommendation: All type of plants and vegetables can be treated with *Trichoderma* for better production such as tomato, potato, pepper, tobacco, sugar beet, sugarcane, brinjal, turmeric, ginger, betel vine, banana, eggplant, cotton, chilies, cardamom, onion, maize, cucumber, peanut, red gram, white gram, Lentil, chickpea, cassava, citrus etc.

Precautions: Some precautionary measures should be kept in mind regarding the application of *Trichoderma* inoculums in the field condition. These are given below:

1. Don't settle treated Farm Yard Manure (FYM) for a longer time.
2. Don't place *Trichoderma* treated seed in direct sunlight.
3. Don't apply chemical pesticides or fungicides after application of *Trichoderma* for 5-6 days.
4. Moisture is an important factor for *Trichoderma* growth and reproduction so don't try to use it in dry soil.

Sensitivity against agrochemicals: The efficiency of the bioagents is hampered due to poisonous nature of fungicides which are used simultaneously in crop production technology. Therefore, the sensitivity and tolerance of *Trichoderma* have been tested by our group and many others (Madhusudhan et al., 2010). The effect of different fungicides together with *Trichoderma* spp. has been studied for integrated disease management. *Trichoderma* spp. have shown greater tolerance for broad spectrum fungicides than many other soil microbes as it has the capacity to colonize the pesticides treated soil more rapidly (Oros et al., 2011). *Trichoderma* alone or their combinations with bacteria or their immobilized formulations can have great potential, as more than a few unusual contaminants can be treated at the same time and will have wider applicability, hence improving the overall cost effectiveness of the technology.

Future prospects: Sustainability is also the major driving force for the investigation of biocontrol with *Trichoderma*. As opportunistic plant symbionts and effective mycoparasites, numerous species of this genus have the potential to become commercial biofungicides. The challenge in this field of research will be the development of reliable screening techniques, which allow for prediction of the biocontrol efficiency of a given isolate by determination of the key factors for this process. Nevertheless, also the ecological effects of the widespread application of a single (or few) fungal species in agriculture remain to be investigated in order to ensure a truly beneficial effect for the environment. *Trichoderma* as biocontrol agent is utmost important part of integrated plant disease management that can be used against soil-borne phytopathogens but its biocontrol potential is yet to be limited to laboratory experiments and very diminutive attention has been paid to its commercial formulation. Moreover, farmers also have lack of information concerning its utilization. So, the concept of *Trichoderma* commercialization needs to be improved and cost-effective production formulation should be popularized. Some biocontrol agents or *Trichoderma* species unsuccessful to compete with phytopathogens that attributed to physiological and environmental parameters influences the effectiveness of BCAs. Thus, molecular tools and genetic engineering need to be performed for improvement of BCAs that can be able to proliferate and compete against a wide range of phytopathogens. So, this is necessary to give the support to agencies that are engaged in this field.

CONCLUSION

Biological control gives the impression of an alternative to chemical-based pesticides for disease suppression and control. Scientists and their research have proved that *Trichoderma* is non-pathogenic to plants and need to be formulated in a way that favors the activity and survival of microbes. Moreover, the novel concept of biocontrol needs a space outside the laboratory to see its fruits in present production systems.

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