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Review

Trichoderma: A significant fungus for agriculture and environment

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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 environment-friendly manner. Thus, there is an immediate need to find eco-friendly solutions such as wider application of biocontrol agents. Among various types of species being used as biocontrol agents, including fungi and bacteria, fungal genus *Trichoderma* produces different kinds of enzymes which play a major role in biocontrol activity like degradation of cell wall, tolerance to biotic or abiotic stresses, hyphal growth etc. The understanding of filamentous fungi belonging to the genus *Trichoderma* has continuously evolved since last two decades, from the simple concepts of biocontrol agents to their recently established role as symbionts with different beneficial effects to the plants. Recent findings from structural and functional genomics approaches suggest the additional use of these microbes as model to study mechanisms involved in multiple player interactions that is, microbes-microbes-plant-environment. In this work, historical development of *Trichoderma* spp., mode of action against different biological agents, potential applications and recent mass production techniques are summarized and discussed in detail with updated advances with their application in the agriculture and sustainable environment.

Key words: Biocontrol agent, mycoparasitism, induced resistance, endophyte, mass production, bioremediation, bioreactors, agrochemicals.

INTRODUCTION

Trichoderma - a multifaceted fungus

Fungi in genus *Trichoderma* (Division - Ascomycota, Subdivision - Pezizomycotina, Class - Sordariomycetes, Order - Hypocreales, Family - *Hypocreaceae*) have been known since 1920s for their capability to function as biocontrol agents (BCA) against plant pathogens (Samuels, 1996). They can be used either to improve

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health of crop plant or to increase the natural ability to degrade toxic compounds by some plants in soil and water. Some species of *Trichoderma* have the multiple interactions (mainly *Trichoderma harzianum* strain T22 and *Trichoderma atroviride* strain P1) with crop plants and soil borne fungal pathogens (Woo et al., 2006). The different species of this genus have long been known not only for the control of plant disease but also for their capability to enhance plant growth and development, elevated reproductive ability, capacity to modify the rhizosphere, capability to grow under adverse conditions, competence in the use of nutrients, strong aggressiveness against phytopathogenic fungi and efficacy in supporting plant growth and enhanced defense mechanisms (Harman et al., 2004; Schuster and Schmoll, 2010; Pandya et al., 2011; Tripathi et al., 2013; Dagurere et al., 2014; Keswani et al., 2014). These properties have made *Trichoderma* a omnipresent genus able to grow in wider habitats and at high population densities (Chet et al., 1997; Chaverri et al., 2011). There is a numerous literature available on *Trichoderma* research but recent updates in cooperation with long-established facts are not summarized in past few years (Gal-Hemed et al., 2011; Sujatha et al., 2013). This review focuses on the occurrence of *Trichoderma* spp., their mode of action, commercial production techniques with applications in agriculture and use in sustainable environmental practices.

The fungus *Trichoderma* has a long history and it was first reported and described in 1794 (Persoon, 1794) and later suggested to have a link with the sexual state of a *Hypocrea* species. However, it was difficult to assign the genus *Trichoderma*/*hypocrea* morphologically. It was even proposed to have only one species, that is *Trichoderma viride*. The first move on development of a particular protocol for species identification was made in 1969 (Rifai, 1969; Samuels, 2006). *Trichoderma* spp. has been known from last 70 years for their ability to produce antibiotics that inhibit growth of pathogenic organisms and used as a biocontrol agents (Harman, 2006). Subsequently, many novel species of *Trichoderma* were revealed and by 2013, the genus already consists of more than 200 phylogenetically defined species based on rpb2 sequence (Atanasova et al., 2013).

PHYLOGENIC EVOLUTION

The genus name *Trichoderma* was first proposed on the basis of macroscopic similarity (Persoon, 1794). The four species categorized in this genus were *T. viride*, *T. nigrscens*, *T. aureum* and *T. roseum* collected in Germany. These species were described as appearing like mealy powder and enclosed by a hairy covering further distinguished from each other by their different colored conditions (Persoon, 1794). However, these four species are now considered to be unrelated to each other and presently known as *Trichoderma viride* (Pers. Ex. Fr.), *Xylohypha nigrescens* (Pers. Ex. Fr.) mason, *Sporotrichum aureum* Pers. Ex. Fr. and *Trichothecium roseum* (Pers.) link ex S.F. Gray. The name *Trichoderma* is now applied to be the most frequently encountered green forms typified by the original *T. viride* species described by Persoon, 1794. The first real generic description of *Trichoderma* was proposed based on colony growth rate and microscopic characters by Rifai, 1969. The genus was sub-divided into nine species, distinguished from each other primarily on the basis of conidiophore branching patterns and conidium morphology. The nine species-aggregates proposed were (1) *T. piluliferum*, Webster and Rifai, (2) *T. polysporum* (link ex Pers.) (3) *T. hamatum* (Bon.) Bain. (4) *T. koningii* Oudemans (5) *T. aureoviride* Rifai (6) *T. harzianum* Rifai (7) *T. longibrachyatum* Rifai (8) *T. pseudokoningii* Rifai and (9) *T. viride* (Pers. Ex. Fr.). However, problem associated with Rifai's key was significant variation which remained to be defined within each of the nine aggregate taxa. During the last couple of decades of the twentieth century, several groups revised and rearranged the *Trichoderma* genus mainly on the basis of morphological characteristics (Bissett, 1984; Bissett, 1991a; Manczinger et al., 2012; Bissett, 1991b; Gams and Bissett,1998; Doi et al., 1987, Samuels, et al., 1998).

There were some earlier reports about false identification of certain species using morphological characteristics, for example name *Trichoderma harzianum* was used for many different species (Kullnig-Gradinger et al., 2002) Recently, methods for safe identification of new species are significantly facilitated by development of and a customized similarity search tool (TrichoBLAST) and an oligonucleotide barcode (TrichOKEY), both available online at http://www.isth.info/ (Druzhinina et al., 2005; Kopchinskiy et al., 2005). Additionally, phenotype microarrays are useful for classification of new species which allow analysis of carbon utilization patters for 96 carbon sources (Bochner et al., 2001). Chaverri and Samuels, 2013 analyzed endophytic species on the basis of their habitat preference and nutrition mode to understand species radiations in diverse groups, and its potential use in development of novel biological control strategies. Several species could be characterized with well-defined isoenzyme patterns during cellulose-acetate electrophoresis, suggesting that this method can be used for the analysis of biochemical diversity between and within particular species of the genus *Trichoderma* (Manczinger et al., 2012). The persistent efforts to clarify variety and geographical incidence of *T. hypocrea* promoted thorough documentations of the genus in many parts of the world (Samuels et al., 2012; Chaverri and Samuels, 2003; Jaklitsch, 2009). Presently, the International Sub commission on *T. hypocrea* assigned 104 species characterized at molecular level

(http://www.isth.info/biodiversity/index.php). A different member of this genus produces a broad array of pigments from bright greenish-yellow to reddish in color but some are colorless.

Trichoderma spp. is ubiquitous in environment

Trichoderma is an asexually reproducing fungal genus most frequently found in soil; nearly all temperate and tropical soils contain 101 to 103 propagules per gram which can be grown in standard laboratory conditions. These species can colonize woody as well as herbaceous plants, in which the sexual teleomorph (genus *Hypocrea*) has observed. Nevertheless, there are many *Trichoderma* strains, including most biocontrol strains with no sexual stages. In nature, vegetative forms of the fungi persist as clonal, often heterokaryotic, individually and in populations that most likely evolve separately in the asexual stage. *Trichoderma* are strong opportunistic invaders, fast growing, prolific producers of spores and also powerful antibiotic producers even under highly competitive environment for space, nutrients, and light (Schuster and Schmoll, 2010; Herrera-Estrella and Chet, 2004; Montero-Barrientos et al., 2011). These properties make *Trichoderma* ecologically very dominant and ubiquitous strains able to grow in native prairie, agricultural, marsh, forest, salt and desert soils of all climatic zones (including Antarctic, tundra, and tropical regions) also found in lake, air, plant biomass, in the vicinity of virtually all types of live plant species, and seeds (Montero-Barrientos et al., 2011; Mukherjee et al., 2013). Recently, marine *Trichoderma* isolates were characterized to evaluate their potential use as halotolerant biocontrol agents and found effective against *Rhizoctonia solani* inducing systemic defense responses in plants (Gal-Hemed et al., 2011).

Trichoderma as a biopesticide in modern agriculture

Trichoderma-based biofungicides are booming in an agricultural market with more than 50 formulations registered products worldwide. Nowadays, there are more than 50 different *Trichoderma*-based agricultural products being produced in different countries and are sold to farmers to get better yields in different crops (Woo et al., 2006). Presently, *Trichoderma* spp. based products are considered as relatively novel type of biocontrol agents (BCAs). The size of current biopesticide market is vague and only scattered information could be obtained based on registered as well as non-registered biofungicides. Recently, *Trichoderma* based BCAs share about 60% of all fungal based BCAs and an increasing number of *Trichoderma* spp. based BCAs products are registered regularly. *T. harzianum* as an active agent in a range of commercially available biofertilizers and biopesticides is being used recently (Lorito et al., 2010; Vinale et al., 2006). The inherent qualities of *Trichoderma* based BCAs are driving factors for their steadily cumulating success (Verma et al., 2007).

There are numerous reports on the ability of *Trichoderma* spp. to antagonize a wide range of soil borne plant pathogens combined with their ability to reduce the incidence of diseases caused by these pathogens in a wide range of crops (Monte, 2001). The mechanisms that *Trichoderma* uses to antagonize phytopathogenic fungi include competition, colonization, antibiosis and direct mycoparasitism (Howell, 2003). This antagonistic potential serves as the basis for effective biological control applications of different *Trichoderma* strains as an alternative method to chemicals for the control of a wide spectrum of plant pathogens (Chet, 1987).

MODE OF ACTION

Trichoderma can work as biocontrol agents in several ways (Figure 1):

1. It may grow faster or use its food source more efficiently than the pathogen, thereby crowding out the pathogen and taking over, known as nutrient competition.
2. A biocontrol agent may excrete a compound that slows down or completely inhibit the growth of pathogens in the surrounding area of such a compound called antibiosis.
3. It may feed on or in a pathogenic species directly known as parasitism.
4. It may promote a plant to produce a chemical that protects it from the pathogen, which is induced resistance.
5. They can grow in an endophytic way in other species and supports plant growth.

Competition

The most common reason for the death of many microorganisms growing in the vicinity of *Trichoderma* strains is the starvation and scarcity of limiting nutrients. This can be effectively used in biological control of fungal phytopathogens. Carbon and iron are two essential elements in most of the filamentous fungi, required for viability. Competition for carbon is effective mode not only in *Trichoderma* but also some other fungi such as strains of *F. oxysporum* (Sarrocco, et al., 2009; Alabouvette et al., 2009). Under iron starving conditions; most fungi produces small size ferric-iron specific chelators to mobilize iron from surrounding environment. *T. harzianum* T35 also controls *Fusarium oxysporum* by competing for both rhizosphere colonization and nutrients (Tjamos et al., 1922). Siderophores produced by some *Trichoderma* isolates are highly efficient chelators for iron

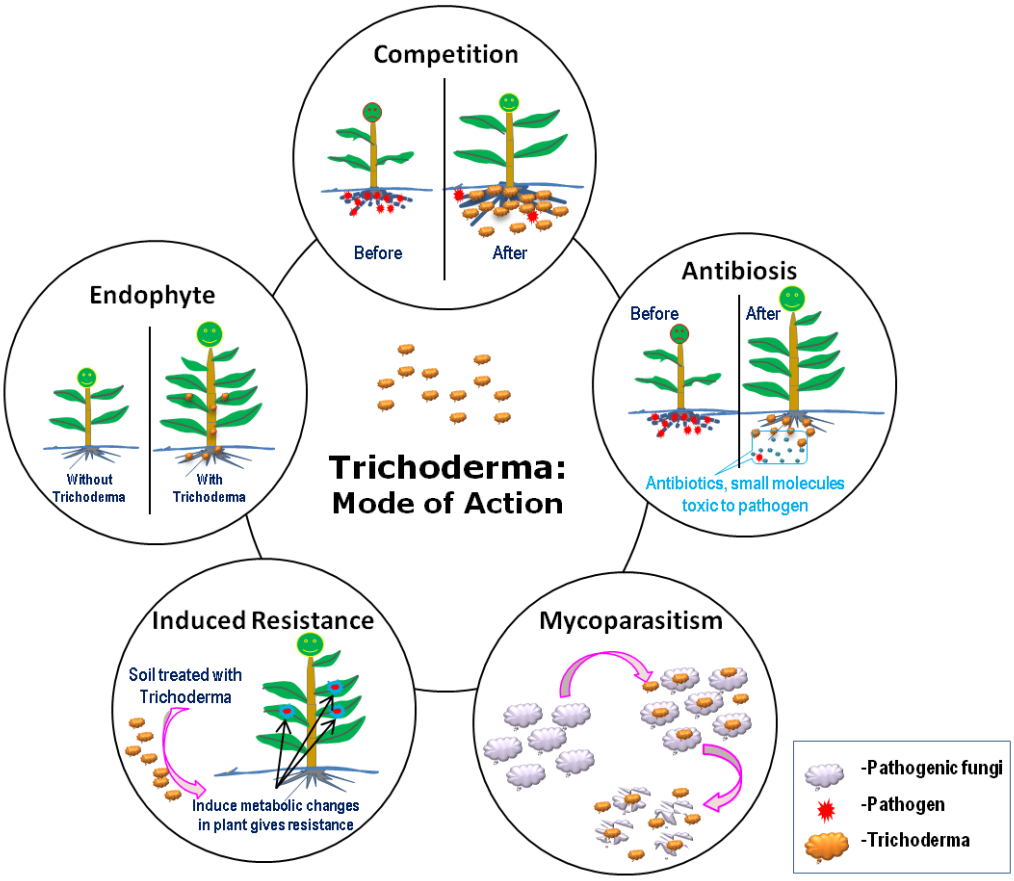

Figure 1. Model depicting mode of action of *Trichoderma* spp. against pathogen and plant growth improvement.

and inhibit the growth of other fungi (Chet and Inbar, 1994). Hence, *Trichoderma* spp. outcompetes with *Pythium* for available iron in soil and effectively controls its growth. There are many more examples about effective application of competition for the biocontrol of pathogens such as *B. cinerea*, which is involved in pre- and post-harvest loss in many countries around the world (Latorre et al., 2001). These reports suggest that the molecular and proteomic assembly of *Trichoderma* is more efficient to mobilize and take up soil nutrients as compared to many other studied pathogens and other organisms.

The proficient utilization of accessible nutrients is resulting from the capability of *Trichoderma* to acquire ATP from the diverse types of sugars, such as those derived from polymers widely available in fungal environments: cellulose, glucan and chitin and others, all of them turning into glucose (Chet et al., 1997). Recently the antifungal properties of filtrates of *Trichoderma* strains were used to control *Ceratocystis paradoxa* responsible for pineapple disease of sugarcane (Rahman et al., 2009). Productions of proteins playing pivotal role in root colonization by *Trichoderma* are also found to be crucial in competition with other root colonizers (Saloheimo et al., 2002; Viterbo et al., 2004; Brotman et al., 2008) and some of them help to establish symbiotic relationship with host plants (Samolski et al., 2012).

Antibiosis

The mechanism of antibiosis is commonly reported among many species including microorganisms and plants. In case of *Trichoderma*, small size diffusible compounds or antibiotics produced by these species inhibit the growth of other microorganisms (Benitez et al., 2004). Production of volatile compounds was not detected in case of four isolates of *T. harzianum* that were tested *in vitro* against *Rhizoctonia solani* (Cumagun and Ilag, 1997). Strains of *T. virens* able to produce gliovirin involved in antibiosis making it efficient biocontrol agent (Howell, 1998). A mutant of *T. harzianum* strain 2413 with elevated levels of extracellular enzymes and of α-pyrone increased resistance than the wild type against *R. solani* and in assays of grape protection against *B. cinerea* under different controlled environmental

conditions (Rey et al., 2001). In tobacco plants, exogenous application of peptaibols activated defense responsive genes and showed reduced susceptibility to *Tobacco mosaic virus* (Wiest et al., 2002). Coconut smell is typical of *T. viride* isolates suggesting the presence of volatile compounds that are inhibitory to pathogen growth. These metabolites include harzianic acid, alamethicins, tricholin, peptaibols, antibiotics, 6-penthyl-α-pyrone, massoilactone, viridin, gliovirin, glisoprenins, heptelidic acid (Vey et al., 2001; Raaijmakers et al., 2009). The different pathways producing secondary metabolites are illustrated and summarized recently by Daguerre et al., 2014, including pyrone biosynthesis pathway, polyketide biosynthesis pathway, peptaibol biosynthesis pathway, flocculosin terpenoid/steroid biosynthesis pathway, gliotoxin and gliovirin biosynthesis pathways.

Mycoparasitism

Mycoparasitism is one of the main mechanisms involved in the antagonisms of *Trichoderma* as a biocontrol agent. The process apparently include, chemotropic growth of *Trichoderma*, recognition of the host by the mycoparasites, secretion of extra cellular enzymes, penetrations of the hyphae and lysis of the host (Zeilinger et al., 1999). *Trichoderma* recognizes signals from the host fungus, triggering coiling and host penetrations. The process of mycoparasitism involves direct attack of one fungal species on another one. This complex process includes sequential events, involving cycle of recognition of fungal strain by *Trichoderma* spp., attack on cellular machinery, and subsequent penetration inside the host and finally killing of the host. *Trichoderma* spp. even can grow towards fungal host by recognizing them. Such remote sensing activity is partially because of the sequential production of pathogenesis related proteins, mostly glucanase proteases, and chitinase (Harman et al., 2004). The response of different *Trichoderma* strains is not similar in the process of mycoparasitism. Constitutive secretion of exochitinases at low level which degrade fungal cell-walls releasing oligomers plays a central role in growth inhibition of pathogenic fungal strains (Gajera et al., 2013). In some cases, the morphological changes like coiling and formation of appressorium containing higher amount of osmotic solutes such as glycerol induces penetration in host cells. *Trichoderma* attached to the pathogen, coils around the pathogen and formed appresoria releases its content. It results in the production of pathogenesis related peptides which helps in both the entry of *Trichoderma* hyphae and the digestion of the cell wall content (Howell, 2003). The cell wall degradation of target fungus by these produced chemical compounds results in the parasitism. There are many factors affecting this process and at least 20 to 30 proteins and other metabolites are directly involved in this interaction. The functions of different glucanases and chitinases in the process of mycoparasitism are well studied from *Trichoderma* spp. using gene-for-gene experiments and future studies will definitely help us to understand this complex process (Daguerre et al., 2014).

Induced resistance

The major focus of *Trichoderma* research was to understand the direct effects on other fungal species, especially mycoparasitism and antibiosis. The first clear demonstration of induced resistance with *T. harzianum* strain T-39 showed that treated soil made leaves of bean plants resistant to diseases caused by the fungal pathogens such as *B. cinerea* and *C. lindemuthianum*, even though T-39 was applied only on the roots and without any on the foliage (Bigirimana et al., 1997). Induced resistance was found to be beneficial in more than 10 different dicots and monocots, to infection by fungi (*B. cinerea*, *R. solani*, *Colletotrichum* spp., *Phytophthora* spp., *Alternaria* spp., *Magnaporthe grisea*, etc.), bacteria (*Xanthomonas* spp., *Pseudomonas syringae*, etc.), and even some viruses like CMV. The soil treated with *T. harzianum* strain T-39 was also effective against fungal pathogens *B. cinerea* and *Colletotrichum lindemuthianum* in bean plants. Similar findings were reported from *B. cinerea* to other dicots (De Mayer et al., 1998).

Similar studies have been conducted with different *Trichoderma* species and strains on different plant species, including both monocots and dicots. *T. harzianum* strain T-22 is the only microbe reported to induce systemic resistance to pathogens in model plants (Contreras-Cornejo et al., 2011; Salas-Marina et al., 2011; Yoshioka et al., 2012) and also in maize indicative of its unique ability (Harman et al. 2012). Induced systemic resistance is believed to be one of the most important mechanisms of biocontrol effects of *Trichoderma* (Harman, 2006). A variety of strains of *T. virens*, *T. asperellum*, *T. harzianum*, and *T. atroviride* stimulate metabolic changes that enhance higher tolerance to many plant-pathogenic microbes including viruses (Table 1). Likewise, this response appears to be broadly useful for many crops; for example, *T harzianum* strain T-22 induces resistance in plants as diverse as tomatoes and maize, suggesting a little or no plant specificity.

Saksirirat et al., 2009 reported that isolate of *T. harzianum* (T9) induced resistance in tomato plant (cv. Sida cultivar) with reducing 69.32% bacterial spot (*Xanthomonas campestris* pv. *vesicatoria*) after 14 days post inoculation. Similarly, on gray leaf spot (*Stemphylium solani*), isolate *T. asperellum* (T18) induced resistance and showed significant reduction in number of spots by 19.23% after 10 days post inoculation. The elicitor filtrate of *T. harzianum* (PDBCTh10 isolate) was found effective against root rot (*Phytophthora capsici*) in pepper plant and induced

Table 1. Induced systemic resistance elicited by *Trichoderma* spp.

Species Plant and strain	Plant species	Pathogens	Outcome	References
T. virens G-6, G-6-5 and G-11	Cotton	*Rhizoctonia solani*	Protected plant by inducing terpenoid phytoalexins toxic to fungi	Howell et al., 2009
T. harzianum T-39	Bean	*Colletotrichum lindemuthianum, Botrytis cinerea*	No infection on leaves when T-39 was applied only on roots	Bigirimana et al., 1997
	Tomato, pepper, tobacco, lettuce, bean	*B. cinerea*	No infection on leaves when T-39 was applied only on roots	De Meyer, 1998
	A. thaliana (L.) Heynh.	*Botrytis cinerea Pers.*	Ecotype Colombia-0 (Col-0) showed resistance leading to reduced grey mold symptoms	Korolev et al., 2008
	Vitis vinifera	*Plasmopara viticola*	Activation of defense related mechanisms	Perazzoli et al., 2012
	Tomato	*Botrytis cinerea*	0.4% T39 drench showed 84% decline in disease severity	Meller et al., 2013
	Cucumber, bean, tomato, and strawberry	*Botrytis cinerea* and *Podosphaera xanthii*	Protected from foliar diseases by direct or indirect effect via stimulation of beneficial microorganisms in the rhizosphere	Levy et al., 2015
T. harzianum T-22; *T. atroviride* P1	Bean	*B. cinerea* and *Xanthomonas campestris* pv. phaseoli	Activation of pathways related to antifungal compounds in leaves when present on roots	Harman et al., 2004
T. harzianum T-1 & T22; *T. virens* T3	Cucumber	*Green-mottle, mosaic virus*	No infection on leaves when strains were present only on roots	Lo et al., 2000
T. harzianum T-22	Tomato	*Alternaria solani*	No infection on leaves when T-22 was applied only on roots	Seaman, 2003
Trichoderma GT3-2	Cucumber	*C. orbiculare*, *P. syringae* pv. lachrymans	Induction of defense related genes related to lignifications and superoxide generation	Koike et al., 2001
T. harzianum	Pepper	*Phytophthora capsici*	Improved production of the phytoalexins capsidiol toxic to pathogen	Ahmed et al., 2009
T. asperellum (T203)	Cucumber	*Pseudomonas syringae* pv. *lachrymans*	Modulated the expression of proteins related to jasmonic acid/ethylene signaling	Shoresh et al., 2005
T. asperellum SKT-1	*A. thaliana* (L.) Heynh.	*Pseudomonas syringae* pv. *tomato* DC3000	Induced systemic resistance to colonization by SKT-1 and its cell-free culture filtrate	Yoshioka et al, 2012
	A. thaliana	*Cucumber mosaic virus*	Improved defense mechanism against infection of CMV	Elsharkawy et al., 2013
T. harzianum Tr6, and *Pseudomonas* sp. Ps14	cucumber and *A. thaliana*	In cucumber- *Fusarium oxysporum* f. sp. *radicis cucumerinum* and in *A. thaliana* against *B. cinerea.*	Ps14 and Tr6 activated the set of defense-related genes	Alizadeha et al., 2013
T. virens and T. atroviride	Tomato	*Alternaria solani, B. cinerea,* and *Pseudomonas syringae* pv. *tomato* (Pst DC3000)	Secreted proteins- Sm1 and Epl1 both induced systemic acquired resistance	Salas-Marina et al., 2015

resistance resulting with 23% less infection (Sriram et al., 2009). At a molecular level, resistance to different pathogens is due to increase in the activity of defensive mechanisms producing higher concentration of related metabolites and enzymes, such as chalcone synthase (CHS) and phenylalanine ammonio lyase (PAL), chitinase, glucanase and some proteins from cerato-platanin (CP) family and

phytoalexins (HR response) synthesizing enzymes such as PKS/NRPS hybrid enzyme (Djonovic et al., 2006; Seidi et al., 2006; Mukherjee et al., 2012). These comprise pathogenesis related proteins (PR) and enzymes involved in the response to oxidative stress (Gajera et al., 2013).

Endophytes

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 (Piotrowski and Volmer, 2006). 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 (Gazis and Chaverri, 2010; Chaverri and Gazis, 2011). Interestingly, strains forming 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 (Chaverri and Samuels, 2013; Harman et al, 2012). Phylogenetic analysis classifies all known endophytic species as a separate taxa 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 (Chaverri et al., 2011; Samuels et al., 2006; Samuels and Ismiel, 2009; Druzhinina et al., 2011).

MASS PRODUCTION

Due to increasing interest in the biocontrol, awareness about pesticide hazards, commercial production and use of biocontrol agents has now come into a reality and there are several reports of successful use of formulations of *Trichoderma* in the green house as well as in the field for control of various diseases, particularly for the soil borne pathogens. For mass introduction of *Trichoderma* in the fields, *Trichoderma* spp. is to be multiplied on some suitable and cheap media which can provide a food base for the initiation of the growth. *T. harzianum* and *T. viride* are the two most commonly used species and have been found effective when applied on about 87 different crops in India (Sharma et al., 2014). Available literature reveals that researchers have attempted for use of varied substrates and techniques for multiplication and introduction of *Trichoderma* into the soil (Sabalpara, 2014). One of the greatest impediments to biological control by *Trichoderma* has been the scarcity of methods for mass culturing and delivering the biocontrol agents. The problem in developing biopesticides, a living system, is during the process of formulation and short shelf life. The most widely used fungal antagonists, *Trichoderma* spp. have been grown on solid substrate like wheat straw, sorghum grains, wheat bran, coffee husk, wheat bran-saw dust, diatomaceous earth granules impregnated with molasses and so forth for their mass multiplication (Table 2).

Papavizas et al. (1984) produced biomass of fungal antagonists by liquid fermentation consisting of molasses and brewer's yeast. Montealegre et al. (1993) proposed liquid fermentation method consisting of molasses, wheat bran and yeast on large scale production of *T. harzianum*. Since *Trichoderma* sporulates relatively poorly in liquid media and sporulates well on various solid substrates, solid substrate fermentation (SSF) process was preferred over the other due to some inherent advantages under Indian conditions. These include utilization of large number of agro wastes as substrate for the en mass production of *Trichoderma*, use of a wide variety of matrices, low capital investment, low energy expenditure, less expensive downstream processing, less water usage and lower waste water output, potential higher volumetric productivity, high reproducibility, lesser fermentation space and easier control of contamination. Fermented biomass of *Trichoderma* consisted mainly of chlamydospores and conidia with some amount of mycelia fragments. The controlled physiological parameters are crucial in production of viable spores suggesting carbon to nitrogen ratio in medium or substrate, pH, and cultivation time are important (Agosin et al., 1997).

Solid state fermentation

Among the grains, sorghum proved very useful and cheaper for the production of nucleus culture while among the organic matter farm yard manure and seasoned pressmud proved superior. Pressmud proved very useful and more applicable source especially in sugar factory area. From the agro wastes tested wheat bran and paddy straw suggested as the most promising source for the mass multiplication of *Trichoderma* (Table 2).

Liquid state fermentation

Liquid state fermentation is generally used to produce spores from fungal strains. Among the liquid media, *Trichoderma* Selective Medium (TSM) along with mannitol, molasses and potato jaggery media were found very effective and suggested for the mass multiplication of *Trichoderma* spp. by many workers. Mass multiplication of *T. viride*, *T. harzianum* and *T. longibrachiatum* using decomposed pressmud was found

Table 2. Substrates successfully used for *Trichoderma* production.

S/N	Species	Substrates	References
Solid based			
I. Grains			
1	*T. harzianum* and *T. viride*	Sorghum	Rini and Sulochana, 2007
2	*T. viride*	Sorghum, wheat	Bhagat et al., 2010
3	*T. harzianum*	Rice, sorghum, pearl millet	Parab et al., 2008
4	*T. harzianum*	Maize	Pramod and Palakshappa, 2009
5	*T. harzianum*	Sorghum	Upadhyay and Mukhopadhay, 2009
II. Organic matters			
6	*T. harzienum* P26	Neem cake, coircompost, FYM, Gliricida leaves	Saju et al., 2002
7	*T. harzianum* (T5), *T. viride, T. hamatum* (T16)	Cotton cake	Sharma and Trivedi, 2005
8	*T. harzianum*	FYM, Local cow dung, Jersey cow dung	Pramod and Palakshappa, 2009
9	*T. harzianum and T. viride*	Cow dung with neem cake, coir pith, coir pith in combination with neem cake	Rini and Sulochana, 2007
10	*T. harzianum* Rifai	Tapioca waste Pigeonpea husk and press mud	Jayraj and Ramabadran, 1996
11	*T. viride*	FYM, vermicompost, poultry manure, goat manure, decomposed coconut, coir pith	Palanna et al., 2007
12	*T. harzianum*	FYM, spent compost	Tewari and Bhanu, 2004
13	*T. harzianum*	FYM, compost	Parab et al., 2008
14	*T. viride*	FYM, Peat	Bhagat et al., 2010
15	*T. harizianum*	Jatropha cake and neem cake	Tomer et al., 2015
III. Agricultural wastes			
16	*T. harzianum*	Rice bran, paddy straw, groundnut shells	Parab et al., 2008
17	*T. harzianum, T. viride* and *T. virens*	Spent Malt	Gopalkrishnan et al., 2003

Table 2. Contd.

18	*T. harzianum*	Wheat straw, paddy straw, shelled maize cob, paper waste, saw dust, sugarcane bagasse, spent straw, wheat bran, rice bran	Tewari and Bhanu, 2004
19	*T. viride* and *T. harzianum*	Tapioca rind, tapioca refuse, mushroom spent straw, paddy chaff, wheat bran, groundnut shell, rice bran, sugarcane baggase, wheat straw, shelled maize cob, paddy straw, chickpea husk	Gangadharan and Jeyrajan, 1990
20	*T. harzianum* and *T. viride*	Saw dust, rice bran	Rini and Sulochana, 2007
21	*T. harzianum*	Shelled maize cobs, paddy straw, paddy husk, wheat bran, baggase, sawdust, groundnut shell	Pramod and Palakshappa, 2009
22	*Trichoderma harzianum, T. virens* and *T. atroviride*	Onion rind (dry onion skin), apple and strawberry pomace, rapeseed meal	Smolinska, et al., 2014
23	*T. harzianum* (T5), *T. viride, T. hamatum* (T16)	Tea waste, sorghum straw, wheat straw, wheat bran	Sharma and Trivedi, 2005
Liquid based			
24	*T. hamatum, T. harzianum*, *T. viride*	Molasses and Brewers yeast	Papavizas, 1984
25	*T. harzianum* strain P1,	Defined basal culture medium with mineral solution	Agosin, 1997
26	*T. harzianum*	RM8	Jin, 1991
27	*T. harzianum* strain 1295-22	Modified RM8	Jin, 1991
28	*T. harzianum*	Czapeck's Dox Broth and V8 Broth	Harman, 1991
29	*T. harzianum* Rifai	Potato Dextrose Broth, V8 juice and molasses yeast medium	Prasad, 2002
30	*T. harzianum* Rifai	Potato Dextrose Broth, Czapeck's Dox Broth and Modified Richards' Broth	Das, 2006
31	*T. harzianum*	Local cow urine, Jersey cow urine, Butter milk, Vermiwash	Parab et al., 2008

most effective as compared to the rest of the substrates tested (Gohil, 1993). In addition, several techniques for the mass production of *Trichoderma* spp. were established and proposed by our group and other researchers based on local conditions and availability of substrates (Pandya et al., 2007; Sabalpara, 2014; Pandya et al., 2012; Sabalpara et al., 2009). A novel technique using talc mixed proportionately with FYM (1:10) was developed for direct soil and nursery bed applications (Ramanujam and Sriram, 2009).

Commercial level production

Bacterial based BCAs are being produced and marketed by many commercial firms and available in global market (Velivelli et al., 2014). In India, there are more than 250 BCA products available in the market. Formulization of commercial BCA for agricultural application should possess several desirable characters and need to have substantial proof in order to convince farmers. These include satisfactory market potential, easy preparation, unfussy application, high stability during transportation as well as storage, abundant viable propagules with good shelf life, sustained efficacy and accepted cost. Various carrier materials proved useful for the preparation of formulation of *Trichoderma* based BCAs because it works as a food base (Table 3). Talc is the most common carrier material suggested for commercial production of *Trichoderma* worldwide.

POTENTIAL APPLICATIONS IN MODERN AGRICULTURE AND SUSTAINABLE ENVIRONMENT

The *Trichoderma* genus can grow in a wide range of habitats and this is achieved by evolved diversified metabolic pathways leading to the production of various enzymes and secondary metabolites. Production of commercially important enzymes such as amylases, cellulases, 1-3 beta glucanases, and chitinases were extensively studied and this technology is continuously being updated (Harman et al., 2004; Ahamed and Vermette, 2008; Sandhya et al. 2004). Recently, they have been found useful in the production of silver nanoparticles (Maliszewska et al., 2009; Vahabi et al., 2011).

Table 3. Various formulations of *Trichoderma* spp.

S/N	Formulations	Ingredients
1	Talc based	Trichoderma culture biomass along with medium: 1 liter, Talc (300 mesh, white colour): 2 kg and CMC: 10 g
2	Vermiculite-wheat bran based	Vermiculite: 100 g, Wheat bran: 33 g, Wet fermentor biomass: 20 g and 0.05N HCL: 175 ml
3	Wheat bran based	Wheat flour: 100 g, Fermentor biomass: 52 ml and Sterile water: sufficient enough to form a dough
4	Wheat flour-kaolin	Wheat flour: 80 g, Kaolin: 20 g and Fermentor biomass: 52 ml
5	Wheat flour-bentomite	Wheat flour: 80 g, Bentomite: 20 g and Fermentor biomass: 52 ml
6	Alginate prills	Sodium alginate: 25 g and Wheat flour: 50 g and Fermentor biomass: 200 ml

Adapted from Pandya, 2012.

Bioremediation technology

Investigations on bioremediation of environmental toxicants are entering in a new era with the application of genetic engineering. However, majority of the studies related to bioremediation have been conducted under the laboratory conditions. The concept of utilizing fungi for bioremediation of soil contaminated with certain pollutants is relatively older. There is liberal evidence of various *Trichoderma* spp. contributing to polycyclic aromatic hydrocarbons (PAHs) degradation, even as affecting native mycorrhizal fungi both positively and/or, negatively (Azcbn-Aguilar and Barea, 1997). Degradation potential of rhizosphere-competent *Trichoderma* strains against several synthetic dyes, pentachlorophenol, endosulfan and dichlorodiphenyl trichloroethane (DDT) were demonstrated previously (Katayama and Matsumura, 1993). Hydrolyses, peroxidase, lactases and other lytic enzymes produced by *Trichoderma* spp. are probable factors aiding indegradation of these contaminants. Therefore, application of some detoxifying agents along with *Trichoderma* spp. would provide healthy soil and environment (Table 4). It may help to improve not only the health of soil and plant, but also a sustained crop yield protection. *Trichoderma* spp. inoculated in the soil can grow rapidly because of naturally resistant ability to many toxic compounds, such as fungicides, herbicides, insecticides and phenolic compounds (Chet et al., 1997).

Trichoderma strains may play an important role in the bioremediation of soil contaminated with pesticides and possess the ability to degrade a wide range of insecticides: organochlorines, organophosphates and carbonates. ABC transporter protein systems in *Trichoderma* strains may be involved in resistance mechanisms against tested noxious compounds (Harman et al., 2004).

Biotic and abiotic stress tolerance

Trichoderma species are good source of natural proteins that may facilitate the plant to survive in the biotic as well as abiotic stress conditions. The *hsp*70 gene from *T. harzianum* T34 was cloned and characterized (Mantero-Barrientos et al., 2008) and encoding protein expression in Arabidopsis showed higher tolerance to heat and other abiotic stresses (Mantero-Barrientos, et al., 2008). The encoding protein product of this gene facilitates higher level of fungal resistance to heat and other stresses such as osmotic, salt and oxidative tolerances. Putative kelch-repeat protein coding gene *Thkel1* isolated from *T. harzianum* regulating the glucosidase activity was able to induce improved tolerance to salt and osmotic stresses in *Arabidopsis thaliana* plants (Hermosa et al., 2011). Number of proteins, for example mitogen-activated protein kinase, Sm1 (Small Protein 1), 4-phosphopantetheinyl transferase, and PKS/NRPS hybrid enzyme from *T. virens* were confirmed and involved in conferring resistance against several soil born and foliar pathogens (Howell et al., 2000; Perazzoli et al., 2012; Viterbo et al., 2005).

Wood preservation

Trichoderma spp. displayed a killing action against these fungi in *in vitro* tests, but *in situ* action was ineffective. Ejechi investigated the ability of *T. viride* to inhibit the decay of obeche (*Triplochiton sceleroxylon*) wood by the decay fungi *Gloeophyllum* sp. and *G. sepiarium* under field conditions under dry and wet season in tropical environment for 11 months. *T. viride* exhibited total inhibition of the decay fungi by means of mycoparasitism and competition for nutrients (Ejechi, 1997).

Industrial bioreactors

Biofuel production is one of the eco-friendly ways to reduce expenditure on energy sector and tackle the global warming effects on environment and human health (Rubin, 1997). *T. reesei*, a non-biological agent is one the

most important genus for industrial purposes as a factory for the production of secreted cellulase in biotechnology and a model for basic studies on protein secretion (Ahamed and Vermette, 2009; Li et al., 2013). Molecular insights into the mechanism of the cellulose degrading pathways and genome sequencing of *T. reesei* provide a platform to explore novel ways of metabolic engineering (Kubicek et al., 2009). *T. reesei* contains the smallest number of genes encoding enzymes responsible for plant cell wall degradation within Sordariomycetes (Martinez et al., 2008). An alternative strategy to the first generation energy sources includes manufacturing of biofuels using agricultural waste products with the help of cellulases andhemicellulases produced by *T. reesei* or other strains and further fermentation by other microbes such as yeast (Schuster and Schmoll, 2010). Nonetheless, the efficiency of this process needs to improve several folds of magnitude to reach final goal of equally compatible energy sources like fossil fuels. Additionally, genus *Trichoderma* is a good source of many secondary metabolites useful in application against phytopathogens, which Keswani and co-workers have recently summarized Keswani et al. (2014) Secondary metabolites inhibiting growth of pathogens can be used irrespective of geographic location and such formulations can be produced with longer shelf life.

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 (Sawant and Mukhopadhay, 1990; Pandey and Upadhyay, 1998; Sharma, et al., 1999; Nallathambi et al., 2001; Sushir and Pandey, 2001; Bhatt and Sabalpara, 2001; Patibanda et al., 2002; Lal and Maharshi, 2007, Madhusudan 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.

CONCLUSIONS

Trichoderma spp. possess many qualities and they have great potential use in agriculture such as amend abiotic stresses, improving physiological response to stresses, alleviating uptake of nutrients in plants, enhancing nitrogen-use efficiency in different crops, and assisting to improve photosynthetic efficiency. The use of this genus has expanded worldwide as general plant protectants and growth enhancers, besides their application in a variety of industrial processes. The genome of *Trichoderma* spp. has been extensively investigated and has proven to contain many useful genes, along with the ability to produce a great variety of expression patterns, which allows these fungi to adapt to many different environments (soil, water, dead tissues, inside the plants, *etc*.). The metabolomics of *Trichoderma* spp. are incredibly complex, especially in terms of secondary metabolites production but with the help of advanced molecular and proteomic approaches, it is possible to explore new pathways, novel functions of compounds produced by this genus and their potential applications. The proteome of *Trichoderma* spp. growing in a variety of conditions and interactions has been mapped, and the information has been used to develop new products based on synergistic combinations of the living fungus with its secreted metabolites. These new formulations, which combine biocontrol with biofertilization, are considered to be more effective than older products and active on a wider range of pathogens.

Conflict of Interests

The authors have not declared any conflict of interests.

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