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Advent of *Trichoderma* as a bio-control agent- A review

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Abstract: *Trichoderma* spp are free living filamentous fungi. They are cosmopolitan and versatile in nature. They have the potential to produce several enzymes that can degrade the cell wall materials. Also, they release a number of fungi toxic substances that can inhibit the growth of the fungal pathogens. Many mechanisms have been described on how *Trichoderma* exert beneficial effects on plants as a bio-control agent. But due to its versatile nature, its potential cannot be explored to its full extent. And it is a developing science in the field of bio-control with its new discoveries adding to the usefulness of the fungi as a bio-control agent. Its development as a bio-control agent passes through many phases and each phase adding novel ideas that will help in the development of an efficient bio-agent which in turn will help in the crop improvement and disease management. The studies on their various aspects responsible for bio-control will open a flood gate to the development of *Trichoderma* as an efficient and reliable bio-agent and provide a better scope for implementation in crop and disease management. The *in vitro* antagonistic activity of *Trichoderma viride* against phytopathogens (*Sclerotium rolfsii, Fusarium oxysporum* f.s.p. *ciceri, Fusarium oxysporum* f.s.p. *udum*) was studied and it was found to be potentially effective against *F. oxysporum* f.s.p. *ciceri* followed by *F. oxysporum* f.s.p. *udum* and *Sclerotium rolfsii.*

Keywords: Antibiosis, Bio-control agent, Mycoparasitism

INTRODUCTION

Trichoderma are free living fungi that are highly interactive in root, soil and foliar environment can parasitize other fungi (Harman *et al.*, 2004)**.** They are asexual filamentous fungi who share its anamorph with the genus *Hypocreae*. They are facultative anaerobes. They are versatile, highly rhizosphere competent, profuse root colonizers and cosmopolitan in nature. They are also opportunistic avirulent plant symbionts. They are known for prolific production of extracellular proteins and fungitoxic substances. They have been utilized extensively as model microorganisms to analyze and improve the understanding of the role that these antagonistic fungi have been playing in biological interactions, for instance with crop plants and phytopathogens (Marra *et al.,* 2006). They have been gaining momentum as an important biocontrol agent (BCA) in control of plant diseases in recent times due to its eco-friendly nature, minimizing the use of chemicals, giving more cheaper and efficient disease control method. Intensive researches have been going on the complex mechanisms, among which, the most important being induction of host defense besides producing large amount of heterologous proteins, effecting plant metabolism and physiology. Because of the certain beneficial effects, *Trichoderma* has been gaining popularity as one of the most important bio-control agent. Their journey towards bio-control passes through many phases with new discoveries adding to the usefulness of this fungus (Lorito *et al*., 2010).

Discovery of bio-control activity mediated by mycoparasitism: *Trichoderma* spp. is known for their ability to attack and control the plant pathogenic fungi. Mycoparasitism is one of the most important mechanisms that impart bio-control activity. The direct attack of one fungus on another or direct antagonism is known as mycoparasitism (Dix and Webster, 1995). This concept dates back to the work of Weindling (1932) who demonstrated the parasitism of *Rhizoctonia solani* hyphae by the hyphae of *Trichoderma virens* in controlling citrus seedling disease. Other researchers also showed the mycoparasitism of *Trichoderma* spp towards *Pythium ultimum* and *Sclerotium rolfsii* (Papavizas, 1985). Mycoparasitism is a sequential and complex process, involving three steps: chemotrophic growth and recognition; coiling and interaction of hyphae and secretion of specific lytic enzymes (Dix and Webster, 1995).

Chemotrophic growth and recognition: The chemical stimuli released by the target fungus are first sense by *Trichoderma* through their specific signaling mechanism. This mediates the growth of *Trichoderma* towards the target fungi. Apparently, *Trichoderma* releases low levels of extracellular exochitinase induced by the cell-wall oligomers of the target fungi and endochitinase gene is activated when interact with the target fungus. Ultimately, all these action induces the released of fungitoxic cell wall degrading enzymes

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(CWDEs) from *Trichoderma viz.* extracellular β-(1, 3)-glucanase, proteases, lipases and chitinases (Viterbo *et al.*, 2007).

Coiling and interaction of hyphae: As *Trichoderma* established contact, it starts coiling around and form appressoria for attachment mediated by carbohydrate (from the cell-wall of *Trichoderma*) and lectin (from the target fungus) complex (Howell, 2003).

Secretion of specific lytic enzymes: Several fungi-toxic CWDEs and peptaibol antibiotics are produced by the *Trichoderma* induced by the cell wall materials of the target fungus. This induces a cascade of physiological changes within the fungus. Due to action of these CWDES holes are produced in the hyphae of target fungus near the site of appressoria. The antagonistic hyphae grow along the host hyphae and secrete different lytic enzymes such as glucanase, chitinase and pectinase that are involved in mycoparasitism. This ultimately leads to the degeneration of the target fungus (Howell, 2003).

Discovery of bio-control activity mediated by antibiosis: Weindling (1934) proposed "lethal principle". This gave a shift to the concept of mycoparasitism. According to which, besides showing mycoparasitic activity, *Trichoderma* do produced certain lethal factors in the soil that inhibits the growth and the development of the pathogenic fungi. Later, in 1941, he showed that the factor behind the lethal principle is Gliotoxin secreted by *Trichoderma virens.* This led to the development of another mechanism behind the bio-control potential i.e. antibiosis. *Trichoderma* produces low molecular weight diffusible compounds or antibiotics possessing antifungal and antibacterial properties.These substances can penetrate the host cell, inhibit cell wall synthesis of the target fungus (Lorito *et al.*, 1996).They also inhibit the growth, uptake of nutrients, sporulation, production of metabolites of the target fungus (Wilcox *et al.,* 1992; Howell, 1998). Antibiosis is species specific i.e., different strains exhibit different antifungal activity (Howell *et al.,* 1993). Howell and Stipanovic (1983) isolated gliovirin from *T.virens* that inhibited *Pythium ultimum* and *Pythophthora.*

However, Howell (1987) showed that mycoparasitism and antibiosis was not the major mechanisms behind the bio-control activity through his studies on cotton seedling disease incited by *Rhizoctonia solani*. He showed that the mutants deficient in mycoparasitism and antibiosis do showed their potential to control the disease. This again points to the involvement of other mechanisms that drives the bio-control activity.

Discovery of competition as a basis of bio-control: *Trichoderma* are good rhizosphere competent and efficient soil colonizers. This makes them an efficient competitor against other soil microflora. Competition as the basis of bio-control was first reported from the controlled of *Chondrostereum purpureum,* the silver leaf pathogen of plum trees due to competition exerted by the *Trichoderma* colonization (Corke and Hunter, 1979).They can proliferate in the soil and out beat the growth of other soil micro-flora. They can compete for nutrients, space, water or oxygen against other soil micro-flora. They have the capacity to mobilise and take up soil nutrients compare to other organisms. Also, they are resistant to a variety of toxins produced by other micro-organisms and other anti-microbial substances produced by the plants due to the presence of ATP-binding cassetts (ABC) transporters that reduce the toxic effects. This enhances the competitive ability against other micro-organisms.Competition for carbon has also been involved in the determination of the antagonism expressed by different strains of *Trichoderma* sp. against several plant pathogens, especially *F. oxysporum* (Sivan and Chet, 1989). Tjamos *et al.* (1992) demonstrated that *T.harzianum* T-35 controls *Fusarium oxysporum* by competing for both rhizosphere colonization and nutrients. Some *Trichoderma* spp produce highly efficient low molecular weight ferric iron specific chelators termed as siderophores to mobilize or chelate environmental iron and stop the growth of other fungi (Chet and Inbar, 1994). Chet *et al.* (1997) reported that starvation of pathogen due to limiting nutrients results in its death. But, cruciality in viewing competion as primary role in bio-control is that *T. koningii*, which is an excellent root colonizer when treated against *R. solani,* there is little or no bio-control (Howell, 2003). These findings again gave an enigma to the Trichoderma researchers on how *Trichoderma* interact with the plant, the target pathogen and its environment.

Discovery of the ability to improve plant resistance to diseases: Strains of *Trichoderma* added to the rhizosphere protect plants against pathogens those that produce aerial infections, including viral, bacterial and fungal pathogens, which point to the induction of resistance mechanisms similar to the hypersensitive response (HR), Systemic acquired resistance (SAR) and Induced Systemic Resistance (ISR) in plants (Harman *et al.,* 2004). The concept of improving plant resistance by *Trichoderma* is the most accepted phenomena by the researchers. The first demonstration of induced resistance by *Trichoderma* was shown by Bigirimana *et al.* (1997) through his studies on the foliage disease of beans caused *by Colletotrichum lindemuthianum* and *Botrytis cinerea.* Yedidia *et al.* (1999) supported the concept of induce resistance through their studies on cucumber seedling disease with *T. harzianum*. A model system for *Trichoderma* induced resistance may be attributed to the potential functioning of MAMP triggered immunity in the host plant. *Trichoderma* strains produce a variety of (MAMPS) such as hydrophobins, expansin-like protein, secondary metabolites and enzymes. They elicit the production of defense metabolites and enzymes such as the enzymes Phenyl Alanine ammonia Lyase (PAL) and Chalcone Synthase (CHS), involved in the biosynthesis of phytoalexins

Table 1. Various diseases controlled by *Trichoderma* spp.

Crop	Disease	Pathogen	References
Rice	Sheath blight	*Rhizoctonia solani*	Biswas and Datta (2013)
	Bakanae	*Fusarium moniliforme*	Ng *et al*., 2015
Wheat	Leaf blight	*Alternaria triticina*	Parveen and Kumar (2004)
	Loose smut	*Ustilago segetum*	Singh (2004)
Chickpea	Wilt,wilt complex	*Fusarium,Sclerotium, Rhizoctonia*	Gupta *et al*., 2005
	Root rot	*Rhizoctonia solani*	Gupta *et al*., 2005
Pigeon pea	Wilt	*Fusarium udum*	Chaudhary and Prajapati (2004)
Apple	Ring rot	*Botryosphaeria beregeriana f.sp. piricola*	Kexiang *et al.*, 2002
	White root rot	*Dematophora necatrix*	Tapwal *et al*., 2005
Guava	Die back	*Lasiodiplodia theobromae*	Yadav and Majumdar (2005)
Chilli	Dry root rot	*Rhizoctonia solani*	Bunker and Mathur (2001)
Tomato	Fusarium wilt	*Fusarium oxysporum f.sp. lycopersici*	Komy *et al*., 2015
	Crown, stem and root rot diseases	*Rhizoctonia solani, Sclerotinia spp.* and *Pythium*	Marzano *et al*.,2013
	Collar rot of tomato	*Sclerotium rolfsii*	Amin *et al*., 2010
Potato	Black Scurf	*Rhizoctonia solani*	Gogoi *et al*., 2007
	Bacterial brown rot	*Fusarium and Phoma spp.*	Gogoi *et al*., 2007
Beans	web blight of beans	*Sclerotinia sclerotiorum*	Amin *et al*., 2010

(HR response), chitinases and glucanases, which are important pathogenesis related proteins (SAR response) and enzymes involved in the response to oxidative stress (Mohiddin *et al.,* 2010). *Trichoderma* produces a number of metabolites that may act as elicitors of plant resistance results in the synthesis of phytoalexins, PR proteins and other compounds and in an increase in resistance against several plant pathogens, including fungi and bacteria (Elad *et al.,* 2000) and also to hostile abiotic conditions (Harman *et al.*, 2004). Under abiotic stressful conditions *Trichoderma* can improve plant growth by lowering deleterious elevated ethylene levels accompanied by an elevated antioxidative capacity (Viterbo *et al.*, 2001) and also by reducing over expression of stress genes (Harman, 2000). Improve plant tolerance against salinity has been reported in *Trichoderma* treated seed. Reduced ascorbic acid has been reported in *Trichoderma* treated plants and this has been attributed to the expression of catalase *(cat)* and Mn/Cu-dependent superoxide dismutase (SOD) genes (Viterbo *et al.*, 2001).

Discovery of the ability to promote plant growth: *Trichoderma* are reported to increase the fertility of soils and significantly improved the plant growth beyond disease control (Harman, 2000). This plant growth promotion may be due to production of plant hormones or increased uptake of nutrients by the plant (Chet *et al.,* 1993). They promote root growth, nutrient availability and uptake for the plant, and released plant growth regulators (Benitez *et al.,* 2004). All these are energy requiring processes and thus, stimulate plant respiration and enhance photosynthesis and photosynthetic efficiency. Root colonization by *Trichoderma* strains frequently enhances root growth, root area, cumulative root length, significant increases in dry weight, shoot length and leaf area (Yedidia *et al.,* 2003) and also promote development, crop productivity, resistance to abiotic stresses and the uptake and use of nutrients (Arora *et al.,* 1992). They stimulate plant growth directly by making available these nutrients to the plants and indirectly by inducing a competitive environment for nutrients against other micro-floras. *Trichoderma* colonization in the roots and soil helps in solubilization of minerals such as rock phosphate, Fe, Mn, Cu and Zn. *Trichoderma* also enhances N-used efficiency (Harman, 2000). Seed treatment with *Trichoderma* spores have shown significant increased in yield (Chet *et al*., 1997).

***Trichoderma* as a bio-control agent- No more a myth**

Disease control: *Trichoderma* is a promising biocontrol agent and extensively used against the soil borne diseases. It has been used against pathogenic fungi such as *Rhizoctonia, Fusarium* and *Colletotrichum* etc. A number of diseases are reported to be controlled by *Trichoderma* species (Table 1). It is effective against both foliar and soil borne pathogens (Ha, 2010).The *in vitro* antagonistic activity of *Trichoderma viride* against phytopathogens (*Sclerotium rolfsii, Fusarium oxysporum*f.s.p. *ciceri, Fusarium oxysporum* f.s.p. *udum*) was studied and it was found to be maximum against *F. oxysporum* f.s.p. *ciceri* (62.85% mycelial growth inhibition) followed by *F. oxysporum* f.s.p. *udum* (59.37% mycelial growth inhibition) and *S. rolfsii* (58.03% mycelial growth inhibition) (Puyam *et al*., 2013). Induced systemic resistance by *Trichoderma* is also one of the important mechanisms of resistance against the pathogens. This has been reported for cucumber, strawberry, bean and tomato against *Botrytis cinerea* and powdery mildew in cucumber (Levy *et al*., 2015). At present, the induced systemic resistance mechanism by Trichoderma has been gaining importance, but still it is a budding science. In depth study is required to construct efficient biocontrol Trichoderma strains, and produce good effect agent of Trichoderma. It is significant for controlling plant diseases.

Plant growth promoter: The initiative to prove the researched findings and promotion of *Trichoderma* as

Table 2. Commercial formulation of Trichoderma used abroad.

Commercial Product	Biocontrol Organism(s)	Formulation and Application	Pathogens controlled	Manufacturer Country
Binab T	*T. harzianum, T. polysporum*	Pellets, wettable powder or granules; spray, drench, mixed in soil	Wood rots causing internal decay, or originating from pruning wounds; *Didymella, Chondrostereum, Heterobasidion, Botrytis, Verticillium, Pythium, Fusarium, Phytophthora, Rhizoctonia*	BINAB Bio-Innovation AB, Sweden (http://www.algonet.se) Henry Doubleday Research Association, United Kingdom
Plant Shield	*T. harzianum*	Granules, wettable powder; soil drench, foliar spray	*Pythium, Fusarium, Rhizoctonia, Cylindrocladiu, Thielaviopsis; suppresses Botrytis*	BioWorks, Inc., USA (http://www.bioworksbiocontrol.com)
Antagon	*Trichoderma* spp.	Powder	damping-off diseases	DeCeusterMeststoffenN.V. (DCM), Belgium (http://www.agreoBiologicals.com)
Promot PlusWP Promot PlusDD	*Trichoderma* spp. *Trichoderma koningii Trichoderma harzianum*		Wilt, Root rot diseases	Tan Quy, Vietnam (Ha, 2010)

a commercial product was done by The Cornell University and BioWorks, Inc., Geneva, NY. They have conducted thousands of assays on crops ranging from ferns to beans to corns and to ornamental flowering plants mainly based on the particular *Trichoderma harzianum* strain-22 (Harman, 2000). And from the experiment, they have found many fascinating results. It was observed that the species colonized all parts of root systems and can displace other micro-flora thereby changing the root micro-floral composition. Also enhances fertilizer used efficiency by the plants and provides long-term protection against diseases from single application at the beginning of the season and increases biomass production. The application of *Trichoderma* enhances root development in field crops induced by *Trichoderma harzianum* strain T-22 in an experiment performed on sweet corn and soybean and enhanced deep rooting in field corn. Their ability to cause the production of more robust and deep roots is likely to be quite profound. These deep roots cause crops, such as corn and ornamental plants, such as pointsettia, to become more resistant to drought. In one of the report, the treatment of corn whose roots are colonized by *Trichoderma* strain T-22 require about 40% less nitrogen fertilizer than in the absence of the organism in the corn root. Another in field report by the Management Collaborative Research Support Program (IPM CRSP), managed by Virginia Tech, *Trichoderma* has been used to combat a range of fungal diseases that affect crops from India to Honduras (www.oired.vt.edu/ipmcrsp/). World wide it has been used to manage a number of diseases. Their survey showed that in Bangladesh and Indonesia it is used against soil-borne diseases of vegetable crops, citrus, oil palm, langsat, durian, vanilla and cacao. In India and Philippines, it is used as sprayed on seedlings as a treatment for vegetable crops. In Indonesia, it is used against clubroot of broccoli. In India, it is used against pythium rot and fusarium wilt and on diseases of horticultural crops. And in the Philippines, it is used to combat anthracnose bulb rot, damping off, and pink rot diseases of onion. And in Honduras, it is being tested on watermelon for the control of fusarium wilt. *Trichoderma*, being a growth promoting agent also helps in increasing yield of crops which has been demonstrated by application of *T. harzianum* (Th3) in irrigated and dry areas of Kota and Jaipur districts of Rajasthan, India (Sharma *et al.*, 2012). Under the project entitled "On Farm Demonstration and Commercial Production of *Trichoderma* as biopesticide and Growth Promoter", they evaluated the rhizospheric competence Index along with its growth promotion effect on tillers rootlets, weight of grains and grain yield by using it at three stages of crop viz., seed, flowering and pre-harvesting. They reported a significant increase in yield of wheat from 36.25 to 46.73Q/ha (29% in Jaipur) and from 36.88 to 50.12Q/ha (36% in Kota) after continuous application for three years (2008-2011).The total income and the benefit cost ratio of farmers increased both at Jaipur (Rs 56242/ha, 1:1.8) and Kota (Rs 60332/ha, 1:1.9). Besides, food crops, four *T. atroviride* isolates have been used as bio-control for soil-borne pathogens of pasture species and also for growth promotion (Kandula *et al*., 2015).

From all these available facts and data, we can now conclude that adoption of *Trichoderma* as a bio-control agent is no longer a myth but a reality that needs to be promoted for the real world success (Harman, 2000).

Drought tolerance: *Trichoderma* has a symbiotic relationship with the host plant. The proteome and transcriptome of plants change as a consequence of the interaction of Trichoderma metabolites or plant coloni-

Table 3. Common commercial *Trichoderma* formulation used in India.

Commercial product	Bio-control organism	Pathogens controlled	Manufacturer/Suplier, Country
Antagon TV	*T.viride*	*Macrophomina* spp	Green Tech, Agroproducts, Rajaji Road Coimbatore
Trichostar	*T.harzianum*	*Macrophomina* spp	Green Tech, Agroproducts, Rajaji Road Coimbatore
Gliostar	*T.virens*	*Fusarium, Rhizoctonia, Sclerotium, Pythium*	GBPUAT, Pantnagar
Monitor	*Trichoderma* spp		Agricultural and Biotech Pvt. Ltd. Gujarat Department of Plant Pathology, MPKV, Rahuri
Bioderma	*T.viride/T. harzianum*		Biotech International Ltd. India
Bio Fit	*T. viride*	*Pythium, Rhizoctonia, Fusarium, Sclerotium, other root rots; for Botrytis in combination with chemicals*	Ajay Biotech (India) Ltd. India (http://www.ajaybio.com
Ecofit	*T.viride*		Hoechst Schering Afgro Evo Ltd, India
Trichoguard	*T.viride*		Anu Biotech Int. Ltd. Faridabad
Biocon	*T.viride*		Tocklai Experimental Station Tea Research Association, Jorhat (Assam), India

zation. The three mechanisms employed by the fungi in enhancing plant growth under drought stress are secretion of phytohormonal analogues, alleviation of damage by reactive oxygen species (ROS) and water-use efficiency. Globally, studies clearly reveal that, the use of Trichoderma spp. under drought stress can effectively augment plant growth (Chepsergon *et al*., 2014).

Mass production: The development of a successful biological control agent depends on the mass culturing and delivery system of the bio-control agent. The most commonly used solid substrate for mass culturing of *Trichoderma* spp are sorghum grain (Upadhyay and Mukhopadhyay, 1986), wheat bran-saw dust (Jogani and John, 2014) and other agro-based waste products. Wastage of potato peel, brinjal, banana, papaya, guava, spinach, sugarcane, used tea leaves and pea husk medium of solid and liquid was reported to as a substrate for the multiplication of *Trichoderma harzianum* and *Trichoderma viride* isolates. The growth media used for production of *Trichoderma* in liquid state fermentation includes molasses and brewer's yeast (Sankar and Jeyarajan, 1996) and Jaggery-soy medium (Prasad *et al.,* 2002). However, sporulation of *Trichoderma* is comparatively high on solid substrates to that of the liquid media.

Formulation: It protects the a.i. (conidia, spores, mycelial germlings) against the adverse environmental conditions like extreme pH, high/low humidity etc. So, standard formulation is needed if one expects the strain to come up to their expectations. A final formulation must have a minimum shelf- life of two years at room temperature, must be easy to handle and stable over a range of 5 to 35ºC. Characteristics of an ideal formulation are as follows:

- Should have increased shelf life.
- Should not be phytotoxic to the crop plants.
- Should tolerate adverse environmental conditions.
- Should be cost effective and should give reliable
- Control of plant diseases.
- Should dissolve well in water.
- Carriers must be cheap and readily available
- Formulation development.
- Should be compatible with other agrochemicals (Kumar *et al.,* 2014).

Developing a safe, cost effective and easy to handle formulation that will retain the viability of the micro-organism are important pre-requisite for developing an efficient and consistent bio-control agent. Formulation is the blending of active ingredients such as fungal spores with inert carrier as fungal spores such as diluents and surfactants in order to improve the physical characteristics (Kumar, 2013). The potential *Trichoderma* isolates are formulated using different organic and inorganic carriers either through solid or liquid fermentation technologies (Kumar *et al.,* 2014).

***Trichoderma* formulations are of two types:**

Solid formulations: These types utilize adhesive substances, inoculation backup, like the arabic gum or carboxymethylcellulose, also clays and compost. They are especially recommended for the inoculation of seeds. They are found as wet dust, dry-dust, granules and capsules. Formulation developed through grounded air dried mats and mixed with the commercially available carrier, contain 10^8-10^9 propagules per g (Kumar, 2013).

Liquid formulations: They are limited in production. They are applied by means of crushing to the aerial part of the plant, or directly to the substrate through the system of irrigation. Tables 2 and 3 describe some commercial formulation of *Trichoderma* that are used worldwide and in India.

Delivery and application system: Delivery and application system also affects the performance of the *Trichoderma* strains. If proper formulations are not applied in a standardized manner then, the efficient strain may fail to show its full potential. So, standardized delivery and application system is important to fully explored the efficiency of the *Trichoderma* strain. *Trichoderma* formulations can be applied either through seed bio-priming, seed treatment, seedling dip, foliar spray and soil application. Application of *Trichoderma* formulations with strain mixtures perform better than individual strains for the management of pest and diseases of crop plants, in addition to plant growth promotion (Kumar *et al.*, 2014).

Some of the suggested delivery and application system (Singh, 2010) are as follows:

Seed treatment: Seed treatment with talc based and wheat bran based (WBB) formulations @ 4 g/kg of seed have been recommended (Jogani and John, 2014).The treated propagules of *Trichoderma* germinates on seed surface. And when they are sown in the soil, the germinating propagules colonize the seedlings roots and rhizosphere (Tewari, 1996). Seed coating with *Trichoderma* is one of the less cumbersome and efficient delivery systems of the antagonist *Trichoderma* for the management of seed/soil-borne diseases. Seed is coated with dry powder/dusts of *Trichoderma* just before sowing. For commercial purpose, dry powder of antagonist is used at 3 to 10 g per kg seed based on seed size (Mukhopadhyay *et al.,* 1992). Seed treatment with *Trichoderma* are found to be effective against the diseases like sheath blight of rice (Das and Hazarika, 2000; Sriram *et al.,* 2000) besides increasing the yield of the crop, loose smut of wheat (Singh and Maheshwari, 2001), *Pythium* spp. and *R. solani* (Mukherjee and Mukhopadhyay, 1995) and oilseed-borne fungi like *Alternaria alternata, Curvularia lunata, Aspergillus flavus, Fusarium oxysporum, F. moniliforme, Rhizopus nigricans, Penicillium chrysogenum* and *Penicillium notatum* which affects oil seed crops like soybean, sesame and sunflower (Jat and Agalave, 2013).

Seed biopriming: Treating of seeds with biocontrol agents and then incubating under warm and moist conditions until just prior to emergence of radical is referred to as bio-primming. In bioprimed seeds, the germinating conidia of *Trichoderma* form a layer around the seeds. Such seeds tolerate adverse conditions of the soil better than the non-primed seeds. This technique has potential advantages over simple coating of seeds as it results in rapid and uniform seedling emergence and also reduces the amount of biocontrol agents that is applied to the seed (Kumar *et al.,* 2014)

Seedling root dip: It is mostly practiced in case of transplanted rice and vegetable crops. The cuttings or seedlings can be treated with the spore suspension prepared by mixing 10g of *Trichoderma* powder with 100g of well rotten FYM per liter of water and dip for 10 minutes before planting. Root dipping in spore suspension before transplantation reduces sheath blight disease of rice (Vasudevan *et al.,* 2002). There are also reports on enhancement of seedling growth in rice, capsicum, chilli, tomato and brinjal (Singh and Zaidi, 2002).

Soil treatment: It is an ideal treatment for nursery and green house. Delivering of *Trichoderma* spp. to soil will increase the population dynamics of augmented fungal antagonists and thereby would suppress the establishment of pathogenic microbes onto the infection court (Kumar *et al.,* 2014). Soil application of *T. viride* either alone and in combination with other treatments significantly reduced red rot caused by *Colletotrichum falcatum* (Reddy *et al.,* 2009), seedling blight, stem rot, color rot and root rot disease of Jute (Srivastava *et al.,* 2010) and against *F. oxysporum, F. solani , Fusarium moniliforme, Alternaria alternata, Botrytis theobromae* and *Rhizoctonia solani* and in seedling establishment of *Dalbergia sissoo* Roxb (Mustafa *et al.,* 2009). Combination of *Trichoderma* treatment with green manuring crops yield good results against the soil borne pathogens besides improving the soil health. For soil treatment, 5 Kg of *Trichoderma* powder per hector is mixed after turning of sun hemp or dhaincha into the soil for green manuring or 1 kg of *Trichoderma* formulation in 100 kg of FYM. The prepared environment is then kept covered with the polythene for seven days and then water is sprinkled intermittently, followed by regular turning of the mixture in every 3-4 days interval and then broadcast in the field (Singh, 2010)

Combination with other bio-control agents: Recently, combination of plant growth promoting *Rhizobacteria, Pseudomonas fluorescens* and *Trichoderma harzianum* have been reported to have greater disease suppression against *Rhizoctonia solani, Sclerotium rolfsii* causing stem and root rot disease of soybean (Kumar, 2013). Another example is the application of three microbial strains, *viz, T. asperellum* T42, *P. fluorescens* OKC and *Rhizobium* sp. RH4, individually and in combination with bioprimed seeds of chickpea and rajma in pots and fields (Yadav *et al.,* 2013). They reported that higher germination percentage and better plant growth in both the crops compared to non-bioprimed control plants. It was also observed that the combined application of the microbes enhanced seed germination and plant growth better than their individual application. Among the combinations, all combinations comprising *Trichoderma* showed better results compared to the others and the triple microbial combination demonstrated best results in terms of seed germination and seedling growth in both chickpea and rajma. Seed treatment with *T. harzianum, A. sativum* and *A. indica* on par with the foliar spray of mancozeb showed results against *Alternaria* blight disease mustard caused by *A. brassicae* and *A. brassicicola* and increasing the yield (Jagana *et al.,* 2013).Is *Trichoderma* bio-control agent a better alternative to chemical fungicides?

Disease control: Regarding disease control, it is quite comparable with the chemicals as denoted by the previous mentioned data and findings but its application to bacterial diseases is limited. If proper formulations are developed, then it will also serve as a good biocontrol agent for the bacterial diseases too (Leelavathi *et al*, 2014). Trichoderma show strong antagonistic activity against *Colletotrichum* causing red rot of sugarcane, *Alternaria* spp. causing Alternaria leaf spot disease, *Paracercospora* spp. causing leaf spot disease of banana, *Fusarium* spp. causing wilt disease in pigeonpea (Kushwaha and Verma, 2014).

Increasing pest resistance: Chemicals are specific in their mode of action. Application of a particular chemical against a particular pathogen over a period of time will induce resistance in the pathogen against the chemicals. So, those which were once effective become ineffective after a period of time. But it is circumvent incase of *Trichoderma* application as it induces resistance against the diseases in plants and provide long-term protection (Yedidia *et al*., 2000).

Sensitiveness towards environment and health: The sensitivity of the purchasers and the growers are also different. Some prefer to use chemicals especially farmers but the green house owners, park owners, household owners prefer to use bio-agents for management of diseases. Moreover, awareness of the environment also increases among the people. The used of specific micro-organisms that interfere with plant pathogens and pest, is a nature friendly, ecological approach to overcome the problems caused by standard chemical methods of plant protection (Harman *et al*., 2004).

Long period of efficacy: A single application of *Trichoderma* will provide long term efficacy as it induces resistance against diseases in plants. They have the ability to proliferate in the soil for a long period of time along with the plant roots through symbiotic association. But, the period of efficacy is limited and effects last for more than a few weeks (Harman *et al*., 2010).

Other effects: It refers to the tolerance induced by *Trichoderma* against the abiotic stresses and also the increased efficiency in the use of nitrogen fertilizer (Shelton, 2014). Trichoderma strains play an important role in the bioremediation of soil that are contaminated with pesticides and herbicides, having the ability to degrade a wide range of insecticides (organochlorines, organophosphates and carbonates) (Jorge, 2014).

Limitations

Less effective when applied to diseases already existed: The establishment of *Trichoderma* in the soil and coming into action takes time i.e., its mode of action slow as compare to chemicals which is quick in action. So, if a disease is build up and the disease pressure is quite high then, application of *Trichoderma* is seem to be less effective and here calls for the chemicals for quick action.

Less obvious visual enhancement of plant improvement: The action of *Trichoderma* is quite slow and so, its results are less obvious to the growers as compare to the chemicals.

Action depends on the environment: We have learned that *Trichoderma* provides tolerance to the abiotic stresses and increases fertilizer used efficiencies. But it fails to show these results if they are grown under near optimal conditions and if no stress occurs in the environment.

Is it safe to regard *Trichoderma* completely as benign?

Certain harmful effects of *Trichoderma* have been reported in human and even some are mushroom pathogens. *T. longibrachiatum* has been reported *as* an opportunistic human pathogen. Eight other species (i.e., *T. atroviride*, *T. citrinoviride*, *T. harzianum*, *T. koningii*, *T. orientale*, *T. pseudokoningii*, *T. reesei*, and *T. viride*) have also been reported occasionally (Hatvani *et al.,* 2013) as human pathogens. Some of the harmful effects are as follows:

Mushroom pathogens: Some species of *Trichoderma* are reported to cause green mold disease of mushrooms thereby affecting commercial production of mushrooms. They are *T. aggressivum, T. pleurotum* and *T. pleuroticola* (Samuels *et al.,* 2002).

Trichothecene antibiotics: *T. viride and T. harzianum* produce antibiotics trichodermin/ harzianum A, which are reported to be trichothecene in action (Degenkolb *et al.,* 2008).

Antibiotic harmful to human cell: Gliotoxin produced by *Trichoderma virens* strain Q is also produced by *Aspergillus* fumigants in maize silage as mycotoxins and are reported to be immunosuppressive in human. So, analysis and identification of the effects of this antibiotic released by *Trichoderma* is required (Richard *et al.,* 2008). Peptaibols produces a rare amino acid –á-aminoisobutyric acid which can lyse red blood cells (Wiest *et al.,* 2002).

Hypersensitive reactions: *Trichoderma* infections in humans have been related with several risk factors, being associated mostly with peritoneal dialysis, organ transplantation, and hematologic disorders (Hatvani *et al.,* 2013). On the other hand, data on animal infections by *Trichoderma* spp. are very limited. They are strong sensitizers and can caused hypersensitive reactions to human and animal tissues. The USA Govt. occupational safety and health Administration (OSHA) classifies *Trichoderma* as allergic and irritant and caused of hypersensitive pneumonitis and dermatitis (Caballero *et al.,* 2007 and Beezhold *et al.,* 2008).

Development and commercialization of Trichoderma formulation for field application should not ignore these harmful effects of *Trichoderma*. Before releasing for farmers used, the product must be tested and should assure that they are not harmful to the environment or human and animal health.

Future prospects

New strains and species are to be genome sequenced and researches are to be done on their metabolomes and expressome.

Targeted genetic improvement and the use of their genes in crop improvement programmes.

Determination of compatibility of effective *Trichoderma* spp with other beneficial fungi or bacteria or with some bio-active compounds will open more scopes and increases the effectivity of the role of bio-control in crop-improvement.

Trichoderma and plant interaction is the another side of the mirror that needs to be explored, given extensive effects on the plant physiology with many more developing molecular mechanisms and this will not only prove *Trichoderma* as an effective bio-control agent but also as a plant health manager too.

Development of quick, suitable and inexpensive methods to monitor *Trichoderma* activity and improved formulations those are easy to adopt by the common farmers.

There are still many diseases without an effective bio-control agent, so it is necessary to increase knowledge about the effects of more enzymes and metabolites of Trichoderma with the aim of extending its spectrum of action as a biocidal agent.

Conclusion

With the increasing threat to the environment and to the food security, adoption of *Trichoderma* spp. as a BCA has been gaining importance which will provide food security in one hand and eco-friendly on the other. To meet this, research on the unexplored part of the world needs to be explored to find a suitable and efficient *Trichoderma* strains that will best serve as a biocontrol agent. In this field many more researches need to be done. Our knowledge till now is still under infantry. In depth study and analysis of the findings about this organism is far more to be done. The novel findings will, then, serve as a milestone for the development and adoption of *Trichoderma* as a BCA which in turn help in the improvement of crop production and disease management.

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