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Evaluation of *Trichoderma* spp., as biological agents in some of plant pathogens

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ABSTRACT

The use of biological agents is becoming an increasingly important alternative to chemical control against insects, weeds and diseases in agriculture. The success of this method depends on the nature antagonistic characters and the mechanisms of action of microorganism. Fungi are the most important biological agents against plant pathogens. Trichoderma species are common filamentous imperfect fungi. The mycoparasite ability of Trichoderma species against some economically important plant pathogens allows for the development of biocontrol strategies. In this research, the effect of biocontrol of Trichoderma species on some plant pathogens was investigated. The results showed a direct inhibition of the pathogenic microorganisms by Trichoderma species. Hence Trichoderma species can be considered as biocontrol agents for control of some plant pathogens.

Keywords: Biological control, *Trichoderma* spp., Plant pathogens.

INTRODUCTION

Biocontrol or Biological control can be defined as the use of natural organisms or genetically modified, genes or gene products the effects of undesirable organisms to favor organisms useful to human, such as crop, trees, animals and beneficial microorganisms [1]. This strategy of control is ecologically clean and compatible with different models of agriculture organic biological and pathogen management [1].

Biocontrol agents are widely regarded by the general public as natural and therefore non-threatening products, although risk assessments must clearly be carried out on their effects on non-target organisms and plants [1]. Moreover, knowledge concerning the behavior of such antagonists is essential for their effective use [1].

One of the most interesting aspects of the science of biological control is the study of the mechanisms employed by biocontrol agents to effect disease control [2].

Bacteria and fungi are the most important biological agents against plant pathogens.

Particular bacterial strains in certain natural environments prevent infectious diseases of plant root. How these bacteria achieve this protection from pathogenic fungi has been analyzed in detail in biocontrol strains of fluorescent Pseudomonads. During root colonization these bacteria produce antifungal antibiotics, elicit induced systemic resistance in the host plant or interfere specifically with fungal pathogenicity factors [3].

2. Description of fungus

Fungal species belonging to the genus *Trichoderma* are worldwide in occurrence and easily isolated from soil, decaying wood, and other forms of plant organic matter [2].They are classified as imperfect fungi, in that they have no known sexual stage. Rapid growth rate in culture and the production of numerous spores (conidia) that are varying shades of green characterize fungi in this genus [2]. The reverse side of colonies is often uncolored, buff, yellow, amber, or yellow-green, and many species produce prodigious quantities of thick- walled spores (chlamydospores) in submerged mycelium. [4]. *Trichoderma* species are fungi with teleomorphs belonging to the Hypocreales order of the Ascomycota division [5]. These fungi colonize woody and herbaceous plant materials, in which the sexual telemorph (genus *Hypocrea*) has most often been found [6]. 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 [6].

They shown high level of genetic diversity, and can be used to produce a wide range of products of commercial and ecological interest. The potential of *Trichoderma* species as biocontrol agents of plant diseases was first recognized in the early 1930s, and in subsequent years, control of many diseases has been added to the list [2]. This has culminated in the commercial production of several *Trichoderma* species for the protection and growth enhancement of a number of crops in the United States, and in the production of *Trichoderma* species and mixtures of species in India, Israel, New Zealand, and Sweden [7]. One of the most interesting aspects of the science of biological control is the study of the mechanisms employed by biocontrol agents to effect disease control. Past research indicates that the mechanisms are many and varied, even within the genus *Trichoderma*. In order to make the most effective use of biocontrol agents for the control of plant diseases, we must understand how the agents work. The selected manuscripts used in this paper were chosen because they illustrate what has been learned about mechanisms involved in biocontrol with *Trichoderma* species.

2.1. The metabolites. They are prolific producers of extracellular proteins, and are best known for their ability to produce enzymes that degrade cellulose and chitin [6]. They also produce other useful enzymes. For example, different strains produce more than 100 different metabolites that have known antibiotic activities [6].

2.2. Telemorphs. Members of the Hypocreales (Fungi, Ascomycota) are common in all types of moist forests [8]. These fungi are easily recognized by their brightly coloured fructifications [8]. The most conspicuous members, species of *Hypomyces* (Hypocreaceae), parasitize mushrooms and bracket fungi [8]. Another commonly encountered genus is *Hypocrea* (*Hypocreaceae*), Species of *Hypocrea* and its anamorph *Trichoderma* Pers. [8] are commonly encountered in humid tropical or subtropical forests although they also occur in arid, temperature or boreal forests, even in the most extreme north and south latitudes [8]. The *Hypocrea* teleomorph can be found on wood, on other members of the Ascomycota, on resupinate basidiomycetes, or on perennial bracket fungi in varying stages of decay, and less commonly on herbaceous substrata. All investigated *Trichoderma* species are intimately related to *Hypocrea*, and increasingly, named *Trichoderma* species are being shown to be the anamorphs of *Hypocrea* species [8]. The *Hypocrea* form is rarely seen in culture. On the other hand, it is in the *Trichoderma* state that members of this genus are recovered in ecological investigations or cultivated in connection with commercial applications. The *Trichoderma* forms are commonly isolated from soils but also sporulate on moist wood, mushrooms, or bracket fungi in forests, where they are easily recognized by their masses of conidia that are usually green, but less commonly white or yellow [8]. They are also found in diverse habitats: for example, they may be found in water-damaged buildings, or as endophytes within the trunks of asymptomatic tropical forest trees [8]. In addition, they may be isolated as etiologic agents of opportunistic infection in immunocompromised humans [8]. The fact that many *Trichoderma* species have demonstrated antifungal or plant-growth-stimulating activities has led to their exploitation as biological control agents, and in this connection some isolates are used in commercially available application [8]. More than 200 species of *Hypocrea* have been described, yet the genus has never been monographed. Recent systematic research suggests that it is not possible to identify a *Hypocrea* species unless its *Trichoderma* anamorph is known [8]. Although only about 50 species of *Trichoderma* have been described, exploration of new geographical locations and ecological niches has revealed many additional undescribed species. Because the anamorph is the form most commonly connected to information on the economic and ecological significance of these organisms, it is essential to clarify the biology of *Trichoderma* and *Hypocrea* in a unitary way. Holomorphs must be studied in order to effectively determine both life cycles and species concepts [8].

Hypocrea was first described by Elias Fries in 1825 based on *Sphaeria rufa* Pers.: Fr., a species with hyaline, ascospores. Currently, the type species of the genus is represented by *Hypocrea rufa* Fr. *Hypocrea* is generally characterized by having perithecia embedded in fleshy stromata formed by pseudoparenchymatous tissue or highly compacted hyphae. *Hypocrea* has eight 1-septate ascospores that disarticulate at the septum early in their development producing 16 part-ascospores in each ascus. Even though the disarticulation of ascospores is one of the distinguishing characters of the genus, other genera such as *Aphysiostroma Arachnocrea*, *Dialhypocrea*, *Podostroma*, *Protocrea*, *Pseudohypocrea*, and *Sporophagomyces* also have disarticulatin ascospores [8]. Recent studies using DNA sequence data suggest that *Podostroma* and *Protocre*a [9] are congeneric with *Hypocrea*.

In several studies, similarities were noted between *Podostroma* and *Hypocrea*, which differ only in the stalked stroma of the former [8]. Other characteristics of *Podostroma*, such as morphology of the stromatal tissue, perithecia, and anamorphs and ecology, are indistinguishable from corresponding features of *Hypocrea*. *Aphysiostroma, Arachnocrea, Dialhypocrea, Pseudohypocrea*, and *Sporophagomyces*, which are similar to *Hypocrea* in the disarticulation of their ascospores, have distinct teleomorph and anamorph morphology [8]. Most notably, ascospores of *Arachnocrea*, *Pseudohypocrea* and *Sporophagomyces* are biconical. *Arachnocrea* and *Sporophagomyces* are mycoparasitic [8]. Recent molecular-phylogenetic studies show that *Aphysiostroma* and *Arachnocrea* are basal to *Hypocrea.*

2.3. Mechanisms and examples of action. *Trichoderma* species have long been recognized as agents for the control of plant disease and for their ability to increase plant growth and development [6]. The ecological role of this genus is that *Trichoderma* strains take part in the decomposition of plant residues in the soil. Some *Trichoderma* species are very good cellulose producers and therefore they are important for the biotechnological industry [10]. Antagonism is based on different mechanisms, like the of antifungal metabolites by *Trichoderma*, competition for production space and nutrients and mycoparasitism. Mycoparasitic *Trichoderma* strains are able to recognize the host hyphae, to coil around them, develop haustoria, penetrate the cell wall of the host with cell- wall degrading enzymes like chitinases, glucanases and proteases, and utilize the contents of the host hyphae as nutrient source [5]. *Trichoderma* strains with effective antagonistic abilities are potential candidates for the biological control of plant diseases [11]. Abiotic and biotic environmental parameters may have negative influence on the biocontrol efficacy of *Trichoderma* strains, therefore it is very important to collect information about the effects of environmental factors on the different activities of *Trichoderma* strains with biocontrol [5]. *Trichoderma* spp. are promising candidates for the biological control of plant pathogenic fungi [5]. When planning the application of antagonistic *Trichoderma* strains for the purposes of biological control, it is very important to consider the environmental parameters affecting the biocontrol agents in the soil [5]. A series of abiotic and biotic environmental parameters has an influence on the biocontrol efficacy of *Trichoderma*. Some important parameters to be considered are the effects of temperature, water potential and pH, and the presence of pesticides, metal ions and antagonistic bacteria in the soil [5]. Most of the *Trichoderma* strains are mesophilic. Low temperatures in winter may cause a problem during biological control by influencing the activity of the biocontrol agent [5]. Another problem emerging during the application of *Trichoderma* strains as biocontrol agents is that they cannot tolerate dry conditions, however, we may need biocontrol agents against plant pathogenic fungi which are able to grow and cause disease even in dry soils [5]. The pH characteristics of the soil also belong to the most important environmental parameters affecting the activities of mycoparasitic. *Trichoderma* strains within the frames of a complex integrated plant protection strategy, we may have to combine *Trichoderma* strains with chemical pesticides or metal compounds, therefore it is important to collect information about the effects of pesticides and metal ions on the biocontrol strains [5]. Antagonistic soil bacteria may also have negative effects on the biocontrol abilities of *Trichoderma* strains, therefore it may be advantageous if a biocontrol strain possesses bacterium- degrading abilities as well [5]. Scientists observed that it was possible to implement biological remediation strategies based on the ability of microorganisms to carry out degradative processes to identify the potential of *Trichoderma* spp. in soil remediation is an important step in the recognition of the value of the genetic resources of biodiversity, in this case, microorganisms. This aspect shows the importance of biological control in soil improvement program [12]. *Trichoderma* spp. attack parasites and possess resistance to most agricultural chemicals. They constitute its living part and are responsible for the dynamics of transformation and development of soil structure.

Trichoderma spp. are agriculturally and industrially important, being the major source of many commercial enzymes and as biofungicides. More than 60% of all registered biofungicides used for plant disease control are *Trichoderma* spp. directly kills and obtain nutrients from other fungi is considered to be one of the most important mechanisms of biocontrol [13]. Others being competition for nutrients, antibiosis and induced resistance in plants against invading pathogen [13]. In addition to use as biocontrol, *Trichoderma* spp., are also used as biofertilizers (plant growth promoters) and for the mitigation of abiotic and physiologic stresses. Earlier, an industrial strain of *Trichoderma reesei* was sequenced and analysed [13]. However, this species is not used as biocontrol, being very weakly mycoparasitic [13].

Some *Trichoderma* species are antagonistic to many soil borne phytopathogenic fungi and significantly decreased infection and disease through antibiosim [12]. *T. harzianum* has been shown to be bioactive against *Armillaria mellea*. Investigations have shown that 6-pentyl-pyrone and other-pyrone analogues exhibit antibiotic activity against the growth of the fungus *Gaeumanmyces graminis* var *tritici.* 6- pentyl-pyrone has been reported to inhibit growth *in vitro* of a number of fungi and that it reduced the rate of damping-off in lettuce by inhibiting the growth of *Rhizoctonia solan*i [14].

Trichoderma harzianum is a fungal biocontrol agent that attacks a range of phytopathogenic fungi. *T. harzianum* alone or in combination with other *Trichoderma* species can be used in biological control of several plant diseases [15]. It has been also shown to be effective in controlling *Fusarium* crown and root rot under greenhouse and field conditions. Although *Trichoderma* spp are ubiquitous, the type of the soil can affect growth, proliferation and effectiveness as biocontrol agent. Because soil ecology is complex, and with year-to-year fluctuations in climate conditions, treatments with microbials are often inconsistent [16]. The study showed that *T. harzianum* strains were effective in reducing disease incidence and severity of *Fusarium* crown and root rot of tomato under greenhouse conditions. The effect of *Trichoderma* on reduction of the crown and root rot disease and on yield of tomatoes has been investigate [15].

3. How *Trichoderma* species work?

Biological control is a promising tool to help maintain current levels of agricultural production while reducing the release of polluting chemical pesticides to the environment. However, more knowledge of the mechanism of action of the biological agents involved is needed to improve [17]. Early in the process of parasitization of *Rhizoctonia solani* by *Trichoderma harzianum,* directed hyphal often in advance of contact then, the hyphae of *T. harzianum* coil tightly around those of *R. solani* [18]. It has recently been shown that a lectin from *Sclerotium sclerotium* induces coiling of hyphae of *T. harzianum* and the formation of mycoparasitism-related structures (e.g., hooks and appressorium-like bodies) around fibres coated with the lectin, and thus simulates the interaction with the host [19]. Similar lectins have been identified in *R. solani* and other fungal host species [20]. Thus, lectins present on the cell wall of the host are suggested to take part in its recognition. The use of light and scanning electron microscopy has demonstrated the penetration of hyphae of *R. solani* by *T. harzianum* [17]. A complex set of extracellular enzymes is produced by mycoparasitic strains of *Trichoderma* when grown on isolated cell walls of *R. solani* [17]. Weindling (1934) reported that a strain of *T. lignorum* produced a "lethal principle" that was excreted into the surrounding medium, allowing parasitic activity by the biocontrol agent. In 1941, he characterized the "lethal principle", demonstrated that it was toxic to both *R. solani* and *Sclerotinia americana,* and named it gliotoxin [2]. Thus, lytic enzymes such as b-1,3-glucanases, chitinases and proteases are probably responsible for hyphal lysis through the digestion of major cell wall components. The chitinolytic system of *T. harzianum* comprises six distinct enzymes, two of which are classified as acetyl glucosaminidases and the rest as endochitinases [21].

Three chitinase-encoding genes have been cloned and characterized [22]. However, the relevance of the corresponding enzymes to mycoparasitism remains to be proven [17]. The gene ech42 coding for the endochitinase [22]. Ech42 is highly expressed when the fungus is grown on media [23]. Another mycoparasitism-related containing chitin or in dual cultures with a host (direct confrontation assays; gene, prb1, encoding a 31-kDa basic proteinase) is strongly induced during direct confrontation experiments [17]. Strong expression of prb1 is also observed when *T. harzianum* is grown in media containing chitin as a sole carbon source [24]. Furthermore, transgenic *Trichoderma* strains carrying multiple copies of prb1 showed a much higher level of control of the disease caused by *R. solani* [25], suggesting that this gene is indeed directly involved in mycoparasitism [17]. It may be expected that the production of hydrolytic enzymes takes place simultaneously with the formation of parasitic structures and that both events might be activated by a common mechanism [17]. However, the recognition events at the molecular level are not yet clear. Oligosaccharides containing N-acetyl-D-glucosamine (GlcNAc), including chitin oligomers, which are components of the potential host, might act as elicitors, enabling the invader to trigger the so-called mycoparasitic reaction [26].Inbar and Chet have shown an increase in the activity of an intracellular 102 glucosaminidase using a biomimetic system based on the binding of a purified lectin from *S. rolfsii* to nylon fibres, suggesting that the recognition event mediated by lectins serves as a signal that triggers a general antifungal response in *Trichoderma* [19]. However not a single mycoparasitism-related gene has been directly associated with the response to the lectin-carbohydrate interaction [17]. The mycoparasitic relationship between *Trichoderma* and its potential host might involve bioterms of its dependence on contact with the host [17]. Mycoparasitic strains of *Trichoderma* were grown on isolated cell walls of *R. solani* [27]. Thus, lytic enzymes such as b-1,3-glucanases, chitinases and proteases are probably responsible for hyphal lysis through the digestion of major cell wall components [17]. The chitinolytic system of *T. harzianum* comprises six distinct enzymes, two of which are classified as N-acetyl glucosaminidases and the rest as endochitinases [17].

4. Mechanisms of plant-disease control by *Trichoderma*

The results presented and discussed in the review allow us to propose a model of the mechanisms by which *Trichoderma* spp. control or reduce plant disease. Other mechanisms also exist, including the inhibition of pathogen enzymes that are necessary for leaf penetration and competition for seed nutrients that are required for pathogen germination in soil [6]. *Trichoderma* spores or other biomass can be added to soil by a variety of methods. If the strain is rhizosphere competent, it colonizes root surfaces and the outer layers of the cortex. This establishes a zone of interaction into which the *Trichoderma* strain releases bioactive molecules [6]. These include elicitors of resistance, such as homologues of avirulence (Avr) proteins and proteins with enzymatic or other functions. The fungi also produce enzymes that release cell-wall fragments, which also enhance plant resistance responses.

The plants produce cell-wall deposits and biochemical factors that limit the growth of the *Trichoderma* strain and cause it to be avirulent [6].

Pathogens can attack roots but, in the presence of *Trichoderma*, infection is reduced by the same or similar molecules and cell-wall alterations that result in the avirulence of the *Trichoderma* strains [6]. Furthermore, several strains induce systemic resistance in plants even though they are localized on the roots, probably through the action of a signalling compound (curved arrow going up from the roots).

Then, when pathogens attack plant leaves or stems, the plant is potentiated to respond rapidly by producing defence-related enzymes and antimicrobial compounds [6]. In addition, *Trichoderma* strains can attack pathogens in the soil by a variety of mechanisms. The strains respond tropically to the presence of the pathogens, and the interaction begins before the two organisms come into contact. The *Trichoderma* produces sensing enzymes that release cell-wall fragments from the hyphae of the target pathogen, which increases the release of additional enzymes [6].Antibiotics can also be produced. The next step of the interaction is the actual parasitism (which frequently results in the coiling of the *Trichoderma* fungus around the pathogen) and the production of a number of synergistic cell-wall-degrading enzymes and other substances, followed by the infection and death of the target fungus. In the absence of *Trichoderma*, root pathogens infect roots and cause disease [6]. Plants are also unprotected against foliar, stem and flower pathogens.

As a consequence of the interactions between *Trichoderma* fungi and plants, a variety of pathogens of roots and the above-ground parts of plants cause less disease in plants in which the roots are colonized by *Trichoderma* [6].

Even in the absence of pathogens or disease, plants frequently have larger roots and higher levels of productivity in the presence of *Trichoderma*. In the absence of *Trichoderma*, either the above-ground or below-ground portions of plants usually have more disease, and are often less robust [6].

Another mycoparasitism-related chemical and physiological reactions that precede the microscopically visible phenomena of hyphal coiling, appressorium formation, penetration and cytoplasmic degradation [17]. In this study, the possible involvement of lectins in the induction of two hydrolytic enzymes in *T. harzianum* during mycoparasitism was investigated [6]. A novel system was used to analyze the parasitic response in gene, prb1, encoding a 31-kDa basic proteinase is strongly induced during direct confrontation experiments. Strong expression of prb1 is also observed when *T. harzianum* is grown in media containing chitin as a sole carbon source [17].

In the recent years, the environmental contamination caused by excessive use of chemical pesticides increased the interest in integrated pest management, where chemical pesticides are substituted by biopesticides to control plant pests and plant diseases [22]. *Trichoderma* based biocontrol agents (BCAs) possess better ability to promote plant growth and soil remediation activity compared to their counterparts (virus, bacteria, nematodes, and protozoa) [22]. Their capability to synthesize antagonistic compounds (proteins, enzymes, and antibiotics) and micro-nutrients (vitamins, hormones, and minerals) enhance their biocontrol activity [28]. Like other fungal BCAs, conidial mass of *Trichoderma* is the most proficient propagule, which tolerates downstream processing despite [22]. The advantages, mass production of *Trichoderma* BCAs is less prevalent, owing to high-cost raw materials like Mendel's medium, molasses, corn steep liquor, and other [29]. *Trichoderma* spp. have gained wide acceptance as effective BCAs against several commercial phytopathogens [22]. These antagonistic fungi are most common among fungal biocontrol agents because of their multiple BCA characteristics, namely, antagonism and plant-growth stimulation [22]. Thus, mass-scale production of *Trichoderma* spp. would have great potential for commercial use. Micropropagules of *Trichoderma* spp. in the form of conidia are preferred over chlamydospores and mycelial biomass because of the viability and stability in field application [22]. Therefore, there are several BCA products of *Trichoderma* spp. in the market containing conidia of *Trichoderma* spp. as active ingredients. Multiple BCA action renders the production of *Trichoderma* spp. conidia of commercial and environmental interest [22]. There is abundant literature on the use of conventional synthetic media like glucose, cellulose, soluble starch, and molasses to produce *Trichoderma* spp. [22]. However, the cost of these raw materials for commercial production of BCAs is one of the major limitations behind the restricted use [22]. To overcome the cost limitation, many researchers have successfully used substrates like corn fiber dry mass and sewage sludge compost [22]. Despite the use alternate sources, the cost of production was still high, as these raw materials need to be supplemented by other nutrients [22].

The density of a pathogen population is one of the most important factors to trigger the initiation of an epizootic [22]. High relative humidity is widely recognized as a critical factor. As such, the objectives of the study comprises the elevation of the use of products of the cereal industry (corn bran and wheat bran) as raw materials to produce *Trichoderma* spp. conidia without the need of the addition of nutrients (C and N sources) to increase spore concentration [22].

CONCLUSION

Biological control is one of the best alternative against some plant pathogens. The limitations to biocontrol use are scarce knowledge on the ecology of rhizosphere and use of in vitro antagonism for selection of biological control agents. But, the advantages of this method is more. *Thichoderma* spp. that are common saprophytic fungi found in almost soil and micro flora, have been investigated as potential biocontrol agents because of their ability to reduce the incidence of disease caused by plant pathogenic fungi.

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