African Journal of Microbiology Research Vol. 6(13), pp. 3348-3353, 9 April, 2012
Available online at http://www.academicjournals.org/AJMR
DOI: 10.5897/AJMR12.247
ISSN 1996-0808 ©2012 Academic Journals

Full Length Research Paper

Antagonistic activity of *Trichoderma harzianum* and *Trichoderma viride* isolated from soil of date palm field against *Fusarium oxysporum*

Kahkashan Perveen* and Najat A. Bokhari

Department of Botany and Microbiology, King Saud University, P. O. Box: 22452, Riyadh-11495, Kingdom of Saudi Arabia.

Accepted 29 February, 2012

Fusarium oxysporum **has a worldwide distribution and is responsible for severe vascular wilt or root rot. Currently, no effective curative treatments to control the fusarium wilt exist. Thus the study was undertaken to find out the antagonistic potential of** ***Trichoderma harzianum*** **and** ***Trichoderma viride*** **isolated from the soil of date palm field against** ***F. oxysporum in vitro.*** **All species of** ***Trichoderma*** **showed the appreciable inhibition of mycelial growth of the pathogen. Results of dual culture interaction test showed that TvDPs (66.3%) was best antagonist in inhibiting the growth of pathogen followed by TDPs (57.4%) and T1s (56.43%) respectively. Volatile metabolites produced by** ***T. viride*** **(TvDPs) exhibited highest growth inhibition (40.91%) followed by T1s (25.97%) and TDPs (7.57%) respectively. The culture filtrate of all antagonists incubated at 25°C showed maximum reduction in radial growth of pathogen. The cell free culture filtrate of** ***T. viride*** **incubated at various temperatures inhibited the radial growth of** ***F. oxysporum*** **at varying degree. Whereas, culture filtrates of** ***T. harzianum*** **isolates incubated at 40°C were unable to control the growth of the pathogen. TvPDs showed best sporulation and mycelial growth on SDA medium, whereas T1s and TDPs flourished well on both PDA and SDA media. On natural media agar (PDA + 1% date palm leaves) the antagonist TvPDs sporulated luxuriously. Present study clearly indicates an excellent antagonistic activity of** ***T. viride*** **(TvDPS) against** ***F. oxysporum in vitro*** **condition.**

Key words: *Fusarium oxysporum*, *Trichoderma harzianum, Trichoderma viride,* antagonist fungi.

INTRODUCTION

Fusarium oxysporum, can be described as a species complex comprising of a collection of several clonal lineages (Michielse and Rep, 2009). It has a worldwide distribution and is responsible for severe vascular wilt or root rot in a wide range of plant families (Enya et al., 2008; Lievens et al., 2008; Michielse and Rep, 2009). It is ubiquitous in both agricultural and non agricultural soils, and is generally found in close association with plant plant species, among which are several economically important crops including banana, bulb flowers, roots (Wunsch et al., 2009). Pathogenic *F. oxysporum* strains can cause vascular wilt or root rot in over 100 cucumber, cut flowers, date palm, melon and tomato (Gordon and Martyn, 1997). Currently, no effective treatments to control *F. oxysporum* exist. Therefore, most efforts are directed towards prevention of the disease. In general, effective means of disease control prior to infection include soil fumigation, disinfestation of plant material or if available, the use of resistant plant cultivars. In addition, bio-control strategies are being developed using either non-pathogenic *F. oxysporum* strains or other

*Corresponding author. E-mail: kperveen@ksu.edu.sa. Tel: 0096614785968 ext. 1222 or 00966503339215.

antagonistic microorganisms (Fravel et al., 2003). Biological control of soil borne plant pathogens is a potential alternative to the use of environment harming chemical pesticides. There is a growing demand of sound, biologically-based pest management practices. Recent surveys of both conventional and organic growers indicated an interest in using bio-control products (Rzewnicki, 2000; Van Arsdall and Frantz, 2001). Research has repeatedly demonstrated that phylogenetically diverse microorganisms can act as natural antagonists of various plant pathogens (Cook, 2000). Interactions that lead to bio-control include antibiosis, competition, induction of host resistance, production of growth stimulating factors and predation (Cook and Baker, 1983).

Bio-control fungi of the genus *Trichoderma* have developed an astonishing ability to interact, both parasitically and symbiotically, with different substrates and living organisms, including plants and other microbes (Harman and Kubicek, 1998; Kubicek and Harman, 1998). These fungi are among the most resistant microbes to natural and man-made chemicals and toxins (Harman et al., 2004). Many strains are strong opportunistic invaders, fast growing, prolific producers of spores and powerful antibiotic. Consequently, these fungi are ecologically very successful since strains have been found in all climatic zones (Monte, 2001). *Trichoderma* spp. are widely used in agriculture, bio-control isolates are used as bio-pesticides, bio-protectants, bio-stimulants and biofertilizers on a wide variety of plants (Harman and Kubicek, 1998). Many of these concepts have been extensively covered in recent reviews (Harman et al., 2004; Woo et al., 2006; Lorito et al., 2010; Druzhinina et al., 2011). The assessment of antifungal activity of antagonist against pathogenic fungi *in vitro* is the initial step towards the selection of most efficient antagonistic strains. Therefore the study was carried out to find out the antagonistic potential of *T. harzianum* and *T. viride* isolated from the soil of date palm field against *F. oxysporum in vitro.*

MATERIALS AND METHODS

Fungal isolates

T. harzianum [(T1s) ITCC, I.D. No. 8192.11] *T. harzianum* (TDPs) and *T. viride* (TvPDs) isolated from the soil of date palm field, Riyadh, Saudi Arabia were used in the present study. Antagonism of these fungi was assessed against the pathogen, *F. oxysporum* (ITCC I.D. No. 7532.09). Stock cultures of test fungi were maintained on PDA slants and stored at 4°C in refrigerator.

Dual culture interaction

Mycelial block of 5 mm diameter were cut from the margin of antagonist (T1s, TDPs, TvPDs) and pathogen (*F. oxysporum*) seven days old colonies. A block of pathogen and one of the antagonists were placed 3 cm apart on potato dextrose agar (PDA) surface. Controls of both the fungi were inoculated separately on a side of the petri plates containing PDA medium.

Effect of volatile metabolites

The effect of volatile metabolites from (T1s), (TDPs), (TvPDs) against *F. oxysporum* was tested in the assemblage described by Dennis and Webster (1971). Two bottoms of Petri dishes containing PDA were individually inoculated with a disc of pathogen and antagonist, and bottoms were adjusted and attached by tape. The control sets did not contain the antagonist.

Inhibitory effect of the culture filtrate of *Trichoderma* spp. incubated at different temperature

Trichoderma species were inoculated into 100 ml sterilized potato dextrose broth and incubated at 5, 15, 25, 35, 40°C without shaking. After 10 days of incubation the culture broth was filtered through Whatman filter paper no.1 and re-filtered through Millipore membrane filter (0.45 µ) to obtain cell-free culture filtrates. Four ml of culture filtrates of each *Trichoderma* species were placed in sterilized petri dish which was immediately followed by pouring 16 ml of PDA, so as to make the final concentration of culture filtrates 20%. After the agar solidified, mycelial discs of the *F. oxysporum* (5 mm in diameter) obtained from actively growing colonies were placed in the centre of the agar plates. The Petri dishes were incubated at 25°C for 4 days and after that the percent inhibition in the radial colony growth was calculated.

The assays of dual culture interaction, volatile metabolites and inhibitory effect of culture filtrate were conducted in triplicates in randomized block design and repeated twice. The per cent inhibition of mycelial growth of the pathogens was calculated using following formula (Singh et al., 2002):

$$I = (C - T/C) \times 100$$

where, I = Inhibition (%), C = Colony diameter in control plate and T = Colony diameter in treated plate.

Mycelial growth rate of *Trichoderma* spp.

Mycelial growth rate of T1s, TDPs, and TvPDs was observed on various culture media; Potato dextrose agar, PDA (Scharlau Chemie, Spain), Czapek dox agar CDA (Oxoid, England), Water agar, WA (2% agar), Sabouraud dextrose agar, SDA (Oxoid, England) and natural media agar (PDA + 1% date palm leaves). Five millimeter mycelial disc of *Trichoderma* spp. were obtained, as mentioned above, and placed on the solidified media. Colony diameter was recorded daily. Average linear growth rate (ALG) was calculated by following formula (Elad et al., 1981):

$$ALG\ (mm/day) = (C3 - C1)/T$$

where C3 = Colony diameter in mm after three days, C1= Colony diameter in mm after one day, T= difference in time (day).

RESULTS

The results of dual culture and volatile metabolites of *Trichoderma* spp. indicated that all species of *Trichoderma* inhibited the growth of *F. oxysporum* at

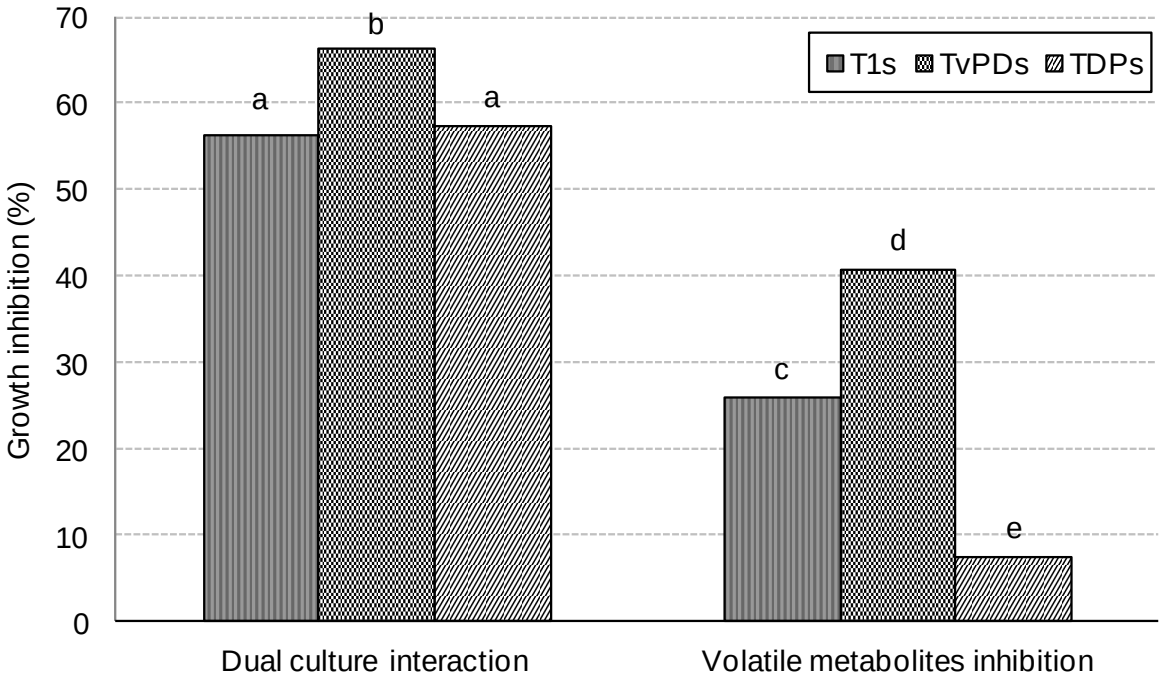

Figure 1. Antagonistic activity of *Trichoderma* species against *F. oxysporum* evaluated by dual culture interaction and volatile metabolites inhibition. Each value is an average of three replicates. Columns' showing different letters are significantly different (P ≤ 0.05) according to Duncan's multiple range test. T1s = *Trichoderma harzianum* isolate T1s, TvPDs = *T. viride* isolate TvPDs, TDPs = *T. harzianum* isolate TDPs.

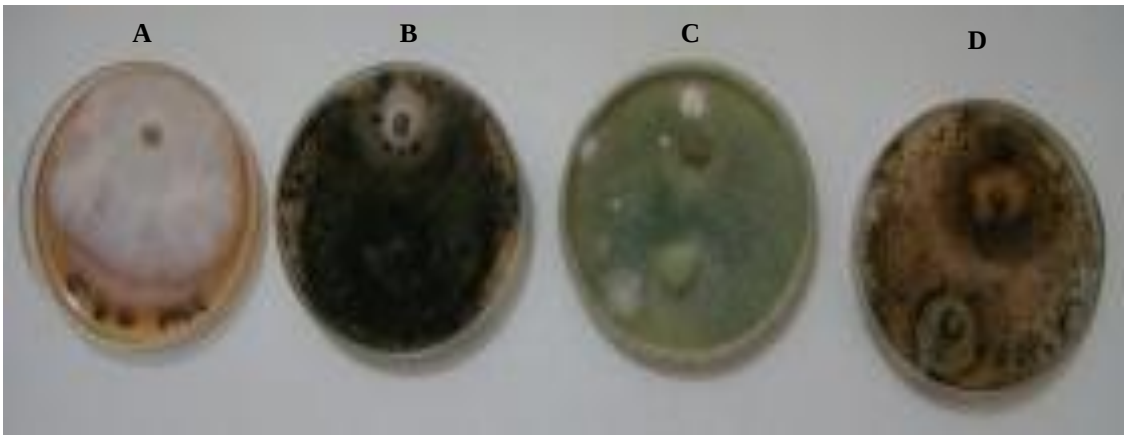

Figure 2. Antagonistic activity of *Trichoderma* species against *F. oxysporum* evaluated by dual culture interaction. (A) *F. oxysporum* alone, (B) *F. oxysporum* + *T. harzianum* (T1s), (C) *F. oxysporum* +*T. viride (*TvPDs), (D) *F. oxysporum* +*T. harzianum (*TDPs).

varying degrees (Figure 1). The results of dual culture show that all antagonists significantly (P ≤ 0.05) inhibited the mycelial growth of the pathogen. It was found that *T. viride* inhibited the growth of pathogen more than the other two isolates of *T. harzianum.* A clear zone of inhibition was observed between T1s and *F. oxysporum,* whereas TvDPs and TDPs overgrew *F. oxysporum* after ten days of incubation (Figure 2). Maximum growth inhibition was recorded by TvDPs (66.3%) followed by TDPs (57.4%) and T1s (56.43%) respectively (Figure 1).

Effect of volatile metabolites on the growth of pathogen presented in Figure 1 show that all *Trichoderma* spp. significantly (P ≤ 0.05) inhibited the mycelial growth of the pathogen. Highest inhibition (40.91%) of the pathogen was reported by *T. viride* (TvDPs). Whereas isolates of *T. harzianum* showed considerable variation in the inhibitory

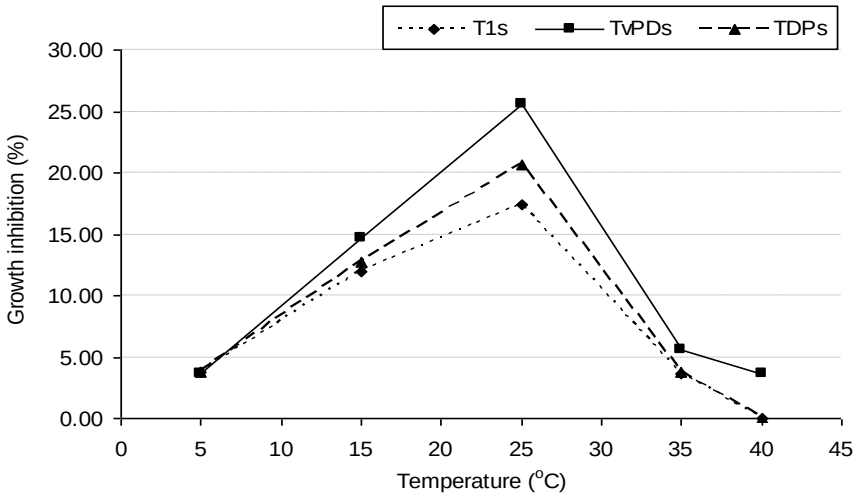

Figure 3. Inhibitory effect of the culture filtrate of *Trichoderma* spp. incubated at different temperature (5, 15, 25, 35, 40°C). Each value is an average of three replicates. T1s = *Trichoderma harzianum* isolate T1s, TvPDs = *T. viride* isolate TvPDs, TDPs = *T. harzianum* isolate TDPs.

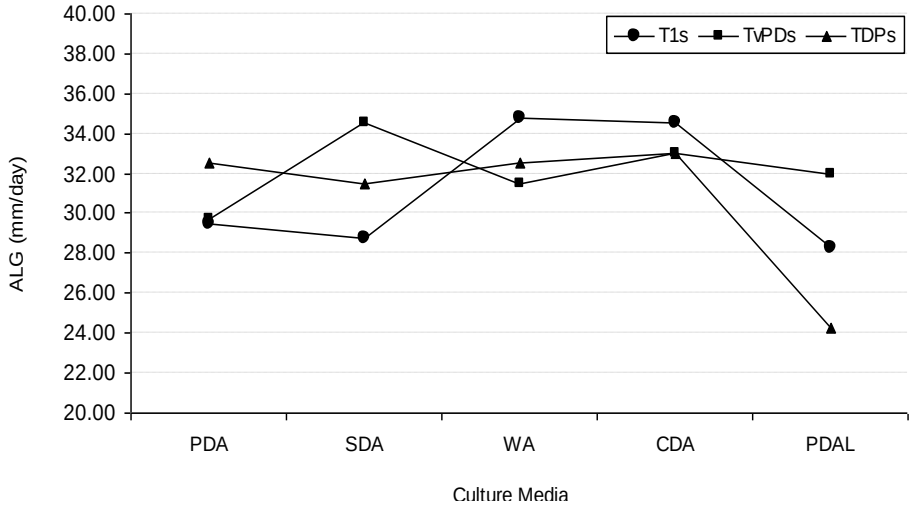

Figure 4. Average linear growth rate (ALG) of *Trichoderma* species on various culture media. Each value is an average of three replicates. T1s = *Trichoderma harzianum* isolate T1s, TvPDs = *T. viride* isolate TvPDs, TDPs = *T. harzianum* isolate TDPs, PDA = Potato dextrose agar, SDA= Sabouraud dextrose agar, WA= Water agar (2% agar), CDA= Czapek dox agar, PDAL= natural media agar (PDA + 1% date palm leaves).

effect, T1s showed 25.97% inhibition of the pathogen while TDPs has only 7.57%.

Figure 3 represent the effect of culture filtrates of *Trichoderma* species incubated at different temperature. It was observed that culture filtrate of *Trichoderma* spp./isolates caused appreciable inhibition of *F. oxysporum* however none was fungicidal to the pathogen. The culture filtrate of all antagonists incubated at 25°C showed maximum reduction in radial growth of pathogen. The cell free culture filtrate of *T. viride* incubated at various temperatures inhibited the radial growth of *F. oxysporum* at varying degrees. Whereas, culture filtrates of *T. harzianum* isolates incubated at 40°C were unable to control the growth of the pathogen. The culture filtrate of *T. viride* (TvDPs) incubated at 25°C showed highest growth inhibition (25.57%) followed by TDPs (20.59%) and T1s (17.43%) respectively.

Mycelial growth rate of T1s, TDPs, and TvPDs was recorded on PDA, CDA, WA, SDA and natural media agar (Figure 4). Average linear growth rate was found to

be more than 24 mm/day on all culture media. Overall, TvPDs showed better growth on all media as compared to other two antagonists. For TvPDs best sporulation and mycelial growth was on SDA whereas T1s and TDPs flourished well on both PDA and SDA culture media. CDA and WA were found to be pretty good media for the mycelial growth of all antagonist. However sporulation on these media was scanty. TvPDs showed 32 mm/day mycelial growth on natural media agar (PDA + 1% date palm leaves) and it sporulated luxuriously, whereas ALG of T1s and TDPs was 28.25 and 24.25 mm/day, respectively.

DISCUSSION

A promising strategy for the replacement of chemical pesticides has been the implementation of biological control. Research has repeatedly demonstrated that phylogenetically diverse microorganisms can act as natural antagonists of various plant pathogens (Cook, 2000).

Trichoderma spp. are common saprophytic fungi which were found in almost any soil and rhizosphere micro flora. They have been investigated as potential biocontrol agents because of their ability to reduce the incidence of disease caused by plant pathogenic fungi, particularly many common soil borne pathogens (Papavizas, 1985; Sivan and Chet, 1986; Calvet et al., 1990; Elad et al., 1993; Spiegel and Chet, 1998; Elad, 2000; Freeman et al., 2004; Ashrafizadeh et al., 2005; Dubey and Suresh 2007), although some have been occasionally recorded as plant pathogens (Menzies, 1993).

The results of dual culture revealed that *Trichoderma* species showed the appreciable inhibition of mycelial growth of *F. oxysporum*. The presence of an inhibition zone in dual culture without the hyphae contact in treatment of *T. harzianum* (T1s), suggests the secretion of diffusible non-volatile inhibitory substance by the *Trichoderma* isolate. Previous studies have demonstrated that before mycelia of fungi interact, *Trichoderma* sp. produces low quantities of extracellular exochitinases (Kullnig et al., 2000; Brunner et al., 2003). The diffusion of these enzymes dissolves cell fragments of host cells. These cell fragments in turn induce the production of further enzymes and trigger a cascade of physiological changes, stimulating rapid and directed growth of *Trichoderma* sp. (Zeininger et al., 1999).

Evaluation of produced volatile components also showed an acceptable performance on inhibiting mycelia growth of pathogens. The results reported here suggest that *T. viride* (TvDPs) was more capable of influencing the growth of pathogen in dual culture and through production of volatile inhibitors under controlled condition, and may be used as a broad spectrum biological control agent under field condition.

Temperature is an important parameter to manipulate growth, sporulation and saprophytic ability as well as production of non-volatile metabolites, involved in nutrition, competition, mycoparasitism and extra cellular enzymes that disintegrate cell wall of fungi. Present results showed that culture filtrate of *Trichoderma* spp. /isolates caused inhibition of *F. oxysporum,* however none was fungicidal to this pathogen. Maximum inhibition was recorded by the culture filtrates of all antagonists incubated at 25°C. The optimum temperature for growth differs among the *Trichoderma* isolates; although most *Trichoderma* strains are mesophilic (Kredics et al., 2003; Hajieghrari et al., 2008). It is well known that *Trichoderma* produces a number of antibiotics such as trichodernin, trichodermol, harzianum A and harzianolide (Howell, 1998; Kucuk and Kivanc, 2004) as well as some cell wall degrading enzymes such as chitinases, glucanases that break down polysaccharides, chitins and -glucanase, there by destroying cell wall integrity (Lorito et al., 1996; Harman and Kubicek, 1998; Kubicek et al., 2001; Woo et al., 2006). The degree of effectiveness of these metabolites varies according to the nature, quality, quantity of antibiotics and inhibitory substances secreted by the antagonists (Harman and Kubicek, 1998). The further chemical investigation using GC-MS for the presence of antifungal metabolites might indicate its potential as a source of novel and useful antifungal antibiotics.

In the present study all culture media supported the growth of antagonist; PDA and SDA are found be the suitable media for the growth and sporulation of antagonist. Our results are in line with other studies (Elad et al., 1981; Azher et al., 2009). TvPDs sporulated luxuriously on natural media agar (PDA + 1% date palm leaves) thus this suggest that palm leaves can be explored further as a cheap material for the large scale propagation of *T. viride*.

Selection of biocontrol agents as well as understanding the mechanisms involved in the antagonistic effect of *Trichoderma* spp. on plant pathogens are important in designing effective and safe biocontrol strategies. Different isolates of *Trichoderma* have different combative ability for pathogen; their indirect effects may also vary. Therefore, one of the most interesting aspects of biology is the study of the mechanisms employed by bio-control agents to effect disease control. Possible mechanisms of antagonism employed by *Trichoderma* spp. includes nutrient and niche competitions, antibiosis by producing volatile components and non-volatile antibiotics that are inhibitory against a range of soil borne fungi, as well as parasitism. Also synergism between different forms of action modes occurs as the natural condition for the biocontrol of fungal pathogens. Many of these concepts have been extensively covered in recent reviews (Harman et al., 2004; Woo et al., 2006; Lorito et al., 2010; Druzhinina et al., 2011).

Antagonistic interactions showed excellent activity of *T. viride* (TvDPS) against *F. oxysporum in vitro* condition. However, antagonist fungi with the highest level of

bio-control *in vitro* may not perform as well as *in vivo* since environmental conditions and competition with other microorganisms are much more restrictive. Therefore, it is important that biocontrol potential in field condition should be further evaluated.

ACKNOWLEDGEMENT

The Authors extend their appreciation to the Deanship of Scientific Research at King Saud University for funding the work through the research group project No. RGP-VPP-066.

REFERENCES

Ashrafizadeh A, Etebarian HR, Zamanizadeh HR (2005). Evaluation of *Trichoderma* isolates for biocontrol of *Fusarium* wilt of melon. Iran. J. Phytopathol., 41: 39-57.

Azher M, Khan MA, Inam HM, Pervez MA, Umar UD (2009). Usefulness of different culture media for *in-vitro* evaluation of *Trichoderma* spp. against seed-borne fungi of economic importance. Pak. J. Phytopathol., 21*: 83-88.*

Brunner K, Peterbauer CK, Mach, RL, Lorito, M, Zeilinger S, Kubicek RL (2003). The Nacetylglucosaminidase of *Trichoderma atroviride* is essential for chitinase induction induction by chitin of and major relevance to bio-control. Curr. Gen., 43: 289-295.

Calvet C, Pera J, Bera JM (1990). Interaction of *Trichoderma* spp. with *Glomus mossaeae* and two wilt pathogenic fungi. Agric. Ecosyst. Environ., 9: 59-65.

Cook RJ (2000). Advances in plant health management in the 20th century. Ann. Rev. Phytopathol., 38:95-116.

Cook RJ, Baker KF (1983). The nature and practice of biological control of plant pathogens. Amer. Phytopathol. Soc. St. Paul MN, pp. 539

Dennis C, Webster J (1971). Antagonistic properties of species groups of *Trichoderma*. II. Production of volatile antibiotics. Trans. Br. Mycol. Soc., 57: 41-48.

Druzhinina IS, Seidl-Seiboth V, Herrera-Estrella A, Horwitz BA, Kenerley CM, Monte E, Mukherjee PK, Zeilinger S, Grigoriev IV, Kubicek CP (2011). *Trichoderma*: the genomics of opportunistic success. Nature Rev. Microbiol., 9:749-759.

Dubey SC, Suresh MS (2007). Evaluation of *Trichoderma* species against *Fusarium oxysporum f. sp. ciceris* for integrated management of chickpea wilt. Biol. Contamin., 40: 118-127.

Elad Y, Chet I, Henis Y (1981). A selective medium for improving quantitative isolation of *Trichoderma* spp. from soil. Phytoparasitica, 9: 59-67.

Elad Y, Zimmand G, Zags Y, Zuriel S, Chet I (1993). Use of *Trichoderma harzianum* in combination or alternation with fungicides to control Cucumber grey mold (*botrytis cinerea*) under commercial greenhouse condition. Plant Pathol., 42: 324-356.

Elad, Y (2000). Biological control of foliar pathogens by means of *Trichoderma harzianum* and potential modes of action. Crop Prot., 19: 709-714.

Enya J, Togawa M, Takeuchi T, Yoshida S, Tsushima S, Arie T (2008). Biological and phylogenetic characterization of *Fusarium oxysporum* complex, which causes yellows on *Brassica* spp., and proposal of *F. oxysporum* f. sp. *rapae*, a novel forma specialis pathogenic on *B. rapa* in Japan. Phytopath., 98:475–483.

Fravel D, Olivain C, Alabouvette C (2003). *Fusarium oxysporum* and its biocontrol. New Phytol., 157:493–502.

Freeman S, Minz D, Kolesnik I, Barbul O, Zreibil A, Maymon M, Nitzani Y, Kirshner B, Rav-David D, Bilu A, Dag A, Shafir S, Elad Y (2004). *Trichoderma* biocontrol of *Colletotrichum acutatum* and *Botrytis cinerea*, and survival in strawberry. Eur. J. Plant Pathol., 110: 361-370.

Gordon TR, Martyn RD (1997). The evolutionary biology of *Fusarium oxysporum*. Annu. Rev. Phytopathol., 35:111–128.

Hajieghrari 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. Afr. J. Biotechnol., 7: 967-972.

Harman GE, Howell CR, Viterbo A, Chet I, Lorito M (2004).*Trichoderma* species opportunistic, avirulent plant symbionts. Nature Rev. Microbiol.*,* 2:43-56.

Harman GE, Kubicek CP (1998). *Trichoderma* and *Gliocladium*, Vol. 2. Enzymes, biological control and commercial applications.Taylor & Francis, London, pp. 393.

Harman GE, Lorito M, Lynch JM (2004). Uses of *Trichoderma* spp. to alleviate or remediate soil and water pollution. Adv. Appl. Microbiol. 56:313-330.

Howell CR (1998). The role of antibiosis in biocontrol. In *Trichoderma and Gliocladium*,. Ed. C. P. Kubicek & G. E. Harman. London; Bristol, PA: Taylor & Francis, pp. 173-184.

Kredics L, Antal Z, Manczinger, Szekeres A, Kevei F, Nady E (2003). Influence of environmental parameters on *Trichoderma* strains with biocontrol potential. In: *Trichoderma* strains with biocontrol potential. Food Tech. Biotech., 47:37- 42.

Kubicek CP, Harman GE (1998). *Trichoderma* and *Gliocladium*. Vol. 1. Basic Biology, Taxonomy and Genetics. Taylor & Francis, London, pp. 278.

Kubicek CP, Mach RL, Peterbauer CK, Lorito M (2001). *Trichoderma*: From genes to biocontrol. J. Plant Pathol., 83:11-23.

Kucuk C, Kivanc M (2004). *In vitro* antifungal activity of strains of *Trichoderma harzianum*. Turk. J. Biol., 28: 111-115.

Kullnig C, Mach RL, Lorito M Kubicek CP (2000). Enzyme diffusion from *Trichoderma atroviride* to *Rhizoctonia solani* is a prerequisite for triggering of *Trichoderma* ech 42 gene expression before mycopariasitic contact. Appl. Environ. Microbiol., 66:2232-2234.

Lievens B, Rep M, Thomma BP (2008). Mini-review recent developments in the molecular discrimination of formae speciales of *Fusarium oxysporum*. Pest Manag. Sci., 64:781–788.

Lorito M, Woo SL, D'Ambrosio M, Harman GE, Hayes CK, Kubicek CP, Scala F (1996). Synergistic interaction between cell wall degrading enzymes and membrane affecting compounds. Mol. Plant-Microbe Interact., 9:206-213.

Lorito M, Woo SL, Gary E, Harman GE, Monte E (2010). Translational research on *Trichoderma*: From 'Omics to the Field. Ann. Rev. Phytopath., 48:395-417.

Menzies JG (1993). A strain of *Trichoderma viride* pathogenic to germinating seedlings of cucumber, pepper and tomato. Plant Pathol., 42: 784-791.

Michielse CB, Rep M (2009). Pathogen profile update: *Fusarium oxysporum*. Mol. Plant Pathol., 10:311–324.

Monte E (2001). Understanding *Trichoderma*: Between biotechnology and microbial ecology. Intl. Microbiol., 4:1-4.

Papavizas GC (1985). *Trichoderma* and *Gliocladium* biology, ecology and the potential for biocontrol. Ann. Rev. Phytopathol., 23: 23-77.

Rzewnicki P (2000). Ohio organic producers: Final survey results. Online. Ohio State University Extension, College of Food Agricultural and Environmental Sciences. Bull. Special Circular, 174.

Sivan A, Chet I (1986). Biological control of *Fusarium* spp. in cotton, wheat and muskmelon by *Trichoderma harzianum*. J. Phytopathol., 116: 39-47.

Spiegel Y, Chet I (1998). Evaluation of *Trichoderma* spp. as biocontrol agent against soil borne fungi and plant parasitic nematodes In Israel. Integr. Pest Manage. J. Rev., 3:169-175.

Van Arsdall RT, Frantz C (2001). Potential role of farmer cooperatives in reducing pest risk: Final report. Online. National council of farmer cooperative. US EPA, Pesticide environmental stewardship program, pp. 105-112.

Woo SL, Scala F, Ruocco M, Lorito M (2006). The molecular biology of the interactions between *Trichoderma* spp., phytopathogenic fungi and plants. Phytopathology, 96:181-185.

Wunsch MJ, Baker AH, Kalb DW, Bergstrom GC (2009). Characterization of *Fusarium oxysporum* f. sp. *loti* forma specialis nov., a monophyletic pathogen causing vascular wilt of birdsfoot trefoil. Plant Dis., 93:58–66.

Zeininger S, Galhaup C, Payer K, Woo SL, Mach, RL, Fekete C, Lorito M, Kubicek CP (1999). Chitinase gene expression during mycoparasitic interaction of *Trichoderma harzianum* with its host. Fungal genet. Biol., 26:131-140.