Chapter

Trichoderma: Invisible Partner for Visible Impact on Agriculture

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Abstract

Species of genus *Trichoderma* may benefit as plant pathogen control agent (mycofungicide) and plant growth promoter (biofertilizer) and their application may lower the production costs and environmental impact. Direct effects of these fungi on plant growth and development are crucially important for agricultural uses and for understanding the roles of *Trichoderma* in natural and managed ecosystems. The *Trichoderma* potential as bioagent is utilized through the commercial production of *Trichoderma*-based product. Commercial products of *Trichoderma*-based biofungicides account for about 60% of the biofungicide market, while the availability and dispersion of *Trichoderma*-based biofertilizers are more widespread than commonly known with a tendency to expand due to the easier registrations. Limiting factors for availability of commercial products are expensiveness of registration requirements as they must be registered as pesticides, especially patenting, efficacy testing, toxicological, and biosafety testing. This chapter intends to give insight into agricultural importance of *Trichoderma* and current status of implementation of *Trichoderma* products in developing and in the developed countries.

Keywords: biocontrol, biotechnological patent, microbial products, pesticide

1. Introduction

The credo of fabulous Spanish architect Antoni Gaudi that *Anything created by human beings is already in the great book of nature* is true for the exploitation of *Trichoderma* benefits. Since the early 1930s, when Weindling reported that *T. lignorum* produces and excretes a "lethal principle" in the surrounding, the scientists become involved in investigation of antifungal ability of various *Trichoderma* species, although *T. harzianum* arisen as the most prominent species of the genus. Today, their agricultural importance is good antagonistic abilities against soil born plant pathogenic fungi, thanks to different mechanisms of antagonism: the production of antifungal metabolites (antibiosis), competition for space and nutrients, induction of defense responses in plant and mycoparasitism. Along with revelation of diverse antifungal mechanisms of *Trichoderma*, the ability to promote plant growth and to increase plant height, leaf area and dry weight were perceived. First, this ability was treated as side effect of suppression of plant pathogenic fungi which leading to stronger root growth and nutrient uptake. Also, positive influence of *Trichoderma* to a faster germination and increase in percentage of emergency were perceived. Nowadays, *Trichoderma* species are considered as opportunistic plant symbionts because they colonize root surface and even penetrate into the epidermis of root tissue and a few cell layers below this level establishing pseudomycorrhizal

relationship with plant host. This intimate relationship is what induces localized and systemic resistance plant responses to pathogen attack. For the *Trichoderma*, abundant healthy roots are environment where it grows and proliferates best owing to the main carbohydrates secreted by plant roots. Furthermore, roots are resort of plant pathogenic fungi and nematodes, the target for *Trichoderma* as mycoparasite and nematophagous. The plants also benefit from this relationship through increased root and shoot growth and increased macro- and micronutrient uptake. Therefore, *Trichoderma* may be benefit as growth promotant (biofertilizer) as well as pathogen control agent (mycofungicide), and their application may lower the production costs and environmental impact. Recently, it is recognized that *Trichoderma* positive effect on plant growth is independent ability and equally remarkable and significant as its antifungal ability because growth enhancement has been observed in the absence of any detectable disease and in sterile soil. Therefore, today is considered that the direct effects of these fungi on plant growth and development are crucially important for agricultural uses and for understanding the roles of *Trichoderma* in natural and managed ecosystems.

To exploit *Trichoderma* benefits, it must be isolated from soil, studied, and encapsulated in formulation which will allow application into soil. But, reintroduction to soil, even the most strongly rhizosphere competent such as *Trichoderma* can be difficult. *Trichoderma* reintroduced into soil must compete with spectrum of rhizosphere microbes while trying to colonize available sites along the plant roots. Therefore, it needs to be applied in low cost but high density inocula engineered to maintain fungal propagule viable during the transport, storage, and application. To accomplished mentioned goals and effective dispersal of fungal inocula, it is necessity to choose the fungal inoculum carrier and the type of formulation. The *Trichoderma* potential as bioagent is utilized through the commercial production of *Trichoderma*-based biofungicides, which account for about 60% of the biofungicide market. The availability and dispersion of *Trichoderma*-based biofertilizers are more widespread than commonly known with a tendency to expand due to the easier registrations because they are not registered as pesticides. Limiting factors for availability of commercial products are expensiveness of registration requirements, especially patenting, efficacy testing, toxicological, and biosafety testing.

2. Historical and commercial background

In 1794 the mycologist C. H. Persoon proposed and named the genus *Trichoderma* after mycelial appearing, like hairy (Gr. thrix, genitive trikhos) cowering on decaying wood surface (Gr. derma). Association with teleomorphs in *Hypocrea* was done by Tulasne brothers in 1865. The genus came in focus after Weindling's article about *T. lingorum* as parasite of soil fungi, followed by another article in 1934 about parasitism on *Rhizoctonia solani* helped by some kind of a toxic compound [1, 2]. In that article, Weindling gave definition of antibiosis as suppressive mechanism based on production on secondary metabolites with antimicrobial effect and also named this *T. lingorum* lethal metabolite, gliotoxin. Further Weindling's papers defined biocontrol of plant pathogens through the *Trichoderma* strains and their following unique mechanisms: mycoparasitism, competition for area and nutrients in the rhizosphere and antibiosis, and production of antibiotics [3–5]. Existence of volatile organic compounds produced by *Trichoderma* that can inhibit the growth of fungi responsible for wood decay was published by Dennis and Webster in 1970 [6]. Since then, species of genus *Trichoderma* become among the most commonly studied biocontrol microbes and are presently marketed as

active ingredients of biopesticides, biofertilizers, plant growth enhancers, and stimulants of natural resistance.

The *Trichoderma* potential as biocontrol agent is utilized through the commercial production of biofungicides and always registers for use as microbial fungicides. *Trichoderma* based biofungicides are today presented with more than 250 products available worldwide and account for about 60% of the international biofungicide market. The leading country in terms of enormous use of *Trichoderma* products is India which comprises 90% of Asian market [7]. Following is Brazil with the greatest production on the South and Central America. For example, in Venezuela and Cuba, the development and use of *Trichoderma*-based products are government supported and officially recommended [8]. Prevalent species in majority of *Trichoderma*-based products is *T. harzianum* (83%), of which 55% of these are combined with *T. viride* and 28% are with *T. koningii* [7]. Widely used *T. harzianum* and *T. viride* are mostly applicate as soil treatment in around 87 various crops against 70 soil-borne and 18 foliar-borne pathogens, mostly fungi [9].

The first *Trichoderma* biofungicide commercialized worldwide was Trichodex produced by Makhteshim Agan Industries (Beersheba, Israel). Trichodex is based on *T. harzianum* isolate T39 studied since 1986 by its creator Dr. Yigal Elad of the Volcani Center, Israel where research and development were carried out [10]. Worked as a biopesticide researcher, Dr. Elad had primarily studying the biological control of economically significant plant pathogens primary gray mold causal, ascomycete fungus *Botrytis cinerea*. Early contact with industry company and sign of agreement with the aim of developing a biocontrol preparation for the control of gray mold were crucial, and Dr. Elad's collaboration with Makhteshim-Agan Industries led to the development and launch of the biopesticide product, Trichodex. Around the world, Makhteshim carried out efficacy trials of Trichodex in controlling of gray mold in vineyards in more than 130 experiments on 34 varieties, under diverse commercial conditions around the world [11]. In 1993, Trichodex has been registered in countries such as Argentina, Australia, Bulgaria, California, Chile, Colombia, Croatia, Cyprus, Greece, Guatemala, Hungary, Israel, Italy, Morocco, Paraguay, Romania, Turkey, Slovenia, South Africa, and the USA. Trichodex was in Croatia registered as contact antibiotic fungicide with enzymatic activity against *B. cinerea* on grapevine and strawberries. In Croatia, microplots with Trichodex were carried out until 1999 in vineyards of famous winegrowing region Kutjevo, situated in the continental part of Croatia were the gray mold disease inflicts damages of 50–60%. Trichodex shown efficacy in interval of 13–55% depending on weather conditions in year which was satisfactory control and occasionally equally efficient as synthetic fungicide Kidan (a.i. iprodione, Bayer, Germany) registered in Croatia at that time [12, 13]. In 1990s, beside Volcani center (Israel) Research institute for Plant Pathology, investigations of *Trichoderma* biocontrol efficacy were conducted at INRA (Paris, France), Institute for Biological Control (Darmstadt, Germany) and in USA at Cornel University (Geneva, NY) Dr. Harman.

Another most famous and useful *T. harzianum* strain was T22 (also known as 1295-22, KRL-AG2, and ATCC 20847). It was produced by Dr. Harman in 1980s and was licensed from Cornell University by the Eastman Kodak Company, which developed the toxicity package and environmental studies and make registration possible. In about 1990, Kodak decided to abandon the agricultural pesticide market and gifted the registration of T22 and other data they had generated to Dr. Harman and his colleagues at the Cornell Research Foundation who founded a company, now BioWorks Inc. This company was founded by Dr. Harman and two of his colleagues previously under name TGT Inc. as their efforts were to develop biocontrol systems for commercial agriculture and to translate biocontrol research into biocontrol

reality [14]. They encapsulated T22 in commercial products RootShield and T22 Planter Box and marketed under new company BioWorks Inc. (Geneva, NY, USA). Sales of those products began in 1993, and in 1998, sales have increased for 20% per year where it was marketing internationally with special consideration for its limited shelf life. Strain T22 was generally promoter of plant growth as well as mycofungicide against soil-borne plant pathogens. In plant, strain T22 enhances expression of proteins involved in photosynthesis and starch accumulation, and supposing its effects are due to increased photosynthetic rates in infected plants [8]. Interestingly, this strain was produced using protoplast fusion in order to be highly rhizosphere competent that also possess substantial ability to compete with spermosphere bacteria. Strains that were fused were *T. harzianum* T-95 and T-12 because first was a rhizosphere competent mutant produced from a strain isolated from a *Rhizoctonia*-suppressive Colombian soil, and second was more capable of competing with spermosphere bacteria than T-95 under iron-limiting conditions [14, 15]. The novel generation of RootShield is based on two *Trichoderma* species and contains strain *T. virens* G-41 together with strain *T. harzianum* T-22. Commercial name of product is RootShield PLUS and some with instruction that should be applied to disease-free plants previously chemically treated with fungicide, because the aggressively growing strains T-22 and G-41 are growing on the outside of roots and do not enter the plant tissue.

The availability and dispersion of *Trichoderma*-based biofertilizers are more widespread then commonly known with the tendency to expand due to the easier registrations. Mostly permitted for use in organic farming in Europe is: RootShield, Plant Box and Bio Trek (northern Europe, USA), Binap (Switzerland, Sweden, UK, USA), Bio fungus (Belgium), Supersivit (Czech Republic), Trichodex (Italy), Trifender (Hungary), and Trianum (Avantagro, Spain) [16]. Novel *Trichoderma* formulations are not based on single culture of one species but come as consortia or mix of at least two or three species or different strains of same species. Compatible consortia of compatible strains with different mechanisms, disease suppressive, or plant growth promoting, which complementary each other were found to be more effective than the application of individual organisms. Mixtures for biocontrol have a broadened range of pathogens against which are effective. Mixtures in plant growth promoters are based on insight that metabolic products of various *Trichoderma* strains are not identical and they have selective character to different plant species and even a variety [17, 18]. Seems this may be due to better interaction of some *Trichoderma* species or some strains with certain plant species because root exudates may induce or inhibit their mycelial growth [19]. In development are other types of consortia, mixtures of *Trichoderma* strains with other organisms, fungi or bacteria that are known as bioagent also. Because knowing of action mechanisms allows combining of strains with different modes of action in order to anticipate efficacy of final product, investigations of effectiveness differences between species and biotypes of same species are in progress.

3. *Trichoderma* as opportunistic plant symbiont

Mycoparasitic and nematophagous *Trichoderma* species found their prey in rhizosphere where roots are resort of plant pathogenic fungi and nematodes. Therefore, it becomes usual to define species of genus *Trichoderma* as free living rhizosphere organisms which colonize plant root surface as opportunistic plant symbionts. Biocontrol of plant pathogens by *Trichoderma* was from the first Weindling report in 1932 [1] considered as the direct ability of these fungi to interact with soil pathogens. Along with revelation of diverse antifungal mechanisms of

Trichoderma, the ability to promote plant growth and to increase plant height, leaf area, and dry weight were perceived. Positive influence of *Trichoderma* to a faster germination and increase in percentage of emergency were perceived also.

Abundant healthy roots are from environment where *Trichoderma* grows and proliferates best. The sucrose that leaks from roots stimulates growth of *Trichoderma* mycelium and leads to interaction with plant. In order to stimulate plant to provide more sucrose, *Trichoderma* has evolved numerous mechanisms for better routing. With its enzyme arsenal, *Trichoderma* enhances solubility of soil nutrients which will be otherwise unavailable to plant. Further, *Trichoderma* enhances nutrient uptake by plant and better plant nourishment that will result in stronger routing which are frequently associated with increase in yield [20]. Special benefit is induction of increased nitrogen use efficiency in plants. This mechanism is not enlightened yet but probably is connected to *Trichoderma* stimulation of deeper rooting, and thereby increasing the volume of soil colonized by plant roots. Plants approximately take up only 33% of the amount of applied nitrogen fertilizer while some field trial data on *Trichoderma* treatments in several different crops indicate possibility of reduction nitrogen application rates by 30–50% with no reduction in yield [21, 22]. Calculation says that if this reduction was applied to the 30 million hectares of wheat in the USA, the savings in nitrogen application would total more than a billion of nitrogen kilos annually.

Sucrose metabolism increased by *Trichoderma* stimulates the resistance response in the leaves that leads to increased photosynthesis and respiration because growth induced by *Trichoderma* plant requires energy, and sunlight energy utilized in increased photosynthesis will be the energy source needed for plant growth enhancement by *Trichoderma* mechanisms. Of course, better photosynthesis enables that more sucrose is translocating to the roots and metabolic circle continues. Increased leaf mass enables increased photosynthesis but the *Trichoderma* has the abilities to increase photosynthetic efficiency [21, 23]. It was demonstrated that electron flow was substantially increased by root colonization and electron transport strongly enhanced [24]. In trial with barley exposed to water deficiency *T. harzianum* substantially increases water deficit tolerance and consequently reduces effects on photosynthetic systems even when plants were at or approaching the permanent wilting point after 2 weeks of withholding irrigation. Therefore, plants benefit from relationship with *Trichoderma* through increased root, shoot, and leaf growth and increased macro- and micronutrient uptake and disease protection as well.

First scientific papers about effects of *Trichoderma* on plant growth promotion, mostly of horticultural crops and conifers, began to appear in 1980s and continuing in 1990s [18, 25–28]. There is ample documentation for the *Trichoderma* influence on plants which are colonized with effective *Trichoderma* strain and that they are substantially different from an uncolonize plants in quality and quantity of yield, withstand to adverse environmental conditions and pathogen attack. Positive influence of *Trichoderma* to a faster germination and increase in percentage of emergency were perceived also [17, 18, 29].

In Croatia, investigations with autochthon *Trichoderma* strains and their influence to growth of fiber flax, lettuce, tomato, cabbage, and red beet were conducted [16, 30–32]. Significant increase of some lettuce quality characteristics was gained with *T. viride* strain TPS applied in the form of alginate-pellets in two frequently used commercial potting compost mixture: Klasmann-Deilmann P 002 (Germany) and Stender A240 (Germany). Except dry weight, TPS enhanced the formation of leaves: at Stender difference against control varies for 1–2 leaves more, at Klasmann for 1 leaf more. Leaf length was longer for 2 cm at Stender and Klasmann amended with TPS-pellets than at control, while leaf width was wider for 3.15 cm at Stender

and for 4.27 cm at Klasmann. Fresh weight was greater for 5.36 g at Stender and for 4.68 g at Klasmann against control. Dry weight was only characteristic on which TPS pellets did not have significant influence perhaps due to the similar nutrient content of Stedman and Klasmann substrates. These substrates are characterized by the use of fine peat with the addition of nutrient specially designed to meet the needs of young plants, so they similar in nutrient content (N 150–260 mg l^{-1}, P 180–280 mg l^{-1}, K 200–350 mg l^{-1}, and Mg 80–150 mg l^{-1}). *Trichoderma* is able to solubilize nutrients but only the ones present in substrate, and as Klasmann and Stender are enriched with the similar nutrients, there were no significant differences among them as trial variants. The differences were bespeaking when the TPS pellets were added against control. In Croatian trial with cabbage and red beet, the indigenous *T. viride* isolates STP16 and STP8 enhanced plant growth in only one trial vegetation season, and results confirm the hypothesis that biotypes of same species differ in their abilities to induce plant growth, so that growth promotion of *Trichoderma* is not species dependent as well as that biotypes of same species differ in their abilities for inducing plant growth. Influence of strains STP16 and STP8 on cabbage growth was estimated by weighing the heads. Fresh weight was greater at STP16 treatment (FW = 1666.5 g) than at STP8 treatment (FW = 1372.5 g) but not statistically different although in comparison to control (FW = 1291 g) significantly increased. Dry weight was slightly but statistically significantly increased at STP16 treatment (DW = 8.2 g) against STP8 treatment (DW = 7.2 g) which was statistically equal to control (DW = 6.3 g). Growth promotion index showed that STP16 treatment promotes fresh weight for 29% while STP8 treatment only for 6.3% and dry weight for 30.16% while STP8 for 14.29%. Influence of those strains on red beet growth was estimated by weighing the root. Fresh weight was increased by both isolates, STP16 (FW = 725 g) and STP8 (FW = 607.5 g). There was no statistically significant difference between isolate influences although STP16 significantly increased fresh weight in comparison to control (FW = 569 g), while STP8 did not. Dry weight was greater at STP16 treatment (DW = 13.1 g) and statistically significant in comparison to STP8 treatment (DW = 12.2 g) and control (DW = 11.6 g). Growth promotion index showed that STP16 treatment increased root fresh weight for 27.42%, while STP8 treatment for only 6.44%. Index calculated for dry weight showed that STP16 increased dry weight for 12.93% and STP8 for 5.17%. In trial with fiber flax indigenous *T. harzianum* strain STP, applied in the form of alginate-pellets and through pelleted seeds, positively influenced germination, seedling emergence and plant growth. Seedling emergence from pelleted seeds were delayed, and on 7th day, after seeding, 16% were emerged, while at STP-pellet treatment, 66% emerged and at control 53%. In the presence of strain STP, plants grow higher (66 cm) than on control (62 cm) and the highest where plants from pelleted seeds (72 cm).

First, this ability was treated as side effect of suppression of plant pathogenic fungi [25, 33–35]. Other possible explanations of this phenomenon include: control of minor pathogens leading to stronger root growth and nutrient uptake [26], secretion of plant growth regulatory factors such as phytohormones [33, 34, 36, 37], and release of soil nutrients and minerals by increased saprophytic activity of *Trichoderma* in the soil. Removing of a toxic and inhibitory material to plant growth from the soil was also presented as interesting explanation of *T. harzianum* plant growth promotion [25].

Today, *Trichoderma* positive effect on plant growth is considered as independent ability and equally remarkable and significant as its antifungal ability because growth enhancement has been observed in the absence of any detectable disease and in sterile soil [17, 28, 31]. Novel genetically analysis shown that the most of *Trichoderma* biocontrol activity is through their abilities to induce plant defense mechanism described as systemic disease resistance [21]. For example, antibiosis of *T. virens* against

Rhizoctonia solani on cotton seedlings and mycoparasitism *T. harzianum* on *Pythium* were found to be due solely to induced resistance [22, 38]. Therefore, the intimate *Trichoderma*-plant relationship is what induces localized and systemic resistance plant responses to pathogen attack, promoting plant growth and support/encourage biocontrol. From now on, the meaning of biological control must be expanded to include induction of plant defense mechanism up to the disease resistance and plant growth promotion along with classical antagonism like antibiosis and mycoparasitism.

Symbiotic *Trichoderma*-plant relationship results with effects that extend beyond biocontrol because *Trichoderma* directly influence plant physiology. That is why it is considered that they establish pseudomycorrhizal relationship with plant host. Species of *Trichoderma* are actually strong plant invaders and can colonize plant internally in endophytic manner with the ability to grow with plants. These allow much longer periods of efficacy than nonendophytic organisms and provide benefits to plants for at least the life of an annual crop [8].

Trichoderma species penetrate into the root cortex, epidermis, and a few cell layers below this level, based upon evidence with *Trichoderma* mutant strains that produce green fluorescent protein and electron microscopy and therefore are similar to endomycorrhizal fungi. Small protein from hydrophobin group is produced on the surface of *Trichoderma* hyphae and facilitates their attachment to root. For penetrations, *Trichoderma* uses appressoria which coil about root hairs and are similar to those observed in mycoparasitism. The *Trichoderma* enables further its entry with help of small protein swolenin TasSwo that recognize cellulose and modify plant cell structure. Entering the cells they have access to plant nutrients, which allows them to proliferate.

Penetration of *Trichoderma* hyphae into plant tissue is infection similar to one of other fungal plant pathogens but does not incite parasitism even though they have enzyme systems fully capable of macerating plant tissue. Although rarely phytopathogenic, one case of *T. virens* pathogenic on cotton seedlings was described and enlightened. It happened because protein responsible for antibiotic production, a single 18 kDa, that induces resistance was not expressed, so resistance was not induced [39, 40]. This nonpathogenic yet plant beneficial life style is a successful strategy for the fungus because it provides *Trichoderma* with more sucrose from enlarged roots due to better plant nourishment and also prey for mycoparasitic and nematophagous strains. Recently, endophytic *Trichoderma* strains are known which colonize vascular systems of certain plants [39] like it is known for other root colonizing biocontrol fungi such as binucleate *Rhizoctonia* and nonpathogenic *Fusarium* species [41, 42]. It was discovered that cocoa permits *Trichoderma* strain ramification throughout their structure. Mostly, plants will not permit that so the same strain will function only as root colonists applied to other plants [43].

After penetration into the root tissue, *Trichoderma* establishes chemical communication with the plant and interact at molecular level. Inside the root cells, *Trichoderma* can modify plant's gene expression to activate its immune system. These result initially in an induction of resistance mechanisms, so plant form thickened cell walls and produce phenolic depositions that intercept the *Trichoderma* to the area of infection and prevent further plant colonization [39, 44]. This is the type of plant localized resistance to *Trichoderma*. Therefore, disease development does not occur and this is asymptomatic infection but plant defense system is triggered.

Plants have two immune systems: basal disease resistance is systemic acquired resistance (SAR) and the other is induced systemic resistance (ISR). SAR is induced by pathogens and follows the salicylic acid pathway to reduce the severity of pathogenicity, while ISR follows jasmonic acid pathway. The first plant response to *Trichoderma* infection was found to increase in salicylic and jasmonic acid levels and typical antipathogenic peroxidase activity. Infected plant cells recognize

that they are under pathogen attack by detecting pathogen-associated molecular patterns (PAMPs), also called microbe-associated molecular patterns (MAMPs) which is more suitable to use for *Trichoderma.* This molecular pattern is essential for the pathogen life which is binding to pattern recognition receptors (PPT) on cell surface, and this triggers basic immunity system SAR. PAMPs are not found in plant, so thus the plant's receptor recognized them as being potentially dangerous and this triggers the plant SAR [45]. Simultaneously, infected plant cell recognizes pathogen through toxins considered being effectors, and this triggers plant's effector-triggered immunity (ETI). ISR is triggered by infection of beneficial microorganisms such as *Trichoderma*, recognized by chitin presence and induced by jasmonic acid and ethylene by wound signals, which they transmit from roots to other plant's part. When *Trichoderma* penetrates root, the ISR is induced by pattern recognition receptors localized in the plasma membrane of plant cell. First response is hypersensitive reaction resulting from production of antimicrobial compounds intent to restrict/restrain the potential pathogen. Plant can identify *Trichoderma* by the following PAMPs: cellulases, chitinases, endopolygalacturonase, peptaibols, and 6-pentyl-α-pyrone [46]. More *Trichoderma* biocontrol compounds that are able to induce plant defenses and are connected with plant-beneficial effects are different proteins, ceratoplatanins, polygalacturonase, cellulose-binding-domain proteins, and nonactive xylanase, and secondary metabolites, peptaibiotics, pyrones, pyridines, and butenolides, The first discovered chemical communicator of *Trichoderma* was a 22 kDa xylanase, protein which induces localized resistance in plants [39]. The peptides or proteins that are effective have masses of 6.5, 18, 20, 32, and 42 kDa. Finding that many of them retained their activity as denatured proteins was lead to premise that particular amino acid sequences are the important factor in their activity rather than enzymatic function.

Once when this *Trichoderma*-root biochemical cross-talk begins, *Trichoderma* may influence plant response to other pathogens attack by increasing SAR and ETI [47]. Effective biocontrol strains of *Trichoderma* changes in the amplitude of plant ETI by using the zig-zag model proposed by Jones and Dangle [48]. *Trichoderma* strains that activate both ISR and SAR or only SAR were also known. In investigation of cucumber root colonization by *Trichoderma* applied at high concentrations, 28 proteins whose expression was affected were identified in cotyledons [49]. All were regulated by *Trichoderma*, and among them, 17 were found to be upregulated, while 11 were downregulated. Proteins differentially regulated by *Trichoderma* were involved in isoprenoid and ethylene biosynthesis, and in metabolism of photosynthesis, photorespiration, and carbohydrate. Important finding is that *Trichoderma* can influence plant's oxidizing, reactive chemical species containing oxygen (ROS). They are a natural by-product of the normal metabolism of oxygen which is important in cell signaling and homeostasis. ROS are influenced by plant responses to stress, mostly to abiotic environmental conditions, so their levels can drastically increase under stress. They have high energy and are unstable so easily react with other species, like biomolecules such as DNA, and oxidizing them. This is known as oxidative stress and may result in significant damage to cell structures. Involvement of *Trichoderma* in ROS scavenging enlightens why *Trichoderma* treated plants are better coping the stress by drought or salinity. One of the pathways is the glutathione-ascorbate cycle, and *Trichoderma* enhancement of enzymes in it will recycle antioxidants more rapidly and thereby reduce stresses effects. In trial with barley exposed to water deficiency, *T. harzianum* substantially increases water deficit tolerance and consequently reduces effects on photosynthetic systems even when plants were at or approaching the permanent wilting point after 2 weeks of withholding irrigation. That shows possible *Trichoderma* influence on photosynthesis. Plants enhanced by *Trichoderma* have increased leaf mass that enables increased

photosynthesis, but *Trichoderma* also have the abilities to increase photosynthetic efficiency [21, 23]. It was demonstrated that electron flow was substantially increased by root colonization and electron transport strongly enhanced [43].

Some *Trichoderma* strains can counteract pathogen toxins (effectors) in two ways: inhibiting pathogenicity factors and influencing pathogen dispersal and nutrition, whereby the ETI is induced as mentioned earlier. *Trichoderma* can improve ETI by releasing compounds that plant receptors will recognize as pathogen effectors or causing faster response. Other strains can induce stronger immunologic plant response than pathogen, meaning they induce ISR more than pathogen induces SAR, by producing a variety of MAMPs such as hydrophobins, expansin-like proteins, secondary metabolites and enzymes having direct antimicrobial activity. There is interesting example of involvement of *T. arundinacea* in induction of plant defense. Its trichothecene toxin harzianum A has been recognized as MAMPs in tomato seedlings and activated both ISR and SAR and primed them against *B. cinerea* and *R. solani* [50]. Trichothecenes are produced by a number of fungal genera like *Fusarium*, *Stachybotrys*, and *Myrothecium*. So far, *Trichoderma* is the only trichothecene producer in which the tri5 gene is not located in the main tri cluster. *Trichoderma* species produces two trichothecene toxins: harzianum A and trichothecene.

Evidently, *Trichoderma* antifungal activity against phytopathogenic fungi and nematodes, as well as competition for nutrients conferring a nutritional advantage, are carried out by production of extracellular hydrolytic enzymes and/or the production of secondary metabolites with antifungal activity. It is postulated that the *Trichoderma* ability to act as soil colonizer, pseudomycorrhizal relationship with plant host with well being for plant health and fitness, mycoparasite and nematofag are to be provided by *Trichoderma* genome. Genome coevolution has been demonstrated in many plant-pathogen interactions, so it can be considered in the case of some plant and *Trichoderma* species. Strong genetic components to the responses of at last maize to *T. harzianum* T22 were confirmed in trials with a series of inbred lines which were preceded by large trial conducted in U.S. corn-belt with 160 maize hybrids and T22 [44]. Today, several hundred separate plant genes or proteins are known whose expression is altered by *Trichoderma* root colonization although the expression consequences are more pronounce in the shoots than in the roots [22]. Considering all described, it is evident that proteomics study is need to give an understanding of how *Trichoderma*-treated plants become more resistant to pathogen attacks.

4. Applying *Trichoderma* in agriculture

Scientific articles describing excellent antifungal *Trichoderma* efficacy against some phytopathogenic fungi or enhancement of plant growth and yield are published worldwide each year. Many originate from countries having economics primarily based on agriculture and describe mostly experimental results achieved by native strain or created product based on it and may not be applicable on large-scale crop production. Furthermore, novel articles summarize biocontrol programs, models of commercialization, and registration requirements. Dominantly is emphasizing that full-scale production, marketing, and registration requirements are unfavorable for products of biocontrol agents and simply too expensive, especially for the agricultural-based countries in which they are needed the most. All those articles clearly show that isolation, experimenting to evaluate biocontrol activity of strain and encapsulating it in formulations for low-scale trial needs are achievable part of developing biocontrol product. To be able to overcome issues on product

industrialization and commercial and registration requirements, *Trichoderma* research community depends on stakeholders for investments in that part of developing process and industrial linkages. That's why the use of *Trichoderma*-products in developing countries falls under local production model for using microbial agents. This model is one of the four economic models for using microbial agents proposed by Hartman et al. [8].

First model is the microbial pesticide model which implies full registration of microbial product as pesticide and marketing worldwide. It is established in developed countries in USA, Canada, and EU; although in USA, it is substantially different. Registration of microbial products as pesticides is based on the interpretation of the term "pesticide." It does not necessarily refer to killing (e.g., fungicide) or inhibiting (e.g., fungistatic) pest only that it is controlled. Therefore, *Trichoderma*-products fall into the scope of pesticide although registration requirements will be substantially less than those for synthetic chemicals, but remain difficult. Good example for this model is T22-product, and data that approximately $12 million were required for registration, development of product facility, formulation, and marketing system before its sales began to grow. In USA, using the microbial pesticide model requires a minimum of $8 million and 3–6 years before highly effective product is established in the market. In Canada in EU, registration regulatory also requires efficacy evaluation with toxicological and environmental testing, while in USA, they are required over time. Efficacy tests are required for almost every crop-pathogen combination and for *Trichoderma*-based products with broad capabilities on many crops and pathogens; this limits or even precludes registration and even makes it almost impossible from a financial standpoint. On the other hand, marketing them as plant-growth enhancements and strengthening agents gives them a market advantage because registration requirements like time and efficacy tests on pathogens are excluded. Although, in the USA, mycorrhizal fungi and rhizobia are not subject to regulatory approval for use while in EU and Canada are needed. Need to overcome the expensive efficacy evaluation of *Trichoderma*-products led to creation of model named *Inoculants, plant strengthening agents and biofertilizers*. This model is used in various agricultural systems where *Trichoderma* products are marketed as plant inoculants for improvement of plant performance, but the pesticidial claims are not made although their diseases control benefits and well known. This gives those product marketplace advantages because many necessities for registration are avoided and therefore takes less time to reach the marketplace. Sales of *Trichoderma*-products may be larger than the sales of registered products. Great example is the product based on strain T22 which was registered as biofungicide in USA but in EU was just the beginning registration process in 2010. Until then, it was sold as plant strengthening agent named Triannum although its biofungicidal activities were known. As reason for delayed EU registration product, authors instigate economic because that the return on investment for full European registration was unlike to occur. In comparison to microbial pesticide model, the plant inoculants model leak two steps required for registration. Both models have followed steps: identification of good agent; development of production and formulation system; patenting of strain and/or process; building large-scale production and nationwide or international marketing, but steps: toxicology and other testing and registration are required only in microbial pesticide model. Local production model has only one step—discovery of good strain. As name tells, the production of autochthon strain is local. Strains are grown and multiplied in order with well-known methods for semi-solid cultivation on wheat or corn bran, rice or similar substrate or in liquid fermentation. Cultivation and growing the required amount of inoculum is timed to be delivered directly before the application, date can be ordered by the grower, what eliminates extensive production and formulating. This

model describes production of plan growth promotion rhizobacteria (PGPR) and *Trichoderma*-formulation at Faculty of Agriculture University of Zagreb in Croatia. The last one is in Croatia produced only by this chapter author. Although it is regulated by the government, local production model is different from model named *Governmental monopolies or state-supported production*. This model is based on Cuban rapidly shift from conventional agriculture to semi-organic farming. After collapse of trade relations in 1989, Cuba sets in economic and food crisis. As the pesticide and fertilizer imports were reduced to more than 80%, they established alternative agricultural technology and urban agriculture (composed of about 8000 gardens nationwide) with biological pest control practices supported with production on biopesticides and biofertilizers on a large scale. During 1996, Havana's urban farms provided the city's urban population with 8500 tons of agricultural produce, 4 million dozens of flowers, 7.5 million eggs, and 3650 tons of meat. This averted the crisis as it was said in Ref. [8, 51, 52] "It thus helps refute the most common argument—that we couldn't "feed the hungry" without pesticides—against taking the "ethical" position in real-world pest management policy debates." In Venezuela, a complete on large-scale implementation of biopesticide/biofertilizer development program has been funded and run through the Instituto Nacional de Investigaciones Agricola. In India, government is promoting all steps connected to adoption of biopesticides through various types of legislative, like the National Farmer Policy.

4.1 Assay for biological activity

Members of genus *Trichoderma* are among the most prevalent cultivable fungi in soils, based upon the frequency of isolation on suitable media. They are present in all types of temperate and tropical soils and constituted up to 3% of the total fungal propagules (mycobiota) in forest and 1.5% in pasture soils, and their populations range from 10^2 to 10^3 per gram of soil. Therefore, *Trichoderma* strains are mostly isolated from soil, dominantly forest soil, and organic substrates form rhizosphere: alive, dead, healthy, or diseased plant tissue (root, green parts), common on wood decaying and fungal structures (fruiting bodies, sclerotia, and mycelial mats) [53]. Mentioned sclerotia and mycelial mats can be used as bates in search for antagonist for specific fungal pathogen. For that purpose, those mycelial structures are burying in natural soil sample. In looking for strain for biocontrol, it is recommended to identify the problem, the target pathogen (soil borne or aerial, source of inoculum, biology, and epidemiology) and its niche, host crop, and environment conditions. The most effective strains are sought in the geographic center of plant origin because as pathogens are coevolving with the host, their antagonist coevolving also. Strain can be isolated from stromata of teleomorph *Hypocrea* which are often found on wood and less frequently on some Basidiomycetous fungi (sedges, bracket). Isolating process is not inexpensive, requires time, and needs labor. When isolating from various sources in nature, one must have in mind that it is easy to obtain mixed cultures of *Trichoderma* species, as well as teleomorphic *Hypocrea* state, because they usually intermingle. To proceed investigation with pure culture, it is necessary to grow culture from single spore, conidia, chlamydospores, or better ascospores if it is possible and even hyphal tips. This can be achieved using dilution series of soils, root, and other plant tissue macerates suspensions of fungal structures which can be then plated on potato dextrose agar (PDA), corn meal agar (CMA) or *Trichoderma* medium E (TME) or other selective methods have been devised by numbered authors. For example, there is selective media developed to distinguish "P" strains of *T. virens* that produce the antibiotic gliovirin and are effective against *Pythium*, than "Q" strains that produce gliotoxin and are effective against *Rhizoctonia*. Cultivation of strain and multiplication for further tests is not difficult, and the methods

described by Rifai (1969) are generally still followed. Recommended are inoculation on oatmeal, cornmeal or malt extract agars, and incubation under daylight for 5–7 days at a temperature of 20–25°C as they allow observation of stable morphological features [54–58]. Less expensive isolation is achieved when strain occurs naturally and is isolated from an area where they will be used after semi-solid cultivation, so it is connected to local production model. Hence, just isolation is not expensive but finding the useful strains in evaluating process is.

After isolation, bioassay for biological activity will be required to determine their nature, whether it is suppressing pathogens or enhancing plant growth, and if it is performed satisfactory. Moreover, the molecular identification of species is required so that the harmful (mushroom pathogens) and dangerous (trichothecene producers) ones can be avoided. Serious mushroom diseases can cause *T. aggressivum*, *T. pleurotum*, and *T. pleuroticola*. This species are genetically distinct from well known biocontrol strains [8, 59, 60]. Trichothecene production and their role in induction of plant defense were discussed earlier but it needs to be emphasizing that for registration and marketing strain must be nontoxic as microbial pesticides model requires toxicity testing. But, as most strains will be marketed under other production model, the potential for harm cannot be avoided entirely. Toxicity information is available for five *Trichoderma* species. Some testing reported acute oral toxicity as being >500 to <2.000 mg/kg, so at highest level, no effects were seen, and further, there have been no reported reactions after many years of extensive use. The species in *T. brevicompactum* complex are trichothecene producers, and they are as well not related to *Trichoderma* strains that are registered as bioagent. Immunosuppressive mycotoxin gliotoxin is produced by *T. virens* "Q" strains earlier mentioned, but they were no reported mycotoxicosis attributed to *T. virens* like it were for *Aspergillus* spp. Antibiotic important for biocontrol is volatile lactone and pyrone as it inhibits spore germination of *Phytophthora*, *Botrytis*, and other fungi. It is produced by *T. viride*/*H. rufa* clade only. Pyrone has pleasant coconut odor and is present in fruits, and it is used as flavoring agent, and therefore not considered as high hazard. In connection to plant defense system, peptaibols were mentioned earlier also. As they can lyses red blood cells that can be potentially harmful but it was reported that the syntheses are induced only by fungal cell walls or other elicitors, so again are connected with *Trichoderma* antifungal activity.

Considering all, finding a biologically useful and perspective strain for encapsulation into successful formulation which will be commercially viable is hard. *Trichoderma* has advantages because it can be easily isolated, grown, and tested for selection of efficient strains, manipulated and encapsulated in various formulations as they have good shelf life, which aids commercialization. Most strains used in bioapplications were local in origin and were used locally or regionally. Only smaller number of strains will show to be useful in various locations and environmental conditions, and therefore they become widely adopted and commercially available, like famous T22 and T39. So, what are the characteristics of perspective *Trichoderma* strain? One of the most important traits of beneficial culture is rhizosphere competence, the ability to survive in the environment, mostly rhizosphere. Rhizosphere competence or competitive saprobic ability is culture ability to compete other microbes in colonizing cellulose-rich substrates or the intercellular spaces of the surface layers of host roots. Therefore, *Trichoderma* culture would require cellulolytic enzymes to occupy this region and interact with the plant at a molecular level, either for plant growth promotion or inducing defense mechanism and inhibiting pathogenicity [57, 61]. Rhizosphere competence is always associated to cellulose production, and this is used for assessment of culture ability to metabolize cellulose. Method is simple as some cellulose materials (straw, cellophane discs) are buried into filed soil, then inoculate with a known quantity of fungal propagule

(conidia, chlamydospores) and after incubation reisolate. The amount of cellulose produced is directly related to the culture rhizosphere competence.

After isolation of strain with high rhizosphere and competitive saprophytic competence which is easily artificially mass multiplicative and safe for the environment, it must be perspective by its broad spectrum of activity. It can provide excellent and reliable control against a more of pathogens or/and can enhance growth of different plant species. The presentation must be of low effective doses. Classical method *in vitro* for assessment of biocontrol efficiency is "dual-culture" method. Probably the oldest one dual culture technique was described in 1955 by Morton and Strouble [62]. In one Petri plate, usually 10 mm diameter one, pathogen and *Trichoderma* are confronted as antagonist in the form of mycelial disc cut from the margin of the 7 days-old culture with a circular cutter. The mycelial disc is 5 mm in diameter but there are variations, so it can be 10 cm in diameter [63]. Both discs are placed on the substrate on the opposite of the plate, usually PDA, in Petri plate in the manner that the mycelium side facing the substrate and gently pressed in. The interspace between them amounted to 5 cm and interspace between micellar discs and edge of plate 2 cm. In control plate, *Trichoderma* disc is replaced with sterile agar disc. Inoculated plates are incubated at 25 ± 1°C for 7 days usually, although it could be 10–14 days depending on the pathogen species. Recommendable is to do readings on 2, 4, and 6 days after the incubation period by measuring radial growth of pathogen. The inhibition is quantified with index of inhibition (I) which represents inhibition percent of average pathogen radial growth (T) in the presence of *Trichoderma* and is calculated in relation to growth of the controls (C) as follows: $I(\%) = [(C - T)/C] \times 100$ [64]. Basic mechanisms can be determined: competition for substrate and nutrients as antagonist grow faster and colonize substrate; mycoparasitism if antagonist colonize pathogen mycelial mat, sclerotia or fruiting body and diminish and decay of those structure can be monitored; antibiosis when inhibition zone is formed because toxins spread through substrate and inhibited growth of pathogen. There is possibility to misinterpret mycoparasitic capability of the antagonist if neglected that it can absorb nutrients from the agar. Antibiosis by production of volatile metabolites can be tested using slight modification of dual culture technique [6]. The mycelial discs are placed centrally, but the *Trichoderma* disc is placed in the lid while pathogen disc on the bottom of the plate sealed together with adhesive paraffin tape.

Furthermore, perspective culture needs to be compatible with other bioagents or tolerant to pesticides commonly used in agriculture which facilitates integrated control. For instance, it is recommended by commercial seed treatment systems to apply a chemical pesticide with the effective *Trichoderma* strains. While chemical will provide short term protection, the *Trichoderma* provides season-long benefits to plants as colonizes roots. Culture should even tolerate oxidizing agent, UV radiations, desiccation, heat, draught, etc. [21, 57, 65, 66]. Because these characteristics are strain depended, fewer than 1% of screened cultures will meet the expectations. Therefore, screening for bioactivity is the critical step when hundreds or thousands of cultures are tested. First, efficacy bioassays are performed in small-scale laboratory trials, *in vitro* and *in vivo,* which are usually random. Recommended is so called three-partner model, a system with potential *Trichoderma* agent, pathogen, and plant. Screening process is obviously time and money consuming as well as requires labor of more than one analyst. Molecular techniques of assisted selection using phenotypic or/and genotypic markers perhaps can improve screening productivity and shorten it but will raise the costs. Moreover, only in few cases, connection with bioactivity and molecular markers was proved. Few perspective cultures from lab-trials are proceeding to greenhouse and small pilot trials at open. At this stage, testing is conducted in different environmental conditions and crops and several

pathogens in the case of biocontrol activity. Satisfaction of these very limiting conditions is important for future possible wide range of applicability of bioagent which is the most important property needed for registration and marketing. For commercialization, the bioagent should be produced on industrial scale.

4.2 Methods of formulation

Trichoderma is mostly fermented in solid state with the aim to achieve highest yield with lower cost of culture medium. Obtained fungal biomass needs to be immobilized in certain carriers in low cost but high density inocula and encapsulated into formulation engineered to maintain fungal propagule viable during the transport, storage, and application. *Trichoderma* has advantages because it can be easily manipulated for encapsulation which means mixing wet or dry fungal biomass with a matrix forming material, such as gelatinized polysaccharide or an oil emulsion. Matrix is serving as carrier of fungal inoculums. Most of the examples on different types of *Trichoderma* inoculum for biocontrol include peat, granular vermiculite or clay mixtures, grains, and alginate pellets. Encapsulation in formulation of alginate pellets has been studied and positively evaluated by various authors as found to be successful for the *Trichoderma* delivery [30–32, 67–72]. In the receipt of chapter's author for small trial purpose, culture is grown on Petri dishes 10 cm in diameter containing 20 ml of PDA and incubated in humid chamber at 25°C for 7 days until conidiation. After incubation, the substrate altogether with hyphal biomass and conidia from two Petri dishes are upraised with spatula and transferred into glass with 50 ml sterile DI water. These are mixed by common blender at low speed for 3–5 min in order to make a suspension. The final concentration to be used contained 4×10^6 spores ml^{-1}. The suspension is mixed with 100 g l^{-1} talcum and 10 g l^{-1} sodium alginate. The formed matrix is placed in a separator funnel modified in order to allow suspension to drip into a 0.1 M suspension of calcium gluconate under stirring on magnetic agitator. Drops of alginate matrix dripped into calcium gluconate suspension transformed to gelatinized spherules or pellets. Pellets were removed from suspension within 10 min, rinsed with distilled water, and allowed to dry on waxed paper under a sterile vertical flow for 12–24 h [16, 30, 31]. In India is quite popular a talc-based formulation of *Trichoderma* developed at Tamil Nadu Agricultural University [64]. There are also oil-based formulations prepared with a combination of vegetable/mineral oils and they are suitable for foliar spraying under dry weather. Some formulations use organic wastes like coffee husk from coffee industry and press mud, byproduct of sugar factory.

Carriers of *Trichoderma* inoculum must be cheap, should dissolve well in water, and preserve fungal viability to insure formulation shelf-life. Formulation good for the commercialization should have increased shelf-life to fulfill requirements for storage and transport, and this is one of the most limiting factors. Further, it should deliver viable propagules in adequate concentration through adequate application. In the case of endophytic strains that grow with plant roots, only small amounts of inoculum are needed to be provided for long-term benefits. The minimal propagule number should not be less than 2×10^6 per milliliter or gram of formulation. Commercial preparations are mostly high concentrated with more than of 10^{10} propagules per gram. Thus, commercial preparations need to be applied in dose of 500 mg per hectare or for addition to greenhouse potting soils only 10^4–10^5 per cm^3 [21].

The most important act before commercialization is that formulation, or even strain/culture should be legally protected by means of patent. It is patented as biotechnological invention because it is based on microbe and its mechanisms or its metabolic products. The strain pure culture needs to be deposited in an officially recognized microbial collection by Budapest treaty signed by all countries

pertaining to the World Intellectual Property Organization. It should be emphasized that patent is not authorization for commercial use, and it does not connote registration for agricultural use. All patents are regulated by legal treaties. There are series of international and national treaties: Union of Paris of 1883, Patent Cooperation Treaty of 1970, and European Patent Agreement of 1973. In the USA is United States Patents while in EU the legal framework is regulated by Directive 98/44CEE on the patentability of biotechnological inventions.

5. Concluding remarks

Present pest control intend to kill, even eradicate organisms but without choosing between harmful and benefit ones. Biocontrol could help in providing low-cost and environmentally safe technologies to farmers especially today when food security and rural livelihood are a key priority. Original paper updates on basic and applied research in all aspects of biological control of invertebrate, vertebrate and weed pests, and plant diseases can be found in the BioControl, the official journal of the International Organization for Biological Control (IOBC). For developing world, biopesticides and biofertilizer are considered extremely important as perceived by Association of Asian Pacific Agricultural Research Institution (AAPARI). Thus are in progress biopesticide researches of the agriculturally important microorganisms led by the *Trichoderma* that can be encapsulated in bioproducts. Although the chemical control of plant diseases differs tremendously from biocontrol by microbial-based biopreparations, registration regulations remain the same or similar depending on country. In USA, the Environmental Protection Agency (EPA) registers pesticides under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA). In EU, registration is defined by Regulatives 91/414/CEE, 2092/91, 1488/97, etc. In Croatia, it is defined by Plant Protection Law, which is harmonized with EU Regulatives. In general, registration of any product making a pesticidal claim is obligation defined by law in order to prevent unreasonable, adverse effects on consumer health, or the environment. Registration requires time, expenses, and efforts for conducting toxicological, environmental, and efficiency testing. Especially is difficult to enlighten excessive specificity of bioagent as success of control by any bioagent depends on three living system: pathogen, plant, and bioagent.

In this new era, the meaning of biological control must be expanded to include plant growth promotion and disease resistance along with classical antibiosis and mycoparasitism because *Trichoderma* biocontrol and plant performances-enhancing activities overlap. Even better, *Trichoderma* research community emphasizes that considering biocontrol as primary ability of *Trichoderma* may influence biocontrol system in development because it means optimizing conditions for wrong mechanism. It must be taking into account that *Trichoderma*, as pseudomycorrhizal plant partner, has effects that extend beyond biocontrol. These fungi produce changes in plant metabolism, which have direct effects on plant physiology, like increasing growth and enhancing resistance, and even reprogramming of plant gene expression owing to coevolution with plants. Thus is especially pronounced that the control of plan response to abiotic and biotic stresses, such as diseases, is only a subset of their activities and benefits to plant. In future can be expected momentum of proteome study in order to help giving an understanding of how *Trichoderma* treated plants become more resistant to pathogen attacks. Also, needed is development of easy and inexpensive screening methods whose test conditions approach as much as possible the real system where biocontrol has to be. Registration requirements need to be revised and include fact that *Trichoderma*-fungicides are entering market as plant growth promoters. There are opinions that future of biopesticides

lies in plant-protecting pesticides or self-protecting plant, a high-value crop plant with embedded genes from bioagent. Yet, are not the same concerns influencing transgenic plant and biopesticide in the light of biosafety? Nontarget effects, toxicity, and possible pathogenicity for plant, animals and humans, allergenicity or horizontal gene transfer to nontarget organisms are exactly limiting issues for both transgenic plant and biopesticide. Further, for both groups are present consumer concerns about living microorganisms in connection with bioterrorism and food-bone diseases. Noticeable is that all that is needed is socially receptive environment and that should be developed and promoted by *Trichoderma* research community.

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