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Review Article

TRICHODERMA: A BIOLOGICAL WEAPON FOR MANAGING PLANT DISEASES AND PROMOTING SUSTAINABILITY

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Trichoderma is a genus of asexually reproducing fungi that is present in all types of soils. Recent discoveries show that they are opportunistic, avirulent plant symbionts, as well as being parasites of other fungi. At least some strains establish robust and long-lasting colonizations of root surfaces and penetrate into the epidermis and a few cells below this level. They produce or release a variety of compounds that induce localized or systemic resistance responses. These root-microorganism associations cause substantial changes to the plant proteome and metabolism. Plants are protected from numerous classes of plant pathogen by responses that are similar to systemic acquired resistance and rhizobacteria-induced systemic resistance. Root colonization by *Trichoderma* spp. also frequently enhances root growth and development, crop productivity, resistance to abiotic stresses and the uptake and use of nutrients. As most of the crops are infected by the soil borne plant pathogens that primarily attack the vulnerable seeds or seedlings, the Trichoderma can be applied directly to target area, i.e., to seeds or seedlings and a single application using an existing delivery system (seed treatment, biopriming, furrow treatment) can significantly reduce crop losses. A number of successful products based on different species of Trichoderma have been commercialized in India and elsewhere. Whatever the limitations these Trichoderma products may have, it can be addressed by enhancing biocontrol through manipulation of the environment, accurate strain identification by molecular approach, using mixtures of beneficial organisms, physiological and genetic enhancement of biocontrol mechanisms, and manipulation of formulations. In some instances, other alternative methods that alone do not provide adequate protection may be integrated with application of biocontrol agents to provide additive or synergistic effects. This review tries to present the approaches adopted by researcher all over the world.

Keywords: Biological control, Disease management, Commercialization, Mode of action

INTRODUCTION

Plant diseases play a direct role in the destruction of natural resources in agriculture. In particular, soil borne pathogens cause important losses, fungi being the most aggressive. Chemical compounds have been used to control plant

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diseases, but abuse in their employment has favored the development of pathogens resistant to fungicides. Unfortunately, the more specific the effect of a chemical on an organism, the greater the probability of decreasing the effect through genetic shift in the population, whereas fungicides of broad spectrum produce undesirable consequences on non target organisms (*Tjamos et al.,* 1992). By contrast, the use of microorganisms that antagonize plant pathogens (Biological control) is risk free when it results enhancement of resident antagonist. Antagonist of phytopathogenic fungi has been used to control plant diseases, and 90% of such applications have been carried out with different strains of the fungus Trichoderma. The most common BCAs of the Trichoderma genus are strains of *T. viride*, *T. virens* and above all *T. harzianum*, which is a species of aggregate that includes different strains used as BCAs of phytopathogenic and viral vector fungi. (Grondona *et al.,* 1997).

WHAT IS *TRICHODERMA* ?

Trichoderma is a genus of asexually reproducing fungi that are often the most frequently isolated soil fungi; nearly all temperate and tropical soils contain 101-103 culturable propagules per gram. These fungi also colonize woody and herbaceous plant materials, in which the sexual Teleomorph (genus *Hypocrea*) has most often been found. However, many strains, including most biocontrol strains, have no known sexual stage. In nature, the asexual forms of the fungi persist as clonal, often Heterokaryotic, individuals and populations that probably evolve independently in the asexual stage .They show a high level of genetic diversity, and can be used to produce a wide range of products of commercial and ecological interest. They are prolific producers of extracellular proteins, and are best known for their ability to produce enzymes that degrade cellulose and chitin—although they also produce other useful enzymes (Harman and Kubicek., 1998). For instance, different strains produce more than 100 different metabolites that have known antibiotic activities (Sivasithamparam and Ghisalberti, 1998). *Trichoderma* species have long been recognized as agents for the control of plant disease and for their ability to increase plant growth and development, high reproductive capacity, ability to survive under very unfavorable conditions, efficiency in the utilization of nutrients, capacity to modify the rhizosphere, strong aggressiveness against phytopathogenic fungi and efficacy in promoting plant growth and defense mechanisms. They are becoming widely used in agriculture, and the most useful strains show a property that is known as 'rhizosphere competence'— that is, the ability to colonize and grow in association with plant roots. These properties have made Trichoderma a ubiquitous genus present in any habitat and at high population densities (*Chet et al.,* 1997). Trichoderma is more efficient in acidic than alkaline soils. Trichoderma BCAs control ascomycetous, deuteromycetous and basidiomycetous fungi, which are mainly soil borne but also air borne pathogens (Monte, 2001).

BIODIVERSITY AND PHYLOGENY OF *TRICHODERMA*

The first description of a fungus named *Trichoderma* dates back to 1794 (Persoon, 1794), and in 1865, a link to the sexual state of a *Hypocrea* species was suggested (Tulasne and Tulasne, 1865). However, the different species assigned to the genus *Trichoderma*/ *hypocrea* were difficult to distinguish morphologically. It was even proposed to reduce taxonomy to only a

single species, *Trichoderma viride*. Hence, it took until 1969 that development of a concept for identification was initiated (Rifai, 1969; Samuels, 2006). Thereafter, numerous new species of *Trichoderma*/ *hypocrea* were discovered, and by 2012, the genus already comprised more than 100 phylogenetically defined species. In some cases, especially in earlier reports, misidentifications of certain species occurred, for example with the name *Trichoderma harzianum* which has been used for many different species (Kullnig *et al*., 2001). In recent years, safe identification of new species was significantly facilitated by development of an oligonucleotide barcode (TrichOKEY) and a customized similarity search tool (TrichoBLAST), both available online at http://www.isth.info/ (Druzhinina *et al.,* 2005; Kopchinskiy *et al*., 2005). A further useful tool for characterization of newly isolated *Trichoderma* species (but also recombinant strains) are phenotype microarrays, which allow for investigation of carbon utilization patters for 96 carbon sources (Bochner *et al*., 2001; Druzhinina *et al*., 2006). 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 (Kredics *et al.,* 2012).The continued efforts to elucidate diversity and geographical occurrence of *Trichoderma*/*Hypocrea* resulted in detailed documentations of the genus in Europe and worldwide (Samuels *et al*., 2002a; Chaverri and Samuels, 2003; Jaklitsch, 2009). At present, the International Sub commission on *Trichoderma*/ *hypocrea* lists 104 species (http:// www.isth.info/biodiversity/index.php), which have been characterized at the molecular level. species of the genus produce a broad array of pigments from bright greenish-yellow to reddish in color, although some are also colorless. Similarly, conidial pigmentation varies from colorless to various green shades and sometimes also gray or brown.

Characteristics of *Trichoderma* spp.

Trichoderma spp. are ubiquitous colonizers of cellulosic materials and can thus often be found wherever decaying plant material is available (Kubicek *et al*., 2008; Jaklitsch, 2009) as well as in the rhizosphere of plants, where they can induce systemic resistance against pathogens (Harman, 2000). Despite the early suggested link between *Trichoderma* and *Hypocrea* (Tulasne and Tulasne, 1865), this anamorph–teleomorph relationship was only confirmed more than 100 years later for *Trichoderma reesei* and *Hypocrea jecorina* (Kuhls *et al*., 1996). Nevertheless, *T. reesei* was then termed a clonal, asexual derivative of *H. jecorina* because all attempts to cross the available strains of this species had failed. It took more than a decade until a sexual cycle was reported in any *Trichoderma* species (Seidl *et al.,* 2009), and a detailed study on molecular evolution of this species led to the discovery of a described sympatric agamo species *Trichoderma parareesei* (Druzhinina *et al*., 2010). Especially because of the industrial importance of *T. reesei*, the availability of a sexual cycle was a landmark discovery and now paves the way for elucidation of sexual development also in other members of the genus. *Trichoderma* spp. are highly successful colonizers of their habitats, which is reflected both by their efficient utilization of the substrate at hand as well as their secretion capacity for antibiotic, metabolites and enzymes. They are able to deal with such different environments as the rich and diversified habitat

of a tropical rain forest as well as with the dark and sterile setting of a biotechnological fermentor or shake flask. Under all these conditions, they respond to their environment by regulation of growth, condition, enzyme production, and hence adjust their lifestyle to current conditions, which can be exploited for the benefit of mankind. One of these environmental factors is the presence or absence of light. *Trichoderma* has a long tradition of research toward the effect of light on its physiology and development, which already started in 1957 and largely paralleled that of *Phycomyces blakesleeanus* (Schmoll *et al*., 2010). Besides effects on growth, reproduction, and secondary metabolite biosynthesis, which are common light responses in fungi, also a surprising influence of light on cellulase gene expression has been found (Schmoll *et al*., 2005). This link between light response and metabolic processes was further substantiated by a study on carbon source utilization using phenotype microarrays in light and darkness (Friedl *et al*., 2008). Studies on the molecular basis of these light effects revealed that interconnections between the signaling pathways of light response, heterotrimeric G-proteins, the cAMP-pathway, sulfur metabolism, and oxidative stress are operative in *Trichoderma* (Schmoll *et al*., 2010).

In recent years, research with *Trichoderma* has been facilitated significantly by sequencing of the genomes of three strains representing the most important applications of this genus: The genome sequence of *T. reesei*, the industrial workhorse (Martinez *et al.,* 2008), surprisingly revealed that, despite its importance in industrial cellulase production, its genome comprises the fewest amount of genes encoding cellulolytic and hemicellulolytic enzymes. Analysis and annotation of the genomes of *Trichoderma atroviride* and *Trichoderma virens*, two important biocontrol species is still in progress. Interestingly, the genomes of *T. atroviride* and *T. virens* are significantly larger than that of *T. reesei*, and they comprise roughly 2000 genes more than does *T. reesei*. It will be interesting to learn the significance of this considerable difference in genome sizes in the physiology of these fungi. These milestones in research with *Trichoderma* enabled detailed studies, which provided intriguing insights into their lifestyle, physiology, and the underlying mechanisms at the molecular level (Brunner *et al.,* 2008; Schmoll., 2008; Seidl *et al.,* 2009).

Trichoderma Species-Opportunistic, Avirulent, Plant Symbionts

Trichoderma spp. have evolved as opportunistic plant symbionts, as they can proliferate, compete and survive in soil and other complex ecosystems. They are also capable of colonizing roots and, in fact, increase in numbers when there are abundant healthy roots in the ecosystem. As they colonize plant roots they invade the superficial layers of the root, but do not penetrate further, at least in part because they elicit plant defence reactions. Therefore, although *Trichoderma* spp. probably have an intrinsic ability to attack plants, they are usually avirulent. The plant defence reactions can become systemic and protect the entire plant from a range of pathogens and diseases, even when *Trichoderma* spp. grow only on the roots. This root colonization also increases the growth of roots and of the entire plant, thereby increasing plant productivity and the yields of reproductive organs. They also help plants to overcome abiotic stresses, and improve nutrient uptake. These findings indicate that *Trichoderma* spp. have developed a symbiotic rather than a parasitic relationship with plants.

Benefits of Trichoderma

Disease Control: Trichoderma is a potent biocontrol agent and used extensively for soil born diseases. It has been used successfully against pathogenic fungi belonging to various genera, viz., Fusarium, Phytopthara, Scelerotia, etc.

Plant Growth Promoter: Trichoderma strains solubilize phosphates and micronutrients. The application of Trichoderma strains with plants increases the number of deep roots, thereby increasing the plant's ability to resist drought.

Biochemical Elicitors of Disease: Trichoderma strains are known to induce resistance in plants. Three classes of compounds that are produced by Trichoderma and induce resistance in plants are now known. These compounds induce ethylene production, hypersensitive responses and other defense related reactions in plant cultivars.

Transgenic Plants: Introduction of endochitinase gene from Trichoderma into plants such as tobacco and potato plants has increased their resistance to fungal growth. Selected transgenic lines are highly tolerant to foliar pathogens such as *Alternaria alternata, A. solani,* and *Botrytis cirerea* as well as to the soil-borne pathogen, *Rhizectonia* spp.

Bioremediation: Trichoderma strains play an important role in the bioremediation of soil that are contaminated with pesticides and herbicides. They have the ability to degrade a wide range of insecticides: organochlorines, organophosphates and carbonates.

Foods and textiles: *Trichoderma* spp. are highly efficient producers of many extracellular enzymes. They are used commercially for production of cellulases and other enzymes that degrade complex polysaccharides. They are frequently used in the food and textile industries for these purposes. For example, cellulases from these fungi are used in "biostoning" of denim fabrics to give rise to the soft, whitened fabric—stone-washed denim. The enzymes are also used in poultry feed to increase the digestibility of hemicelluloses from barley or other crops.

Plant growth promotion: For many years, the ability of these fungi to increase the rate of plant growth and development, including, especially, their ability to cause the production of more robust roots has been known. The mechanisms for these abilities are only just now becoming known.Some of these abilities are likely to be quite profound. Recently, we have found that one strain increases the numbers of even deep roots (at as much as a meter below the soil surface). These deep roots cause crops, such as corn, and ornamental plants, such as turfgrass, to become more resistant to drought.Perhaps even more importantly, our recent research indicates that corn whose roots are colonized by *Trichoderma* strain T-22 require about 40% less nitrogen fertilizer than corn whose roots lack the fungus. Since nitrogen fertilizer use is likely to be curtailed by federal mandate to minimize damage to estuaries and other oceanic environment, the use of this organism may provide a method for farmers to retain high agricultural productivity while still meeting new regulations likely to be imposed.

Modes of Action of *Trichoderma*

The following are the modes of biocontrol action of *Trichoderma*.

Mycoparasitism

Trichoderma spp. parasitize a range of other

fungi. The events leading to mycoparasitism are complex, and take place as follows: first, *Trichoderma* strains detect other fungi and grow tropically towards them (Chet *et al.,* 1981). Once the fungi come into contact, *Trichoderma* spp. attach to the host and can coil around it and form APPRESSORIA on the host surface. Attachment is mediated by the binding of carbohydrates in the *Trichoderma* cell wall to lectins on the target fungus. Once in contact, the *Trichoderma* produce several fungitoxic cell-wall-degrading enzymes , and probably also peptaibol antibiotics (Benhamou and Chet, 1997)). The combined activities of these compounds result in parasitism of the target fungus and dissolution of the cell walls. At the sites of the appressoria, holes can be produced in the target fungus, and direct entry of *Trichoderma* hyphae into the lumen of the target fungus occurs. There are at least 20-30 known genes, proteins and other metabolites that are directly involved in this interaction, which is typical of the complex systems that are used by these fungi in their interactions with other organisms.

Antibiosis

Antibiosis occurs during interactions involving low molecular weight diffusible compounds or antibiotics produced by *Trichoderma* strains that inhibit the growth of other microorganisms. *Trichoderma* known for its mycoparasitic activity against several fungal plant pathogens is aided by the production of different chitinases, ß-1, 3-glucanases and proteases and cellulase. These extra cellular enzymes such as ß-1,3-glucanase, chitinase and cellulase are effective in disrupting the mycelium of plant pathogenic fungi (Samuels *et al.,* 2002). Production of volatile compounds was not detected on the four isolates of *T. harzianum* that were tested *in vitro* against *R. solani* (Cumagun *et al.*, 1997b). Coconut smell is typical of *T viride* isolates (Rifai, 1969), suggesting the presence of volatile compounds that are inhibitory to pathogen growth (Dennis and Webster, 1971). The isolation and over expression of tri5 (trichodiene synthase) gene in *T. brevicompactum* Tb41tri5 transformant increased the trichodermin production and fungal activity against *Aspergillus fumigates* and Fusarium spp. (Tijerino *et al*., 2011). The greater efficacy of antibiotic engineered biocontrol strains raises the scope of designating effective biocontrol strains against various crop diseases.

Competitive Saprophytic Ability

Competition for substrates is the most important factor for fungi as is competition for light in the case of evolution of plants (Garrett., 1956). Competition is the phenomenon in which the pathogen and the introduced *Trichoderma* compete for the availability of space and nutrients. *Trichoderma* is capable of degrading straw with mycoparasitic ability against several plant pathogenic fungi. Cumagun *et al.,* (2009) found that the higher the amount of inoculum of *T. harzianum* strain no 94-016, the higher percent colonization of rice straw in the soil. Further tests were carried out to compare the decomposition of rice straw on the surface and buried in the soil and the effect of moisture on soil decomposition. The depth of rice straw and moisture content are factors identified as significant in affecting crop residue decomposition. Buried rice straw inoculated with *T. harzianum* strain no 94-016 with watering on a daily basis provided better decomposition (Cumagun *et al.,* (2009). Knowledge gained from this study could help better understand and design field application of the beneficial microorganisms for disease management.

Induced Resistance

Induced systemic resistance is believed to be one of the most important mechanisms of biocontrol effects of *Trichoderma* (Harman *et al.*, 2006). A variety of strains of *T. virens*, *T. asperellum*, *T. atroviride* and *T. harzianum* induce metabolic changes in plants that increase resistance to a wide range of plant-pathogenic microorganisms and viruses (Table 1). Moreover, this response seems to be broadly effective for many plants; for example, *T harzianum* strain T-22 induces resistance in plants as diverse as tomatoes and maize, which indicates that there is little or no plant specificity. When spores or other propagative structures are added to soil and come into contact with plant roots, they germinate and grow on root surfaces, and at least some infect the outer few root cells. They produce at least three classes of substance that elicit plant defence responses that prevent further infection of roots by plant pathogens. These elicitors include peptides, proteins and low-molecular weight compounds. In some cases, the resistance is localized—as seems to be the case with *T. virens* on cotton—but in most plant–*Trichoderma* systems, the resistance is systemic. At least for a short period of time, increased expression of defence-related genes occurs throughout the plant. This process can be transitory, but strongly potentiates the expression of defence-related proteins when plants are challenged by pathogens at sites distant from the location of the *Trichoderma*

Table 1: Evidence for, and Effectiveness of, Induced Resistance in Plants by *Trichoderma* Species

Species and Strain	Plant	Pathogens	Evidence or Effects	Time after application	Efficacy	References
T. harzianum T-1&T22; T *virens* T31	Cucumber	Green mottle mosaic virus	Protection of leaves when Trichoderma strains were present only on roots	7 days	Disease induced reduction in growth eliminated	Lo *et al.,* 2000
T. harzianum T-22	Tomato	Alternaria solani	Protection of leaves when T-22 was present only on roots	3 months	Up to 80% reduction in early blight from natural field infection	Seamon, 2003
Trichoderma GT3-2	Cucumber	C. orbiculare p. syringae pv. lachrymans	Protection of leaves when *Trichoderma* strains were present only on roots; induction of lignification and superoxide generation	1 day	59% and 52% protection from disease caused by *C. orbicular*epv. lachrymans present only on roots; or *P. syringae*, respectively	Koike, 2001
T. harzianum	Pepper	*Phytophthora capsici*	Protection of stems when *Trichoderma* strains were present only on roots;	9 days	~40% reduction in lesion length	Ahmed *et al.,* 2000

strain. These results indicate that the initial reactions, which include production of pathogenesis-related proteins, have features in common with SAR. At least for the interaction between *T. asperellum* and cucumber, a longer-term response includes low levels of expression of pathogenesis-related proteins before pathogen infection. This response therefore has elements in common with ISR.

Enhancement of Biocontrol Ability of *Trichoderma*

There are constraints in using *Trichoderma* as biocontrol agents. *Trichoderma* colonizes in the spermosphere effectively but they do not survive well in the rhizosphere (Deacon, 1994). The same author observed that *Trichoderma* spp. are active only in some types of soil and season thus achieving only transitory localized dominance of the rhizosphere. For this reason, another constraint is the quiescent and inactive nature of *Trichoderma* spores in the soil and because of this, *Trichoderma* cannot be added as spores (Vidhyasekaran, 2004). Furthermore, numerous mechanisms were discovered to improve efficacy of Trichoderma for desirable characteristics like better saprophytic ability, survival ability in the environment, rhizosphere competence, wider host range, tolerance to pesticides, tolerance to adverse environmental conditions, vigorous growth and long shelf life, the method used are as follows:

Protoplast Fusion

Trichoderma is known as a producer of glucanses, chitinases, cellulases and various other mycolytic enzymes. Combination of these desirable traits form different strains can give rise to superior strains. The strain improvement of Trichoderma by protoplast fusion is one of the ways. The advantage of protoplast fusion is that the possibility to obtains recombinants is higher, which also allows testing of a large number of recombinanats in a short time. Sundaram (1996) developed fusants of two isolates of *T. harzianum* (Th-1 and Th-3), among them, some showed mycological characteristics intermediate between Th-1 and Th-3. In majority of fusants, dominance of characteristics of Th-1 over Th-3 was observed. When *T. harzianum* (Th-3) was fused with *T. virens*, many fusants were developed and few of them exhibited improved biocontrol potential over parent isolates. Protoplast fusants developed by taking isolates of Trichoderma spp., as parent strains exhibited an enhanced antagonistic potential against *Fusarium oxysporum, Rhizoctonia solani, Venturia inaequalis, and Cochliobolus miyabeanus* (Lalitha kumari, 2000).

Mutation

Mutation are employed to generate variability in populations, which give an opportunity to select the desirable type. Criteria defined for selecting a particular phenotype from the population, N-methyl-N- nitro nitrosoguanidine (NTG) has been the most widely used chemical for inducing mutations in fungal Trichoderma. By exposing the conidia of Trichoderma spp, to NTG, Ahmad and Baker (1990) generated mutants that were rhizosphere competent and superior to wild type in respect of controlling *Pythium ultimum*. These mutants were insensitive to up to 100 µg/ml of benomyl. Mukhopadhyay (1993) developed seven stable mutants of *T. virens* by exposing the cultures to 125 k rad of gamma radiation. The mutants differed from the wild type strains in phenotype, growth rate, sporulation and antagonistic potential. One of the mutants (M- 7) that produced profuse chlamydospores but no

conidia was more aggressive than the wild type in antagonizing *Sclerotium rolfsii* and *Fusarium oxysporum f. sp. ciceri*. Selvakumar (2000) developed carboxin tolerant mutant *Trichoderma viride* by exposing the culture to UV – light and ethyl methane sulfonate , Triazole tolerant biotypes of *T. viride* developed by UV- irradiation showed wide variation in enzyme activity especially β-1,3-glucanase and chitinases .

Genetic Engineering

The biosynthesis of cell wall degrading enzymes like glucanases, chitinase and protease which are involved in mycoparasitism, is controlled mainly at transcriptional level and responsible genes are present as single copy genes. To overproduce these enzymes, their gene copy number has been increased by transformation (Harmon and Bjoorman, 1998). Transformants overproducing these enzymes are more efficient as biocontrol agents (Table 2). Biocontrol efficiency of Trichoderma has been improved by transformation with genes prbl (protease), egll (β-1,4-glucanase) and chit 33(chitinase). Lo *et al* ., (1998) developed transformant of *T. harzianum* strain 1295-22 by integrating *β*-glucuronidase (GUS) and hygromycin B (hyg B) phosphor-transferase genes, that exhibited increased biocontrol activity *R. solani* as compared with the wild type.

Commercialization of Trichoderma

Before a biological control agent can be successfully introduced into the market, a series of studies must be carried out. Concomitantly , production and formulation of an inoculums with an acceptable shelf life had to be achieved which is frequently a bottleneck. With the availability of large quantities of inoculums, field and glass house test on relevant crops are to be done to determine the reproducibility of control. In past, commercialization of Trichoderma spp. as a BCA had been hampered by the lack of a cost effective way of producing sufficient amounts of fungal material to be used in field and glass house trials. However, in recent years, many small and large entrepreneurs have entered into commercial production of Trichoderma spp. as BCA resulting in development of several products into world market. Commercialization of biopesticides is a multistep process involving a wide range of activities depicted below.

Table 2: Genetically Modified Strains With Biocontrol Properties Against Various Plant Pathogens

Source Organism (gene)	Transformed	Crop/Pathogenic organism	Remarks	Reference
Trichoderma brevicompactum (tri 5-trichodiene synthase gene)	*Trichoderma brevicompactum* Tb41tri5	Aspergillus fumigatesFusarium spp.	Overexpression of tri5 gene Enhanced trichodermin activity	Tijerino *et al*., 2011
Trichoderma harzianum(ech42 chitinase gene)	T. virens	*R. solani in vivo*	Improved lytic activity	Baek *et al*., 1999
T. atroviride (prb l proeinase gene)	*T. atroviride* SJ3-4	Bean/*R. solani, Pythium ultimum. Botrytis cinerea*	Glucose gene insertion enhances the ISR activity in bean plants	Brunner *et al.,* 2005

Discovery

Development of bioagents begins with discovery of a useful naturally occurring microorganism. The search involves extensive screening of natural soil isolates for Trichoderma strains having the desired traits. Isolates are then screened for activity against the pathogen in laboratory assay and green house condition. Once an isolate with desirable traits is obtained. It is evaluated under field condition. Many of the promising soil isolates of Trichoderma in recent years have been obtained in the laboratories of India and abroad.

Mass Production

One of the greatest impediments to biological control by *Trichoderma* has been the paucity 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. 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 masse production of Trichoderma, use of a wide variety of matrices, low capital investment, low energy expenditure, less expensive down stream processing, less water usage and lower waste water output, potential higher volumetric productivity, high reproducibility, lesser fermentation space and easier control of contamination (Singh, 2006). Fermented biomass of *Trichoderma* consisted mainly of chlamydospores and conidia with some amount of mycelia fragments. Air dried mats were grounded and mixed with a commercially available carrier. The formulation thus developed contained 10^8 to 10^9 propagules/g.

Formulation

Developing a safe, easy to use, cost effective formulation that will keep the micro organism alive is one of the most important steps in developing a biological product. Formulation is the blending of active ingredients such as fungal spores with inert carriers such as diluents and surfactants in order to improve the physical characteristics. A final formulation must have a long shelf life, at room temperature, be east to handle, insensitive to abuse and must be stable over a rage of –5 to 35^0C. Failure to meet these grid standards, however, should not stop the commercialization bioagents. What is needed urgently is the development of drying techniques, which allow retention of maximum number of viable propagules in dried product. At PDPC, Bangalore, three carrier materials namely talc, kaolin and bentonite were tested for their effect on shelf life of *T. harzianum*. Kaolin, and talc were identified as best carriers of *T. harzianum.* (Prasad and Rangshwaran, 2000). Combination of *Trichoderma harzianum* and plant growth promotory rhizobacteria *Pseudomonas fluorescens had* greater disease suppression

and enhanced consistency against the pathogens, viz., *Rhizoctonia solani, Sclerotium rolfsii* and *Macrophomina phaseolina* responsible for root and stem rot disease of Soybean. Application of more than one antagonists of diverse origin is suggested as a reliable means of reducing the variability and increasing the reliability of biological control. (Mishra *et al.*, 2011) So consortium of Trichoderma with other biocontrol agents also need to be developed.

Shelf Life

One of the major impediments to commercialization of a biopesticidal preparation is the loss of viability of the biocontrol agents over time. A talc based preparation of *T. virens* conidia retained 82% viability at 5^0C in refrigerator after 6 months while at room temperature (25-30^0C) same level of viability was observed only upto 3 months. Shelf life was same, when *T. virens* treated chickpea or soybean seeds were stored at room temperature. Viability was better for CMC based formulations (Tewari, 1996). Conidia of Trichoderma in pyrophyllite survived better than fermentor biomass propagules alone at –5^0C to 30^0C. The most suitable temperature to prolong shelf life of conidia and fermentor biomasss propagules in pyrophyllite were –5 to 5^0C. (Mukherjee, 1991). Seed coating with biocontrol agents has emerged as a feasible way of delivering the antagonist, i.e., supplying the coated seed to the farmers directly by the seed companies/agencies. Mukherjee (1991) quantitatively assessed the viability of *T. virens* on coated chickpea seeds when the seeds were stored at low temperature (5^0C) and at room temperature (15-35^0C), 88% of propagules remained viable for up to 4 months.

Chlaymydospres based formulations of Trichoderma sp. exhibited longer shelf life (80% viability for 9 months) than conidia based formulations (80% viability for 4 months) at room temperature. A preparation of *T. virnes* (mainly in the form of chlaymydospores was from peat moss czapeks broth culture) stored at 25^0C for six months without loss of viability,

Green House and Field Testing

Testing of Trichoderma begins in growth chambers or green chambers. Although green house testing provide a useful preliminary screening for selecting Trichoderma for further testing , it is very difficult to mimic true farming conditions. Therefore, well designed field trials are necessary to establish the efficacy of any by-product. Once promising results are obtained they are tested under field conditions. It is important that field tests ultimately be conducted at several locations to prove efficacy under varying climatic conditions.

Market Potential

Presently, *Trichoderma* spp. based products are considered as relatively novel type of BCAs. In comparison to *B. thringiensis* biopesticides, their market size is quite small (Bt shares about 97% of overall pesticides), they fall in remaining 3% bracket, which also comprises viral and nematode based biopesticides. However, a general consensus is that Trichoderma spp. based BCAs share about 60 % of all fungal based BCAs and an increasing number of Trichoderma spp. based BCAs products are registered regularly. Moreover, field application/trials through out the world is being accepted and many biopesticide companies are endorsing these products on regular basis. The innate qualities (e.g., simultaneous biocontrol and growth promotion) of Trichoderma spp. based BCAs are driving factors for their steadily cumulating success.

Constraints in commercialization of *Trichoderma* spp. BCAs.

Despite all the acquired understanding about antagonistic action and growth promotion of the Trichoderma spp., there are nevertheless some hurdles to their wide spread success:

1. Raw material like, glucose, sucrose , corn steep liquor, wheat bran, soya meal, fish meal, used in culture media for production of the fungi are very costly.
2. Low efficacy.
3. Low spore yield.
4. Difficulties in quantification of BCA activity.
5. Most of the Trichoderma based BCAs are unregistered and are being marketed simply as soil enhancers , probably due to lack of well defined modes of action of these fungi and their underdeveloped bioassay methods.
6. Furthermore, the registration of Trichoderma spp. based BCAs as fungicides anf growth promoters is time consuming , expensive and frequently without well defined protocols.

Trichoderma Formulations

Important commercial formulations are available in India and Abroad(Table 3) .These formulations contain 3 x10^6 cfu per 1 g of carrier material. Talc is used as carrier for making powder formulation

Trichoderma Formulations: Important commercial formulations are available in India and Abroad (Table 3). These formulations contain 3 x10^6 cfu per 1 g of carrier material. Talc is used as carrier for making powder formulation.

Uses: Used in Damping off caused by *Pythium* sp. *Phytophthora* sp., Root rot caused by *Pellicularis filamentosa*, Seedling blight caused by *Pythium*, Collar rot caused by *Pellicularia rolfsii*, Dry rot caused by *Macrophomina phaseoli*, Charcoal rot caused by *Macrophomina phaseoli*, Loose smut caused by *Ustilago segetum*, Karnal bunt diseases, Black scurf caused by *Rhizoctonia solani*, Foot rots of Pepper and betel vine and Capsule rot of several crops. Effective against silver leaf on plum, peach and nectarine, Dutch elm disease on elm's honey fungus (*Armillaria mellea*) on a range of tree species, Botrytis caused by *Botrytis cinerea*, Effective against rots on a wide range of crops, caused by *fusarium, Rhizoctonia*, and *pythium*, and sclerotium forming pathogens such as *Sclerotinia* and *Sclerotium.*

Recommended For: Trichoderma is most useful for all types of Plants and Vegetables such as cauliflower, cotton, tobacco, soybean, sugarcane, sugarbeet, eggplant, red gram, Bengal gram, banana, tomato, chillies, potato, citrus, onion, groundnut, peas, sunflower, brinjal, coffee, tea, ginger, turmeric, pepper, betel vine, cardamom, etc.

Precautions

- Don't use chemical fungicide after application of Trichoderma for 4-5 days.
- Don't use *trichoderma* in dry soil. Moisture is a essential factor for its growth and survivability.
- Don't put the treated seeds in direct sun rays.
- Don't keep the treated FYM for longer duration.

Compatibility

- *Trichoderma* is compatible with Organic manure *Trichoderma* is compatible with biofertilizers like *Rhizobium, Azospirillum, Bacillus subtilis* and *Phosphobacteria*.
- *Trichoderma* can be applied to seeds treated with metalaxyl or thiram but not mercurials. It can be mixed with chemical fungicides as tank mix.

Table 3: Some Commercial Products of *Trichoderma* Spp. Available in India and Abroad

Species/strain of Trichoderma	Product	Agency/Company
Trichoderma viride	Ecofit	Hoechest and Schering Agro. Evo. Ltd., Mumbai, India
Trichoderma viride	Funginil	Crop Helath Bioproduct Research Centre, Gaziabad (UP)India
Trichoderma viride	Trichogourd	Anu Biotech international Ltd. Bangalore, India
Trichoderma viride	Defense SF	Wockhardt Life Science Ltd., Mumbai India
Trichoderma viride	Tricho-X	Excel Industries Ltd., Mumbai,India
Trichoderma viride	Biogourd	Krishi Rasayan Export Pvt. Ltd., Solan (HP), India
Trichoderma viride	Biocon	Tocklai Experimental Station Tea Research Association, Jorhat (Assam), India
Trichoderma viride	Bip T	Poland
Trichoderma harzianum	Pant biocontrol agent-1	Deptt. of Plant Pathology, GB pant University of Agriculture &Technology, Panatnagar, Uttarakhand
Trichoderma harzianum T-22	Top shield, Root shield	Bioworks , Geneva (Switzerland) & New York (USA)
Trichoderma harzianum T-22	T-22 and T-22 B	TGT Inc., New York USA
Trichoderma harzianum	F-Stop	Eastern Kodak Company TGT Inc. New York
Trichoderma harzianum	T-35	Makhteshim- Agan Chemical , Israel
Trichoderma harzianum	Harzian 10 and 20	Natural Plant Protection , Noguerres, France
Trichoderma harzianum strain T-39	Trichodex	Makhteshim- Agan Chemical , Israel
Trichoderma viride+ Trichoderma harzianum	Bioderma	Biotech international Ltd., new Delhi, India
Trichoderma viride+ Trichoderma harzianum	Ecoderma	Morgo Biocontrol Pvt. Ltd., Bangalore, India
Trichoderma harzianum +Trichoderma viride	Trichodowel, Trichoject, Trichopel	Agrimm, Technologies Ltd., New Zealand
Trichoderma harzianum+ T. polysporum	Binap- T&W	Bio Innovation AB, Toreboda, Sweden
Trichoderma virens	Gilogard and Soil guard	Greece- Sierre Company, Maryland

Need of Future

- Highly effective strains of *Trichoderma* spp. should be obtained or produced which contains several genes encoding for the products inhibitory to the plant pathogens.
- The gene/genes conferring biocontrol like activities in the Trichoderma spp. should be cloned in the crop plants that may become basis for "nature organic" crop protection and production.
- The strains of Trichoderma used for biocontrol purpose should be such that compete, proliferate, colonize and can protect the roots for the life of the crop plants from a wide range of pathogens.
- Inexpensive production and formulations of Trichoderma should be popularized.
- Base materials used for the development of formulations must ensure high viability and long lasting storage capacity.
- Delivery system adopted should ensure good growth of bioagents and high field performance.
- Public sector should also come forward to support the agencies engaged in this field.
- Intellectual property right regarding the production and development of new strains and formulations of Trichoderma always be protected.

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