From Biological Control to Bioactive Metabolites: Prospects with *Trichoderma* for Safe Human Food

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ABSTRACT

*Trichoderma*, a commonly known mycoparasite inhabiting nearly all agricultural soils, has shown outstanding biological properties in controlling growth of other less desirable or more harmful (pathogenic) types of fungi. What makes *Trichoderma* very interesting is that it uses local materials (decaying products) for proliferation, is non-toxic and biodegradable, produces numerous useful metabolites with complex chemistry and performs diverse biological activities. What is more intriguing, however, is its ability to target a specific mechanism rather than killing or repelling organisms indiscriminately. Although the biological control ability of *Trichoderma* has been studied and proven for many years, the ability of these fungi to increase the rate of plant growth and development, particularly to enhance the production of more robust roots, is now being documented. While working on more than 260 strains of *Trichoderma* collected from different habitats, the researchers have documented the bio-control ability of these organisms, not only at laboratory level but also at the field level, as well as up to the extent of commercialization. Based on the study, the researchers also discovered that several strains increased the size and numbers of deep roots which were quite below the soil surface. These deep roots cause crops, such as corn, fruit crops and ornamental plants, to become more resistant to drought. Besides such potentialities, certain *Trichoderma* species are highly efficient producers of many extracellular enzymes and are used commercially for the production of cellulases and other enzymes which degrade complex polysaccharides. They are frequently used in the food and textile industries. In particular, *Trichoderma* protease appears to exhibit excellent mechanisms of action in controlling grey mold on the surface of bean leaf by preventing germination of mold spore and deactivating harmful mold enzymes. The researchers’ recent interests warrant the use of secondary metabolites as potential biopesticidal and biofungicidal agents. From the local free-living *Trichoderma* isolates, different chemical fractions are extracted and separated in search of novel bioactive metabolites for their *in vitro* testing against phytopathogenic fungi, bacteria and pests. In addition to this, the researchers are now planning to extend such studies to the marine-derived *Trichoderma* species, such as *Trichoderma reesie*, with an aim to evaluate their biological activity and ability to be successfully used in field trials to control many crop pathogens.

Keywords: *Trichoderma*, biological control, bioagent, bioactive metabolites

INTRODUCTION

Access to food remains a central issue to every civilization from the time immortal, and agriculture, as a whole, is the only basic activity performed by the humans to feed all civilizations. However, with the increasing need and greed for acquiring more and more and global commercialization of the agricultural products to gain maximum economic returns, even at the cost of deviation from the natural way of growing food crops, the griming scenario of agriculture all over the world has started. Huge industrialization, chemicalization in farm inputs, unscientific farming practices and vastly expanding population have worsened the situation even more. Therefore, the concept of sustainability in agriculture is being advocated in farming communities to re-establish the losing
potential of farms, natural quality of foods and feeds, as well as to regain the decline in human health, due to the consumption of contaminated food. All these motives can only be achieved with the dependency of low-input agriculture practices, by recycling agriculture wastes into the farms, minimizing the use of chemical farm inputs, promoting bio-inputs such as microbes, beneficial fungi and nematodes, bioagents, worms, etc., to keep the soil and plant in good health, and over and above, harmonizing the natural ways of producing crops.

Pesticide consumption in India amounts to 288 g/ha, and crops produced for agricultural exports worth US$ 25 million are being rejected yearly due to the presence of high pesticide residues. There is a growing movement in many countries to mitigate the amount of chemicals being released into the environment or stop its use altogether. The persistence and accumulation of these chemicals in the food chain and environment (especially in the soil and aquatic ecosystem), over a period of time, are major causes for concern due to their inherent hazards to plants and human life. It is a fact that world food production needs to be increased to feed the ever-increasing population. However, it would be better if such practices, which enhance the quality of food in terms of less residual or phytotoxic effects of chemicals, are adapted. It is for this reason that the natural ways of pest and disease control, using natural enemies of the pest or disease-causing agents, have been emerged as a potential alternative to hazardous farm chemicals. For such an approach, the use of biofertilizers and biopesticides is being recommended, along with the use of other practices of organic agriculture. When human civilization started cultivation of crops approximately 10,000 years ago, it encountered the losses in crop yields due to plant diseases, decreased soil fertility, unsuitable soil, and moisture conditions. The early farmers observed that when the same land was used continuously for the cultivation of the same crop, the yields decreased in subsequent years and that by shifting to new fields for cultivation, the yield could be increased in many folds. The management of plant pathogens by the use of crude chemicals, like Bordeaux mixture, was started in the nineteenth century and their use kept on increasing in the ensuing years. Nevertheless, to maintain sustainability, the use of microbes as fertilizers and pesticides has become instrumental in keeping plant healthy and enhancing the yield qualitatively and quantitatively.

Trichoderma as Biocontrol Agent

Trichoderma, a genus of asexually reproducing fungi, is present in nearly all temperate and tropical soils. The strains of Trichoderma spp. are strong opportunistic invaders, fast growing, prolific producers of spores and powerful antibiotic producers. These properties make these fungi ecologically very successful and are the reasons for their ubiquitousness (Kubícek et al., 2002). They show a high level of genetic diversity and can be used to produce a wide range of products of commercial and ecological interest. Much of the known biology and many of the uses of these fungi have been documented recently (Buhariwalla, 2005; Druzhinina, 2006).

Trichoderma as a Source of Potential Bioactive Metabolites

With the growing interest in the biological properties of Trichoderma species, several bioactive metabolites have been isolated and identified from different strains. These compounds possess diverse structures which belong to different classes of molecule and accordingly possess a range of bioactivities (Table 1). Trichoderma species produce different volatile and non-volatile compounds which inhibit the growth of fungal phytopathogens. The mechanism of antibiosis constitutes a much more complex system, leading to the phenomenon of biological control. Among these antibiotics, the production of gliovirin, gliotoxin, viridin, pyrones, peptabiols and others have been described extensively (Vey et al., 2001). Besides, the production of a large variety of volatile secondary metabolites by Trichoderma (e.g. ethylene, hydrogen cyanide, alcohols, aldehydes and ketones up to C4 chain-length) also plays an important role in biocontrol. Another antibiotic compound, i.e. peptabiols, which are non-ribosomally synthesized antimicrobial peptides and exhibit antibacterial and antifungal properties, represented by alamethicin (Reusser, 1967; Landreau et al., 2002), do play an important role in antagonism. To date, 309 peptabiols have been sequenced, among them more than 180 are synthesized by Trichoderma. Xiao-Yan et al. (2006) studied antimicrobial metabolites, produced by Trichoderma koningii SMF2, and
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<th>Principles constituents/metabolites</th>
<th>Isolated from</th>
<th>Biological activity</th>
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<tr>
<td>Trichodermin, Trichodermol</td>
<td>Trichoderma polysporum and T. sporulosum</td>
<td>Growth inhibitors</td>
<td>Adams and Hanson. Sesquiterpenoid metabolites of <em>Trichoderma polysporum</em> and <em>T. sporulosum</em>. <em>Phytochemistry</em>, 1, 92, 423.</td>
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<td>Sesquiterpenoids</td>
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TABLE 1 Cont.

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<th>Compound</th>
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<td>Bisorbicillinoid</td>
<td><em>Trichoderma</em> sp. USF-2690</td>
<td>Free radical scavenger</td>
<td>Abe, N., Sugimoto, O., Arakawa, T., Tanji, K., Hirota, A. Sorbicillinol, a key intermediate of bisorbicillinoid biosynthesis in <em>Trichoderma</em> sp. USF-2690, School of Food and Nutritional Sciences, University of Shizuoka, Japan. <a href="mailto:abe@fns1.u-shizuoka-ken.ac.jp">abe@fns1.u-shizuoka-ken.ac.jp</a></td>
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showed them to be effective against a wide range of fungal phytopathogens. Although production of antibiotics by *Trichoderma* involved in biocontrol is a well-documented phenomenon, clear identification and understanding of the role of antibiotics in disease control lags far behind than that in bacteria and therefore needs to be addressed (Whipps, 2001; Singh *et al*., 2002). Further research is needed to study the toxicity of antibiotic compounds and the mechanisms of their biocontrol activity against phytopathogens.

**Trichoderma - Selection of Potential Strains for Biocontrol**

The discovery of fungal antagonists has led to new challenges in research, development, and registration of biocontrol products in a market where chemical pesticides dominate. Bringing a biocontrol product into market is a multilayered process including discovery, efficacy trials, toxicological testing, mass production, formulation, and registration (Singh *et al*., 2001, 2002, 2004). Four steps mark the process of strain selection viz., the selection of effective strains in relation to plant pathogens; screening of isolates with high biotechnological indices, analysis of properties specific for plants in pathogens, useful insects, animals and humans, as well as search for economically viable substrates, convenience for mass multiplication of fungus which maintain high colony forming units (cfu) for longer periods (Singh *et al*., 2002, 2003, 2007). The first step in developing a fungal biocontrol agent is the discovery, through empirical or targeted screening. Isolation and screening of potential fungal biocontrol agents have been identified in numerous cropping systems or natural ecosystems (Baker and Cook, 1982). Following isolation of potential strain of *Trichoderma* spp. for a particular or broad range of diseases, screening of the fungal isolates for biocontrol activity is generally performed *in vitro*, along with certain tests to establish the identity and relatedness of the newly found strains. This is most often accomplished using various available standard techniques. The relative ease of finding the right antagonist strain of *Trichoderma* and other biocontrol micro-organisms under laboratory and field conditions is an added advantage, but it does ensure that the agent will work consistently and effectively in the field.

The development of a highly successful strain, T-22 of *T. harzianum*, by Harman and his colleagues took more than a decade and it was only in later part of the last century that the sale of *Trichoderma*-based products started to pick up from the scratch (Mathre *et al*., 1999; Harman, 2000). Similarly, the researcher also had gone through a similar period of time in their endeavour to isolate the potential strain of *Trichoderma*. In the study, several strains of *Trichoderma* of different species (such as *T. harzianum, T. viride, T. atroviride, T. koningii*) were isolated. They have been found effective against a large number of fungal phytopathogens affecting several economically important crops (Singh *et al*., 2006). Kalra *et al*., (2002) developed a potential strain of *Trichoderma harzianum* (ATCC-PTA3701) which is useful as a nematode inhibitor, biofungicide and plant growth promoter (US Patent No. 6,475,772). The application of this strain, as a biocontrol agent of soil-borne fungal pathogens, had additional advantages in improving the growth of plants and economic yields of crop plants, contribution to the reduction of deleterious nematode population in the host tissue and rhizosphere, and thereby, reducing the severity of root knot disease. Moreover, its use as soil amendment in reducing the application of hazardous chemical fungicides and nematicides has been found to disturb the natural beneficial soil micro-flora and pollute the soil and soil water. The application of this strain in nursery has also been found to reduce the input of chemical fungicides, which sometimes inhibit the rooting of cuttings. Similarly, Singh and Singh (2004) screened two strains, viz, *T. harzianum* (IMI No. 359869) and *T. virens* (ITCC No. 1066.95) having the potential to control the collar rot disease of *Mentha* species caused by *Sclerotium rolfsii* and also increasing the oil yield significantly, which was otherwise drastically reduced in diseased plants. It would be boon to the industries associated with the development of biocontrol products, if such potential strains could be produced successfully at large scale and marketed for use by the farmers.

Once the identity of the fungus had been established, the researchers screened different isolates to assess the diversity within the species. This further added in selecting the most efficient and antagonistic strains for biocontrol ability *in vivo*. Overall, ecological fitness is a fundamental
requirement for bio-control agents (BCAs) because of the relatively narrow window of parameters, particularly relative humidity, type of soil, osmotic potential, and temperature. The adaptability to these factors is a major requisite for *Trichoderma* strains to grow efficiently in the natural environment. Due to this reason, it is therefore essential to select fungal isolates under a range of conditions.

**Biological Control: How Does Trichoderma Offer Disease Protection?**

The antagonistic nature of *Trichoderma* had been demonstrated more than seven decades ago (Weindling, 1934), although the first report on the biological control experiment using *Trichoderma* under natural field conditions came much later (Chet et al., 1997). Furthermore, progress has been made towards the improvement of *Trichoderma*, as biological control agents, in the last 15 years than in the previous 60 years. Since then, many potential strains of *Trichoderma* have been isolated and characterized from different natural habitats and used in biocontrol experiments against several plant pathogenic fungi.

The prevention of infection or suppression of disease by *Trichoderma* is based on hyperparasitism, antibiotic, reduction of the saprophytic ability, induced resistance in the host plant, competition for nutrients and space, as well as reducing spore dissemination and/or restraining of pathogenicity factors of the pathogens, which may act co-ordinately and whose importance in the antagonistic process depends on several parameters (Elad and Freeman, 2002; Howell, 2003; Harman et al., 2004).

Its mycoparasitism involves a complementary action of antibiotics, nutrient competition and cell wall degrading enzymes, such as chitinases, β-1, 3-glucanases and proteases. Since chitin is the major component of most fungal cell walls, a primary role has been attributed to chitinases in the biocontrol activity of *Trichoderma* (Harman, 2000). Therefore, studies on the molecular structure and characteristics of genes encoding enzymes of the chitinase complex in *Trichoderma* will contribute to a better understanding of the relationships between the different enzymes involved in the biocontrol mechanisms.

**Competition and Rhizosphere Competence**

For a *Trichoderma* species to be rhizosphere-competent, it must colonize the rhizosphere beyond 2 cm depth from the seed (Chao et al., 1986; Ahmad and Baker, 1987) or proliferate to a concentration which exceeds the initial population coated on the seed (Beagle-Ristaino and Papavizas, 1985). Rhizosphere competence of a particular isolate of *Trichoderma* makes it a successful bio-control agent. *Trichoderma* species, either added to the soil or applied as seed treatments, grow readily along with the developing root system of the treated plant (Zhang et al., 1996; Harman, 2000; Howell et al., 2000). Although competition through rhizosphere competence may not be among the principal mechanisms which drive biological control, it is certainly a valuable adjunct to those that do. One concept associated with competition and rhizosphere competencies is the replacement of indigenous fungi on the root surface (Harman, 2001). Possibly, the ability of *Trichoderma* to colonize rhizosphere leads to increased levels of defence-related plant enzymes viz., peroxidases, chitinases and β-1,3-glucanases. In addition, root colonization by these beneficial fungi also induces significant changes in the plant metabolic machinery. Biochemical analysis of sunflower, soybean, mustard and maize plants treated with *Trichoderma* formulations at different intervals of time after sowing, shows substantial increase in the level of proteins, phenols, antioxidants, total chlorophyll and vitamins.

**Induced Resistance**

The treatment of plants with various agents (e.g., virulent or avirulent pathogens, non-pathogens, cell wall fragments, plant extracts, and synthetic chemicals) is now well-documented and this leads to the induction of resistance to subsequent pathogen attack, both locally and systemically (Walters et al., 2005). The ability of *Trichoderma* spp. to induce local and systemic resistance has been shown with *T. harzianum* in agricultural crops such as bean, cotton, tobacco, lettuce, tomato and maize (De Meyer et al., 1998; Ahmed et al., 2000; Hanson and Howell, 2004; Harman et al., 2004), with *T. asperellum* in cucumber (Yedidia et al., 2000, 2003), and with *H. virens* (*T. virens*) in cotton (Howell et al., 2000). According to Harman et al. (2004), on the contrary to the previously held opinions on biocontrol mechanisms, direct effects on plant pathogens are only one mechanism of biocontrol, and are perhaps less important than induced resistance. With rhizosphere-competent strains which grow
continuously with the plant, long-term systemic resistance is a possibility. The molecular basis of resistance has been partially elucidated with the fact that *Trichoderma* strains produce a set of ATP-binding cassette (ABC) transporters (Lanzuise *et al.*, 2004). These include resistance to environmental toxicants which are produced by soil micro-flora or introduced by human activity and secretion antibiotics and cell-wall-degrading enzymes necessary for the establishment of a compatible interaction with a host fungus.

According to Harman *et al.* (2004) and Viterbo *et al.* (2005), at least three classes of substances which elicit plant defense responses have been identified (e.g. peptides, proteins and low-molecular weight compounds). The systemic response in plants occurs through the JA/ethylene, signaling a pathway in a manner similar to the rhizobacteria-induced systemic resistance (Sarma *et al.*, 2002). These are proteins with enzymatic or other functions, homologues of proteins encoded by the avirulence (*Avr*) genes, oligosaccharides and other low-molecular-weight compounds which are released from fungal or plant cell walls by the activity of *Trichoderma* enzymes (Zeilinger *et al.*, 1999).

**Commercialization of Trichoderma Based Biopesticide**

In India, the majority of farmers continue to express interest in biologically-based pest management strategies of all types as the central components of an IPM approach. Such market realities promote the development of biocontrol products. However, the path to developing and applying effective biocontrol methods is still a long one, fraught with many difficulties. In addition to this, scientific, regulatory, business

![Diagram of the development process](image_url)

**Scheme 1**: Sequence of events in the development of commercial *Trichoderma* formulation.
management and marketing issues, all must be handled effectively for a biocontrol product to be successful in the marketplace.

For successful commercial production of *Trichoderma* based biopesticide, the following properties are essential:

1. Abundant and cost effective production of microbial propagules
2. Optimization of culture conditions to produce high yield and high quality conidial biomass
3. Development of low cost production, storage, and distribution systems
4. Ability to survive downstream processing, particularly drying, which is required to avoid contamination
5. Stability and adequate shelf-life of the final product upon storage, preferably without refrigeration
6. Ability to withstand environmental variations in temperature, desiccation, relative humidity in order to survive and establish active populations in soil
7. Consistent efficacy under varying agro-climatic conditions at commercial feasible states
8. Integration of biocontrol into current agronomic practices.

**Biopesticides - Where Do We Stand?**

Despite slight reluctance to promote the use of biofertilizers and biopesticides till recently, its use seems to be emerging as an alternative to the use of chemicals; this is rather slowly but surely. This is evident from the recent trend among an increasing number of farmers in the Green Revolution belts of the country to voluntarily switch over to organic agriculture using various ecofriendly means as well as their willingness to gain knowledge about more and more products based on microbes and plants. Organic agriculture requires less financial input and places more reliance on the available natural and human resources. It has the potential to become a viable alternative for the resource-poor farmers of the country. In fact, a switch-over to organic farming can go a long way in improving the economic well-being of these impoverished cultivators if they can take advantage of the rapidly growing global markets for organic products which offer handsome premiums. It will expand more if home grown products could be developed using the available resources, and for this to happen, knowledge dissemination is needed on the benefits of shift to the use of biofertilizers and biopesticides.

The government has to play a very important and proactive role, if scientists, industrialists and farmers have to come together to change the way the agriculture is being practiced. It is the most appropriate time that the Government of India and different states make a structural shift in their current policy stance of promoting agriculture based on the natural resources and the industrial products. The Ministry of Agriculture will have to devise a full-fledged long-term policy framework to create an environment conducive enough for organic agriculture to flourish. Firstly, grants for the development of technologies based on microbial- or plant-based products should be given to the specialist institutions and rigorously monitored. Moreover, efforts must be made for knowledge dissemination through print and audio visual media. Organic certification processes must be made less tardy and faster, while maintaining their integrity. Market development needs to be hastened with the help of the governments for various products such as biofertilizers, biopesticides or bio-control agents. They should be made available in adequate quantities and at reasonable prices. For this purpose, production units may be established for large-scale. For this, Cuba can be a model, i.e. a country where organic conversion has been successfully undertaken on a nation-wide scale, as a consequence of a conscious policy decision on the part of the Fidel Castro government.

**Conclusions and Future Prospects**

For industrialization of any product, all the requisite parameters should be such that the chances of failure at any stage are non-existent or minimal. The solid state fermentation technology, for the production of *Trichoderma*-based biopesticides, is one such technology. The researchers have successfully standardized the technology and several industries have successfully marketed their products in India. This particular research group is very much interested in developing new methods of biological control which are more suitable to agriculture as what has been practiced in our country due to small landholdings and which may be industrialized profitably. In particular, the researchers are interested in developing consortium of the different species or isolates...
of *Trichoderma* itself and *Trichoderma* as well as other microbes which can be effectively integrated in different integrated management modules currently used by farmers. In addition, we would also like to focus genes encoding on specific proteins in *Trichoderma* species. These proteins degrade chitin, a structural component of most pathogenic fungi and herbivorous insects. Furthermore, there is also a need for tolerant strains of *Trichoderma*, which could withstand high temperature encountered during summer in the country. Moreover, cooperative efforts from the researchers at the Banaras Hindu University and elsewhere should lead to a series of next generation of biocontrol products for commercialization.

REFERENCES


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