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Biological Control with *Trichoderma* Spp. With Emphasis on *T. harzianum*

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Abstract: Biocontrol with antagonistic microbes such as the fungus *Trichoderma* is one area of research. *Trichoderma* spp. are among the most common saprophytic fungi. *Trichoderma* spp. are well documented as effective biological control agents of plant diseases caused by both soilborne fungi and leaf- and fruit-infecting plant pathogenic fungi. *Trichoderma* spp. are often very fast growing and rapidly colonize substrates, thus excluding pathogens such as *Fusarium* spp. Several of these fungi are also parasitic to other fungi including plant pathogens. *Trichoderma harzianum* Rifai is an efficient biocontrol agent that is commercially produced to prevent development of several soil pathogenic fungi. *T. harzianum* alone or in combination with other *Trichoderma* species can be used in biological control of several plant diseases. An additional advantage for *T. harzianum* is that it increases growth in various plants. This review discussed biological control with *Trichoderma* spp. with emphasis on *T. harzianum* Biological Control with *Trichoderma* spp. with emphasis on *T. harzianum* and numerous mechanisms that *Trichoderma* spp. have evolved in attacking other fungi and enhancing plant and root growth.

Key words: Trichoderma harzianum, biological control, antibiosis, competition, induced resistance, mycoparasitism

INTRODUCTION

Several species of *Trichoderma* have been extensively studied for their biological control effects against fungal plant pathogen[1-5]. In fact, the antifungal abilities of these beneficial microbes have been known since the 1930s, and there have been extensive efforts to use them for plant disease control since then[6]. Weindling[7,8] and Weindling and Fawcett[9], perhaps, were the first investigators to demonstrate the potential of *Trichoderma* spp. to control plant disease. Wendling[7] ascribed biocontrol by *Trichoderma lignorum* of citrus seedling disease, incited by *Rhizoctonia solani*, to mycoparasitism. Wendling described in detail the mycoparasitism of *R. solani* hyphae by the hyphae of the biocontrol agent, including coiling around pathogen hyphae, penetration, and subsequent dissolution of the host cytoplasm.

Many *Trichoderma* strains have been identified as having potential applications in biological control of plant pathogenic fungi on many crops including strawberries (Fragaria Vesca), beans (*Phaseolus vulgaris)*, peas (*Pisum sativum*), cucumbers (*Cucumis sativus*), tomatoes, radishes (*Raphanus sativus*), sugar beets (*Beta vulgaris*), cotton (*Gossypium hirsutum* L.). A partial list of plant pathogenic fungi affected by *Trichoderma* includes: *Armillaria*, *Botrytis*, *Chondrostereum*, *Colletotrichum*, *Dematophora*, *Diaporthe*, *Endothia*, *Fulvia*, *Fusarium*, *Fusicladium*, *Helminthosporium*, *Macrophomina*, *Monilia*, *Nectria*, *Phoma*, *Phytophthora*, *Plasmopara*, *Pseudoperonospora*, *Pythium*, *Rhizoctonia*, *Rhizopus*, *Sclerotinia*, *Sclerotium*, *Venturia*, *Verticillium*, and wood-rot fungi[10-16].

T. harzianum, a member of the fungal genus *Trichoderma,* has been extensively studied as biological control agent[17,18] due to its ability to successfully antagonize other fungi including plant pathogenic species. It has been commercially labelled to prevent development of several soil pathogenic fungi. Strains of *T. harzianum* are marketed in a number of products; such as PlantShield7 / RootShield7 from the U.S., Trichodex7 from Israel, Binab T7 from Sweden, and Supresivit7 from the Czech Republic[19].

Mechanisms of biological control activity of *Trichoderma* spp.: *Trichoderma* spp. have evolved numerous

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mechanisms that are involved in attacking other fungi and enhancing plant and root growth. These mechanisms include competition for space and nutrients[20], mycoparasitism[21-23], production of inhibitory compounds[24], inactivation of the pathogen's enzymes[25] and induced resistance[26,27]. These mechanisms are going to be discussed in detail with examples.

Competition: Competition is one of the mechanisms of biological control activity of *Trichoderma spp.* against phytopathogenic fungi. *Trichoderma* species are generally considered to be aggressive competitors[6]. *Trichoderma* spp. are often very fast growing and rapidly colonize substrates, thus excluding pathogens such as *Fusarium* spp.[4]. Rhizosphere competence by biocontrol agents, described in a previous section, is important in the mechanism of competition, especially with seed treatments[28]. This is the zone where protection against pathogens is critical[28]. It is important for control of root diseases because a biocontol agent cannot compete for space and nutrients if it is unable to grow in the rhizosphere[29]. *Trichoderma* species, either added to the soil or applied as seed treatments, grow readily along with the developing root system of the treated plants[30,31,32,29].

Soil treatments with *T. harzianum* spores suppressed infestations of *Fusarium oxysporum* f. sp. *vasinfectum* and *F. oxysporum* f. sp. *melonis.* Competition was a proposed mechanism, although it was not proven to be the main activity[33]. The study by Utkhede *et al.*[34] demonstrated that applications of RootShield 7 (*T. harzianum*) and yeast strain (S33) of *Rhodosporidium diobovatum* Newell and Hunter applied as a postinoculation foliar sprays were effective to control tomato stem canker caused by *B. cinere*a. They concluded that the mechanism could be competition for space. Competition between *Trichoderma* and *Botryris* is especially active during the colonization of floral debris, and the supply of the antagonist at the end of the flowering is of prime importance[35]. Competition at the atmosphere level as biocontrol mechanism in *Trichoderma* spp. was suggested by Marchetti *et al.*[36]. In *in vitro* experiments, Rhizoctonia *solani*, *Pythium ultimum* and *Chalara elegans* were strongly inhibited by *Trichoderma* while *Fusarium oxysporum* and *Cytospora* sp. showed tolerance to the antagonistic activity of four species of *Trichoderma.* Both the pathogens and the *Trichoderma* were grown in pairs on the same agar medium in a confined environment on separate plates. The growth inhibition efficacy of *Trichoderma* on the pathogen seems to perform not only at medium, but also at atmosphere level. The observed inhibiting action of *Trichoderma* was associated with a high rate and extent of CO_2 accumulation. The plant pathogenic fungi that were characterized by slow rates of CO_2 production were more sensitive to the antagonists.

Antibiosis: Many isolates of *Trichoderma* spp. produce volatile and nonvolatile antibiotics[37,38]. Howell and Stipanovic[39] isolated and described an antibiotic, glovirin from *Gliocladium (Trichoderma) virens* that was strongly inhibitory to *Pythium ultimum* and *Phytophthora* species. Sivan *et al.*[2] reported that growing *Trichoderma*, which is antagonistic to *Pythium aphanidermatum,* produced inhibitory compounds. When *Trichoderma* was grown on a cellophane membrane placed on agar and the membrane was removed and the agar was inoculated with *Pythium*, they showed that the growth of the pathogen was partially inhibited by substances produced by the *Trichoderma.* Liftshitz *et al.*[40] showed that control of *Pythium* species on peas by *T. harzianum* strain T12 and *T. koningii* strain T8 was not due to either competition or mycoparasitism. They ascribed biocontrol to the production of a toxic factor by the biocontrol organism in the spermosphere, which inhibited growth of the pathogens. However, most attempts to correlate *in vitro* antibiosis by *Trichoderma* against fungal pathogens with what actually happened in natural systems have failed[41]. Examination of the literature has shown that *Trichoderma* spp. secrete a number of antifungal antibiotics including pyrones, isocyanates, peptides, peptaibols and trichothenes[42,43]. Whether or not these antifungal metabolits are relative to biocontrol under field conditions is a point of speculation[39,42,44,45].

Mycoparasitism: Another mechanism involved in the antagonistic activity of members of the genus *Trichoderma* against phytopathogenic fungi is mycoparasitism. *Trichoderma* spp. are active as mycoparasites and, therefore, can serve as potential biocontrol agents. The mode of hyphal interaction and parasitism of *Trichoderma* spp. with several soilborne pathogenic fungi has been reported[46,37,12,40]. *Trichoderma* grows tropically toward hyphae of other fungi, coil about them in a lectin-mediated reaction, and degrade cell walls of the target fungi by the secretion of different lytic enzymes. This process (mycoparasitism) limits growth and activity of plant pathogenic fungi. *Trichoderma* attaches to the host hyphae via coiling, hooks and appressorium like bodies, and penetrate the host cell wall by secreting lytic enzymes. The interaction is specific and not merely a contact response. *Trichoderma* recognizes signals from the host fungus, triggering coiling and host penetration[33,47]. These enzymes have been reported mainly in isolates of *T. harzianum*[33,23,48].

Trichoderma harzianum is known to produce relatively high concentrations of cell-wall degrading enzymes such as *â*-1,3-glucanases and different chitinolytic enzymes[49,50]. These enzymes have been suggested as the key enzymes in mycoparasitism[49,50]. Several enzymes have been purified and characterised, and their ability to inhibit the germination of spores and elongation of the hyphae belonging to the pathogenic fungi has been shown *in vitro*[51]. For instance, scanning electron microscopy and fluorescence microscopy showed that both *T. harzianum* and *T. hamatum* were mycoparasites of both *Sclerotium rolfsii* and *Rhizoctonia solani*. The antagonist attached to the pathogen and secreted glucanase and chitinase enzymes that ate through the cell wall[12]. *T. harzianum* strain T24 showed a potential for control of the pyhtopathogenic basidiomycete *Sclerotium rolfsii*. Inhibition of *S. rolfsii* correlated with both chitanase and â-1,3-glucanase activities in the culture filtrate of *T. harzianum* strain T24, suggesting the involvement of thsese enzymes in the biocontrol process[52].

Trichoderma produces cellulose, â-1,3-glucanase, and chitinase and degrades the glucans in the walls of *Pythium* spp. and the chitin and glucans in the walls of *Rhizoctonia solani*[53,54]. Papavizas *et al.* [69] reported that seed treatment with *T. harzianum* reduced Pythium seed rot of pea and Rhizoctonia damping-off of cotton. Application of *Trichoderma lignorum* as a wheat-bran preparation, conidial suspension, or seed coating greatly decreased the number of seed infested by *R. solani* as well as damping-off percentages and hence controlled the fungal disease[55]. Varaschin *et al.*[56] reported that *Trichoderma* spp. strains, SC164, SC167 and SC168, selected *in vitro* were good biocontrol agents against the disease caused by *R. solani* in tomato under greenhouse conditions. One of the strains improved plant growth. Lewis and Lumsden[57] showed *T. hamatum* and *T. virens* reduced damping-off of eggplant *(Solanum melongena* L.), zinnia (*Zinnia haageana*) pepper (*Capsicum annum* L.), cucumber (*Cucumis sativus* L.) and cabbage (*Brassica oleracea*) caused by *R. solani*. On the other hand, some controversial experimental results also have been reported with regard to the potential role attributed to chitinases, since no correlation was found between antagonism against *R.solani* and the hydrolic activity of *Trichoderma* spp.[58]. Also, *T. virens* mutants lacking *in vitro* mycoparasitism maintained biocontrol activity[59] .

Induced resistance: Specific strains of fungi in the genus *Trichoderma* colonize and penetrate plant root tissues and initiate a series of morphological and biochemical changes in the plant, considered to be part of the plant defense response, which in the end leads to induced systemic resistance (ISR) in the entire plant[60]. Application of *Trichoderma harzianum* to bean roots resulted in a 25 to 100% reduction in the severity of the foliar disease, gray mold, caused by *Botrytis cinerea*[61]. Biocontrol fungus *T. harzianum* T39 and a chemical BTH (benzothiadiazol) were tested for induction of resistance in tomato to *B. cinerea*. In these experiments, it became clear that resistance-inducing strains stopped the fungus in a very early stage, and the number of spreading lesions declined to about 30 %[62]. The involvement of locally and systemically induced resistance was demonstrated with *T. harzianum* isolate T39. Cells of the biological control agent applied to the roots, and dead cells applied to the leaves of cucumber plants induced control of powdery mildew. The activation of plant defense systems in association of roots treated with *T. harzianum* strain T-203 was suggested by Yedidia *et al.*[26]. The roots of cucumber plants inoculated with T-203 exhibited higher activities of chitinase, â B1,3-glucanase, cellulase and peroxidase when compared to an untreated control 72 hours post inoculation. Scanning electron microscopy revealed typical fungal structures previously associated with mycoparasitic interactions of *Trichoderma* spp. Treatment of cucumber plants with 2,6-dichloroisonicotinic acid, an inducer of the plant defense response, displayed responses that were similar but not identical to those of plants inoculated with *T. harzianum.* Khan *et al.*[63] reported that *T. hamatum* 382 (T382) induced systemic resistance in cucumber against Phytophthora root and crown rot as well as leaf blight. *Pseudomonas aeruginosa* 7NSK2 and *Trichoderma harzianum* T39 induced systemic resistance against *B. cinerea* on bean and tomato and stopped spread of the pathogen at a very early stage[64]. When the infection pressure was very high, however, *B. cinerea* spread could not be controlled effectively by induced resistance[64].

Inactivation of the pathogen's enzymes: Inactivation of the pathogen's enzymes is another biocontrol mechanism by *Trichoderma* spp. Enzymes of *Botrytis cinerea*, such as pectinases, cutinase, glucanase and chitinase, were suppressed through the action of T39 secreted protease on plant surfaces[20]. The *in vitro* inhibitory ability of *Trichoderma harzianum* on the phytopathogen *Alternaria alternata* (Alternaria alternata (Fr.) Keissl.) was investigated in the presence of growth regulators. *A. alternata* is a pathogenic fungus that can secrete endo-polygalacturonase (endo-PG) and pectate lyase (PL). These enzymes are responsible for the hydrolysis of pectic components of the plant cell wall. The presence of *T. harzianum* decreased endo-PG secretion of *A.*

alternata by about 50%. This inhibitory effect was independent of the presence of growth regulators[25].

Strains of *T. harzianum* are marketed in a number of products. Commercial products currently on the open market or under registration include[16]:

- Bio-Fungus (Belgium) labelled for use against *Sclerotinia*, *Phytophthora*, *Rhizoctonia solani*, *Pythium* spp., *Fusarium*, *Verticillium*
- Trichodex (Israel) labelled for use against *Botrytis* of vegetables and grapevines
- Binab-T (Sweden) labelled for use for control of wound decay and wood rot
- Root Pro (Israel) labelled for use against *R. solani*, *Pythium* spp., *Fusarium* spp., and *Sclerotium rolfsii*
- RootShield (also sold as Bio-Trek T-22G) (USA) labelled for use against *Pythium* spp., *R. solani*, *Fusarium* spp.
- SoilGard (formerly GlioGard) (USA) labelled for control of damping-off diseases caused by *Pythium* and *Rhizoctonia* spp.
- Supresivit (Denmark) labelled for use against various fungi
- Trichoject, Trichopel, Trichodowels and Trichoseal (New Zealand) labeled for control of *Armillaria*, *Botryosphaeria*, *Chondrosternum*, *Fusarium*, *Nectria*, *Phytophthora*, *Pythium*, *Rhizoctonia*
- TUSAL (Spain) labeled for control of damping-off diseases caused by *Pythium*, *Phoma* and *Rhizoctonia* species, rhizomania disease of sugar beet and drop of lettuce
- Trichoderma 2000 (Israel) labelled for use against *R. solani*, *S. rolfsii*, *Pythium* spp., *Fusarium* spp.
- Trieco (India) labelled for use against *Rhizoctonia* spp., *Pythium* spp., *Fusarium* spp., root rot, seedling rot, collar rot, red rot, damping-off, Fusarium wilt.

Adverse effect of *Trichoderma* spp. in biological control: Although *Trichoderma harzianum* is an effective biocontrol agent against several fungal soilborne plant pathogens, possible adverse effects of this fungus on arbuscular mycorrhizal (AM) fungi might be a drawback in its use in plant protection. AM fungi are obligate biotrophic endosymbionts in roots of most herbaceous plants. These fungi grow from the roots out into the surrounding soil, forming an external hyphal network, which increases uptake of mineral nutrients[65] and consequently promotes plant growth. The results from pot experiments suggest that *Trichoderma* species suppress AM root colonization[66,67,68]. The presence of *T. harzianum* in soil reduced root colonization by *G. intraradices*. The external hyphal length and density of *G. intraradices* was reduced by the presence of *T. harzianum* in combination with wheat bran. On the other hand, adverse effects of AM fungi on the population density of *Trichoderma koningii* also have been observed[67].

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