

In Vitro Antagonism of *Trichoderma viride* against Five Phytopathogens

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ABSTRACT

Fungal diseases are of concern in forest regeneration as they occasionally pose a serious threat causing considerable mortality in nurseries. Seedlings are generally susceptible to diseases due to the tenderness of their tissues and are often difficult to establish. The use of biocontrol agents is an important alternative to conventional chemicals in the protection of crops against weeds, insects, and diseases in both agriculture and forestry. The success of biocontrol and increase in the yield depends on the nature of antagonistic properties and the mechanisms of action acquired by the organisms used. Both fungi and bacteria are able to synthesise a wide range of metabolites with fungicidal and bactericidal ability. In this study, *Trichoderma viride*, evaluated under laboratory conditions against some common phytopathogens belonging to different groups of fungi, effectively inhibited the growth of the tested pathogens in dual cultures by hyperparasitism and by secretion of volatile and non-volatile metabolites. In the dual culture experiment, maximum inhibition was recorded for *Fusarium oxysporum* followed by *Rhizoctonia solani* and least for *Alternaria zinniae*. The volatile metabolites of *T. viride* were most effective against *F. oxysporum* and least effective against *R. solani*; an almost reverse situation was observed when non-volatile metabolites were examined.

Keywords: biocontrol, non-volatile metabolites, volatile metabolites

Abbreviations: PDA, potato dextrose agar; PDB, potato dextrose broth

INTRODUCTION

Plants are a major source of food, fibre, fodder, medicines and many other useful products for mankind. Various insects, bacteria, viruses, fungi and other pests attack plants at various stages of their development. This reduces their productivity and leads to huge losses. The most common and popular method of disease control is the use of synthetic pesticides. However, due to the polluting and non-biodegradable nature of such pesticides and the development of resistance by pathogens, biological control offers an important alternative to synthetic chemicals for disease management. The goal of biocontrol research is to provide additional tools for disease management. Biocontrol can be used in situations where no chemical control is available, where conventional pesticides cannot be used due to re-entry or residue concerns, or where the product must be certified as organic. Biocontrol involves harnessing disease-suppressive microorganisms to improve plant health. Disease suppression by biocontrol agents is the sustained manifestation of interactions among the plant, pathogen, biocontrol agent, microbial community on and around the plant, and the physical environment (Handelsman and Stabb 1996). In recent years, there has been a worldwide swing towards the use of eco-friendly methods for protecting crops from pests and diseases (Rao *et al.* 1998). Biological control of plant disease by microorganisms, especially of soil-borne plant pathogens and nematodes, has been considered as a more natural and environmentally acceptable alternative to existing chemical treatment methods (Barker and Panlitz 1996; Eziashi *et al.* 2007).

There are a variety of fungal species and isolates that have been reported as biocontrol agents, although *Trichoderma* species clearly dominate, perhaps due to their ease of

growth and wide host range (Whipps and Lumsden 2001). Several strains of *Trichoderma* have been reported to be effective biocontrol agents for various soil-borne plant pathogenic fungi under greenhouse and field conditions (Akrami *et al.* 2009; Morsy *et al.* 2009; Ajith and Lakshmidevi 2010; Dev and Dawande 2010). The genus *Trichoderma* is a common filamentous imperfect fungi (Deuteromycetes, Dematiaceae), the most common saprophyte in the rhizosphere and is found in almost all soils. It is a very effective biological means of plant disease management, especially for soil-borne diseases. It is highly interactive in root, soil and foliar environments. Several *Trichoderma* species were reported to restrict plant pathogenic fungi under laboratory and natural conditions. They secrete a variety of volatile and non-volatile compounds with antibiotic properties (Papavizas 1985; Tapwal *et al.* 2004, 2005; Dubey *et al.* 2007; Ajith and Lakshmidevi 2010). *Trichoderma* and *Gliocladium* are closely related fungal biocontrol agents. Antimicrobial compounds suppress disease by diverse mechanisms, including the production of structurally complex antibiotics gliovirin and gliotoxin (Howell *et al.* 1993). Production of antibiotics by fungi exhibiting biocontrol activity is common for *Trichoderma* and *Gliocladium* isolates (Howell 1998).

Although the number of biocontrol products is increasing, they still represent only about 1% of agricultural chemical sales (Fravel 2005). In comparison, fungicides represent approximately 15% of pesticide sales (Fravel 2005). Before assessing the practical implementation of a biocontrol agent through its antagonistic activity, it is essential to determine the effects of changing environmental conditions on biological control. It is important to learn the ecology of these organisms and their interactions with the pathogen, host plant, soil and rhizosphere microbial communities, and

their surrounding environments as much as possible (Handelsman and Stabb 1996). With this in view the present investigation was carried out to examine the efficacy of volatile and non-volatile metabolites produced by *Trichoderma viride* against five fungal plant pathogens of economic importance under *in vitro* conditions.

MATERIALS AND METHODS

Isolates

Cultures of selected phytopathogens and *T. viride* were obtained from the Department of Botany, Shoolini Institute of Life Sciences and Management, Solan, Himachal Pradesh. Potato dextrose agar (PDA; Merck, Mumbai, India) was the choice of medium for sub-culturing and experiments.

Dual culture technique

T. viride was evaluated against five selected phytopathogens by the dual culture technique (Morton and Strouble 1955). Mycelial discs 5 mm in diameter were excised from the edge of an actively growing antagonist and the pathogen was cultured on opposite ends of a Petri dish equidistant from the periphery. A completely randomized experimental design was used with three replicates for each isolate. In control Petri dish, in place of antagonist, a sterile agar disc was inoculated on the side opposite pathogen. Inoculated plates were incubated at $25 \pm 1^\circ\text{C}$ for 5 days. After the incubation period, radial growth of pathogens was measured and the percent inhibition of average radial growth was calculated relative to the control as follows:

$$L = [(C - T)/C] \times 100$$

where L is the percentage inhibition of radial mycelial growth, C is radial growth of the pathogen in the control; T is radial growth of the pathogen in the presence of *T. viride* (Edington *et al.* 1971).

Evaluation of volatile metabolites

The effect of the volatile metabolites released by *T. viride* on the mycelial growth of the pathogens was evaluated by the 'inverted plate technique' as described by Dennis and Webster (1971). The 5-mm mycelial discs of *T. viride* obtained from the margin of young cultures were placed centrally on the PDA glass dish (Riviera, 100×20 mm) and incubated in $25 \pm 1^\circ\text{C}$ for 0, 24, 48 and 72 h. In the control plates, sterile PDA media discs 5 mm in diameter were placed on the plates as mentioned above. At the end of the incubation period, the top of each Petri dish was replaced with the bottom of the Petri dish inoculated with pathogen and sealed together with adhesive tape. Sealing Petri dishes avoided the escape of volatile compounds of the antagonist and ensured their inhibitory effect on the pathogen. A completely randomized experimental design was used with three replicates. Radial growth of the pathogens was recorded on the 5th day of incubation and L was calculated, as described above.

Evaluation of non-volatile metabolites

The food poisoning technique was used to evaluate the effect of non-volatile metabolites produced by *T. viride* on the growth of pathogens. 5 mm discs of young mycelial agar plugs were taken from the edge of young cultures and were inoculated in 100-ml sterilized potato dextrose broth (PDB) in 250-ml conical flasks and incubated at $25 \pm 1^\circ\text{C}$. After incubating for 7 days, the culture filtrates were collected and centrifuged at 3000 rpm for 20 min; the supernatants were filtered through 45- μm Millipore filters using a vacuum pump assembly under aseptic conditions. Just before pouring, a predefined amount of culture filtrate was mixed with molten PDA to obtain a final concentration of 5, 10, 15 and 20%. 5-mm discs of the pathogens were inoculated to the Petri dishes amended with culture filtrate and incubated at $25 \pm 1^\circ\text{C}$. The colony diameter of the pathogens was measured after 5 days and compared with the growth of the pathogen in the control. In control, the PDA was amended with the same amount of sterilized

Table 1 Growth inhibition of five phytopathogens by *Trichoderma viride* in dual culture.

Pathogens	Percent growth inhibition	SEM
<i>Alternaria solani</i>	34.76	2.23
<i>Alternaria zinniae</i>	32.44	2.01
<i>Curvularia lunata</i>	44.32	2.67
<i>Fusarium oxysporum</i>	67.96	2.06
<i>Rhizoctonia solani</i>	48.67	1.94

distilled water as mentioned above. The experiment was carried out in triplicate.

Experimental design and statistical analysis

Statistical analysis was performed following a completely randomized design (CRBD) with three replicates in each treatment. The data was subjected to analysis of variance (ANOVA) using statistical software MINITAB 11.2 (<http://www.minitab.com>) and significance of various treatments were evaluated by *F*-tests ($P < 0.05$).

RESULTS AND DISCUSSION

Growth inhibition of phytopathogens by *T. viride* in dual culture

In the dual culture experiment, *T. viride* had a marked significant inhibitory effect on the growth of selected phytopathogens compared to their respective controls (Table 1). Growth inhibition decreased as follows: *F. oxysporum* (67.96%) > *R. solani* (48.67%) > *Curvularia lunata* (44.32%) > *A. solani* (34.76%) > *A. zinniae* (32.44%). Since *Trichoderma* is a fast-growing fungus, it reached the pathogen within 3-4 days and overgrew them in 8-10 days. The presence of an inhibition zone in dual culture without hyphal contact in treatments advocates the secretion of some diffusible non-volatile antibiotics by *T. viride*. Similar findings on the interaction of *Trichoderma* species (*Trichoderma hamatum* T614, *T. hamatum* T612, *Trichoderma harzianum* T447, *T. harzianum* T969, *Trichoderma virens* T523 and *Trichoderma* sp. T) were recorded by Hajieghrari *et al.* (2008) for *F. graminearum*, *R. solani* and *Macrophomina phaseoli*; *T. viride* for *Fusarium oxysporum* f.sp. *radicis-lycopersici* (Kerkeni *et al.* 2007) and *T. harzianum* for *Phytophthora capsici* (Shashidhara *et al.* 2008). The antagonist restricted the growth of all pathogens and in most cases, *T. viride* grew over the zone of inhibition, overgrowing the pathogen and sporulating there after 10 days. The inhibition in radial growth of two interacting organisms in dual culture has been attributed to secretion of extracellular hydrolytic enzymes (Schirmböck *et al.* 1994), by the production of antibiotics (Howell 1998) or as well as some cell walls degrading enzymes such as chitinases, glucanases that break down polysaccharides, chitins and β -glucanase, thereby destroying cell wall integrity (Elad 2000). These may play a key role in mycoparasitism because of changes in cell wall integrity prior to actual physical contact.

Effect of volatile metabolites from *T. viride* on the radial growth of phytopathogens

To study the effect of volatile compounds released by *T. viride* on the growth of pathogens, four experiments were conducted. In the first, the antagonists and the pathogen of the same age (0 day old antagonist) were used. The antagonist culture was one day (24 h) older than that of the pathogen in the second experiment. In the third experiment, the antagonist was two days (48 h) older than the pathogen and in the fourth experiment the antagonist was three days (72 h) older than the pathogen. The growth of the pathogen was measured on the 5th day of incubation and compared with the control. The gaseous metabolites released by *T. viride* diffused and inhibited the growth of the pathogen inocu-

Table 2 *In vitro* growth inhibition of five phytopathogens by volatile compounds of *T. viride*.

Age of <i>T. viride</i>	Percent growth inhibition				
	<i>Alternaria solani</i>	<i>Alternaria zinniae</i>	<i>Curvularia lunata</i>	<i>Fusarium oxysporum</i>	<i>Rhizoctonia solani</i>
0 days old	7.69	18.00	3.50	27.61	1.78
1 day old	11.28	22.00	12.50	37.73	4.44
2 days old	15.38	26.80	17.50	44.79	8.89
3 days old	24.62	38.00	21.50	53.99	19.56

F = 8.85 (rows); F = 3.84 (columns); SEM = 1.02; CD = 3.07; P = 0.05

Table 3 *In vitro* growth inhibition of five phytopathogens by non-volatile compounds of *T. viride*.

Conc. of culture filtrate (%)	Percent growth inhibition				
	<i>Alternaria solani</i>	<i>Alternaria zinniae</i>	<i>Curvularia lunata</i>	<i>Fusarium oxysporum</i>	<i>Rhizoctonia solani</i>
5	7.66	18.42	7.32	9.09	25.56
10	19.15	19.74	21.95	10.61	37.78
15	31.91	23.68	26.83	17.27	48.33
20	41.70	27.63	36.59	21.21	55.00

F = 55.38 (rows); F = 316.37 (columns); SEM = 0.46; CD = 1.39; P = 0.05

lated in the inverted face of the Petri dish. The growth of all pathogens was significantly inhibited and it increased with the age of the antagonist (Table 2). On the 5th day of incubation, a 3-days-old culture of *T. viride* inhibited the growth of antagonists as follows: *F. oxysporum* (53.99%) > *A. zinniae* (38.00%) > *A. solani* (24.62%) > *C. lunata* (21.50%) > *R. solani* (19.56%). The volatile compounds of *T. viride* have a marked inhibitory effect on the growth of pathogens in comparison to their respective controls. Growth was significantly inhibited in *A. solani*, *A. zinniae* and *C. lunata* but not significantly so for *R. solani* and *F. oxysporum*. A comparison of growth inhibition among pairs of pathogens revealed that for *A. solani* and *F. oxysporum*, *A. solani* and *R. solani*, *C. lunata* and *F. oxysporum*, and *C. lunata* and *R. solani*, there were significant differences ($F = 8.85$, SEM = 1.02, CD = 3.07, $P = 0.05$). The volatile compounds of *T. viride* of all ages significantly inhibited the growth of all tested pathogens. However, within-growth inhibition was only significant between the age of 0-3 and 1-3 days old cultures of *T. viride*. But it was non-significant for lower age differences of 0-1, 1-2 and 2-3 days ($F = 3.84$, SEM = 1.02, CD = 3.07, $P = 0.05$).

Padmodaya and Reddy (1996) reported the antagonistic efficacy of volatile compounds of *Trichoderma* spp. against *Fusarium oxysporum*. *Trichoderma* species are known to produce a number of antibiotics such as Trichodermin, Trichodermol, Harzianum A and Harzianolide (Dennis and Webster 1971; Kucuk and Kivanc 2004). The major advantage of antibiosis via volatile compounds is that the antibiotic substances produced by the antagonists may diffuse through air-filled pores in soil and help to check the root rot pathogen without establishing actual physical contact with them.

Effect of non-volatile metabolites (culture filtrate) from *T. viride* on the radial growth of phytopathogens

The food poisoning technique was used to study the efficacy of non-volatile metabolites on the growth of pathogens. The *T. viride* secreted antibiotic metabolites into the liquid media (PDB) and its filtrate inhibited the growth of pathogen inoculated in the amended media. The culture filtrate of the antagonist restricted the growth of all pathogens with better efficacy at higher concentrations (Table 3). On the 5th day of incubation at 20% (v/v) of culture filtrate, growth inhibition was greatest against *R. solani* (55.00%), followed by *A. solani* (41.70%), *C. lunata* (36.59%), *A. zinniae* (27.63%) and least against *F. oxysporum* (21.21%). Compared to the control, the non-volatile metabolites secreted by *T. viride* in culture medium significantly restricted the growth of all pathogens at all concentrations of culture filtrate. Within treatment comparisons also recorded significant differences [$F = 55.38$ (rows), $F = 316.37$ (columns), SEM = 0.46, CD = 1.39, $P = 0.05$].

T. viride may have secreted some antibiotic metabolites like trichodermin, dermadin, trichoviridin and sesquiterpene, heptalic acid (Nakkeeran *et al.* 2002), nutrient impoverishment and pH alteration in the medium (Maheshwari *et al.* 2001), which inhibited the growth of pathogens. Several groups have reported the production of antibiotics from the culture filtrate of *T. viride* (Ooka *et al.* 1966; Eziashi *et al.* 2006; Hajieghrari *et al.* 2008; Siddiquee *et al.* 2009; Rajendiran *et al.* 2010). Hence, *T. viride* has a potential to develop as a biological agent to control the common phytopathogens.

Biological control of plant diseases is an eco-friendly approach that utilizes antagonistic microorganisms as a potential, non-chemical means of disease management. One of the goals for the use of biocontrol in agriculture is to avoid the pitfalls associated with overuse of synthetic pesticides, including the development of resistance amongst pest populations. An attractive feature of biocontrol strategies is that the populations of pathogens developing resistance to antagonistic products produced by biocontrol agents is likely to be very slow (Jenkins and Grzywacz 2000). This may happen because most biocontrol agents produce more than one antimicrobial component and resistance to multiple antimicrobial factors should occur only at a very low frequency. Weller (1988) proposed the manipulation of the rhizosphere to benefit micro-organisms, which produce antagonistic compounds that will protect roots from the deleterious effect of soil-borne pathogens. Support for this concept came from the discovery of suppressive soils in which the active microflora naturally controls the disease-causing activities of pathogen populations (Weller *et al.* 2002).

In this study, *T. viride* restricted the growth of selected pathogens in all treatments, but there was no definite trend. In our case, maximum growth inhibition by *T. viride* volatile compounds was observed for *F. oxysporum* followed by *A. zinniae* and least for *R. solani*. In the case of non-volatile compounds, the trend was almost reversed i.e. minimum inhibition was observed for *F. oxysporum* followed by *A. zinniae* and maximum for *R. solani*. It may not be necessary that the pathogen, which was more susceptible to volatile metabolites of the antagonist, may also be sensitive to non-volatile compounds of the same antagonist. In conclusion, *T. viride* was quite effective *in vitro* in restricting selected pathogens. Further research on field applications and isolation and identification of these antibiotic compounds is required.

REFERENCES

- Ajith PS, Lakshmidivi N (2010) Effect of volatile and non-volatile compounds from *Trichoderma* spp. against *Colletotrichum capsici* incitant of anthracnose on bell peppers. *Nature and Science* 8, 265-269
- Akrami M, Sh. Ibrahimov A, Zafari DM, Valizadeh E (2009) Control *Fusarium* rot of bean by combination of by *Trichoderma harzianum* and *Trichoderma asperellum* in greenhouse condition. *Agricultural Journal* 4, 121-123
- Barker R, Paulitz TC (1996) *Theoretical Basis for Microbial Interactions*

- Leading to Biological Control of Soil Borne Plant Pathogens: Principals and Practice of Managing Soil Borne Plant Pathogens, The American Phytopathological Society, St. Paul, Minnesota, USA, pp 50-79
- Dennis C, Webster J** (1971) Antagonistic properties of species groups of *Trichoderma*-II. Production of volatile antibiotics. *Transactions of the British Mycological Society* **57**, 47-48
- Dev N, Dawande AY** (2010) Biocontrol of soil borne plant pathogen *Rhizoctonia solani* using *Trichoderma* spp. and *Pseudomonas fluorescens*. *Asiatic Journal of Biotechnology Resources* **1**, 39-44
- Dubey SC, Suresh M, Singh B** (2007) Evaluation of *Trichoderma* species against *Fusarium oxysporum* f.sp. *ciceris* for integrated management of chickpea wilt. *Biological Control* **40**, 118-127
- Edington LV, Khew KL, Barron GI** (1971) Fungitoxic spectrum of benzimidazole compounds. *Phytopathology* **61**, 42-44
- Elad Y** (2000) Biological control of foliar pathogens by means of *Trichoderma harzianum* and potential modes of action. *Crop Protection* **19**, 709-714
- Eziashi EI, Omamor IB, Odigie EE** (2007) Antagonism of *Trichoderma viride* and effects of extracted water soluble compounds from *Trichoderma* species and benlate solution on *Ceratocystis paradoxa*. *African Journal of Biotechnology* **6**, 388-392
- Eziashi EI, Uma NU, Adekunle AA, Airede CE** (2006) Effect of metabolites produced by *Trichoderma* species against *Ceratocystis paradoxa* in culture medium. *African Journal of Biotechnology* **5**, 703-706
- Fravel DR** (2005) Commercialization and implementation of biocontrol. *Annual Review of Phytopathology* **43**, 337-359
- Hajjehghari B, Torabi-Giglou M, Mohammadi MR, Davari M** (2008) Biological potential of some Iranian *Trichoderma* isolates in the control of soil borne plant pathogenic fungi. *African Journal of Biotechnology* **7**, 967-972
- Handelsman J, Stabb EV** (1996) Biocontrol of soilborne plant pathogens. *Plant Cell* **8**, 1855-1869
- Howell CR** (1998) The role of antibiosis in biocontrol. In: *Trichoderma and Gliocladium: Enzymes, Biological Control and Commercial Applications* (Vol 2), Taylor and Francis, London, pp 173-184
- Howell CR, Stipanovic RD, Lumsden RD** (1993) Antibiotic production by strains of *Gliocladium virens* and its relation to the biocontrol of cotton seedling diseases. *Biocontrol Science and Technology* **3**, 435-441
- Jenkins NE, Grzywacz D** (2000) Quality control of fungal and viral biocontrol agents – assurance of product performance. *Biocontrol Science and Technology* **10**, 753-777
- Kerkeni A, Daami-Remadi M, Tarchoun N, Khedher BN** (2007) *In vitro* and *in vivo* suppression of *Fusarium oxysporum* f.sp. *radicis-lycopersici* the causal agent of *Fusarium* crown and root rot of tomato by some compost fungi. *International Journal of Agricultural Research* **2**, 1022-1029
- Kucuk C, Kivanc M** (2004) *In vitro* antifungal activity of strains of *Trichoderma harzianum*. *Turkish Journal of Biology* **28**, 111-115
- Maheshwari DK, Dubey RC, Sharma VK** (2001) Biocontrol effects of *Trichoderma virens* on *Macrophomina phaseolina* causing charcoal rot of peanut. *Indian Journal of Microbiology* **41**, 251-256
- Morsy EM, Abdel-Kawi KA, Khalil MNA** (2009) Efficiency of *Trichoderma viride* and *Bacillus subtilis* as biocontrol agents against *Fusarium solani* on tomato plants. *Egyptian Journal of Phytopathology* **37**, 47-57
- Morton DT, Stroube NH** (1955) Antagonistic and stimulatory effects of microorganism upon *Sclerotium rolfsii*. *Phytopathology* **45**, 419-420
- Nakkeeran S, Krishnamoorthy AS, Ramamoorthy V, Renukadevi** (2002) Microbial inoculants in plant disease control. *Journal of Ecobiology* **14**, 83-94
- Ooka T, Shimojima Y, Akimoto T, Takeda I** (1966) A new antibacterial peptide 'Suzukacillin'. *Agricultural and Biological Chemistry* **30**, 700-702
- Padmodaya B, Reddy HR** (1996) Screening of *Trichoderma* spp. against *Fusarium oxysporum* f.sp. *lycopersici* causing wilt in tomato. *Indian Journal of Mycology and Plant Pathology* **26**, 266-270
- Papavizas GC** (1985) *Trichoderma* and *Gliocladium* biology, ecology and the potential for biocontrol. *Annual Review of Phytopathology* **23**, 23-77
- Rajendiran R, Jegadeeshkumar D, Sureshkumar BT, Nisha T** (2010) *In vitro* assessment of antagonistic activity of *Trichoderma viride* against post harvest pathogens. *Journal of Agricultural Technology* **6**, 31-35
- Rao MS, Reddy PP, Nagesh M** (1998) Evaluation of plant based formulations on *Trichoderma harzianum* for the management of *Meloidogyne incognita* on egg plant. *Nematologia Mediterranea* **26**, 59-62
- Schirmbock M, Lorito M, Wang YL, Hayes CK, Arisan-Atac I, Scala F, Harman GE, Kubicek CP** (1994) Parallel formation and synergism of hydrolytic enzymes and peptaibol antibiotics, molecular mechanisms involved in the antagonistic action of *Trichoderma harzianum* against phytopathogenic fungi. *Applied and Environmental Microbiology* **60**, 4364-4370
- Shashidhara S, Lokesh MS, Lingaraju S, Palakshappa MG** (2008) *In vitro* evaluation of microbial antagonists, botanicals and fungicides against *Phytophthora capsici* Leon. the causal agent of foot rot of black pepper. *Karnataka Journal of Agricultural Sciences* **21**, 527-531
- Siddiquee S, Yusuf UK, Hossain K, Jahan S** (2009) *In vitro* studies on the potential *Trichoderma harzianum* for antagonistic properties against *Ganoderma boninense*. *Journal of Food, Agriculture and Environment* **7**, 970-976
- Tapwal A, Sharma YP, Lakhanpal TN** (2004) Effect of volatile compounds released by *Gliocladium virens* and *Trichoderma* spp. on the growth of *Dematophora necatrix*. *Indian Journal of Mycology and Plant Pathology* **34**, 37-40
- Tapwal A, Sharma YP, Lakhanpal TN, Anju** (2005) *In vitro* interaction of *Phytophthora cactorum* with *Gliocladium* and *Trichoderma* species. *Journal of Basic and Applied Mycology* **4**, 119-122
- Weller DM** (1988) Biological control of soil borne plant pathogens in the rhizosphere with bacteria. *Annual Review of Phytopathology* **26**, 379-407
- Weller DM, Raaijmakers J, McSpadden Gardener B, Thomashow LM** (2002) Microbial populations responsible for specific soil suppressiveness to plant pathogens. *Annual Review of Phytopathology* **40**, 309-348
- Whipps JM, Lumsden RD** (2001) *Commercial Use of Fungi as Plant Disease Biological Control Agents – Status and Prospects: Fungi as Biocontrol Agents – Progress, Problems and Potential*, CABI Publishing, Wallingford, pp 9-22