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Table 2. Glasshouse evaluation of mycoparasitic efficacy of *T. harzianum* against *F. oxysporum* f.sp. *lycopersici*

Application Method	Wilt Incidence %	Wilt reduction %
Seed coating with		
T. harzianum in sick soil	37 [a]	23.7 [b]
Carbendazim (2g/kg) treated in sick soil	35 [a]	28.9 [b]
Carbendazim (2g/kg) treated in sick soil		
50 g/kg soil in sick soil	4.2 [c]	90.1 [a]
65 g/kg soil in sick soil	3.1 [c]	92.2 [a]
Cheek		
Control	63.4 [a]	
The treated seed in natural soil	0	
Untreated seed in natural soil	13.8 [b]	
The biomass applied 50 g/kg in natural soil	1.3 [c]	

Different alphabets in column represent insignificant different at $p < 0.05$ employing DMRT [15].

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al. (1996). The present investigation showed that *T. hamatum*, *T. harzianum* and *T. longiconis* were more or less equally effective, *T. koningi* was less effective, whereas the least was noticed in case of *T. viride*. It was reported that isolates of the *T. harzianum* collected from different soil samples was not equally effective to inhibit the growth of *F. udum* (Bell *et al.*, 1982 and Biswas, 1992). It was found that one isolate (T1) among the 10 isolates of this antagonist was effective. So, there is need to search a very specific isolate(s) of *Trichoderma* sp. for successful control of *Fusarium oxysporum* f.sp. *lycopersici* (Biswas, 1992).

Further, it was reported that a *Trichoderma* isolate having no antagonistic effect in *in vitro* test may prove effective in glasshouse and field trial when delivered suitably. Thus, in dual culture of *F. oxysporum* f.sp. *vasinfectum* in cotton wilt and *F. oxysporum* f.sp. *niveum* in water melon wilt were not reduced by *T. harzianum* but the same isolate caused disease reduction in field trial Siven & Chet (1987). So *in vitro* less effective isolate, T. *koningi* and the least effectiveness of *T. viride* could be effective in glasshouse or field trial to reduce the wilt disease. *Trichoderma* after augmentation into soil reduced successfully *Fusarium* wilt of pigeon pea up to 84% by biomass formulation and 68.7% by seed treatment (Montealegre *et al.*, 2005). In the present study significant disease reduction up to 92.2% was obtained by direct application of *T. harzianum* biomass into soil. No significant effect was noticed in case of seed coating as compared to soil application of *T. harzianum*; it may be due to low population of *T. harzianum* applied into soil as compared to biomass application. Disease reduction was insignificant by sowing carbendazim treated seed (Table 2). It was also reported that carbendazim by seed treatment, soil drenches and combination of both did not reduce disease of some soil-borne pathogens (Biswas, 1992 and Sumita & Gaikwad, 1995). On the base of present study the bioagents of fungi, might be exploited for future plant disease management programs (DMP) to save environmental risk.

Table 1. *In vitro* efficacy of bioagents against *F. oxysporum* f.sp. *lycopersici**

Antagonist	Percent over growth of *Trichoderma* after days	Antagonistic interaction after days	
	4	4	6
T. hamatum	100[a]	An. started reduction growth of Pa	An. reduced 28% growth of Pa
T. harzianum	100[a]	An. started reduction growth of Pa	An. reduced 55% growth of Pa
T. koningi	27[b]	-	An. started reduction growth of Pa
T. longiconis	95[a]	-	-
T. viride	3[c]	-	-

* Data are average of four replications; Different alphabets in column represent insignificant difference at $p< 0.05$ employing DMRT [15]; An.: Antagonist (*Trichoderma*); Pa: Pathogen (*F. oxysporum* f.sp. *lycopersici*).

growing plants and incorporation of chopped infected plant into the same pot soil was repeated twice. Then the pots were used to test the efficacy of *Trichoderma* to control *Fusarium* wilt of tomato.

Seed coating with T. harzianum:

Tomato seeds washed with sterilized water were air dried and dipped into 2% carboxyl methyl cellulose (CMC) solution for 2 min. The seeds were taken from CMC solution and rolled over the *Trichoderma* spores on Petri-plats and the *Trichoderma* coated seeds were sown into previously infested soil.

Soil infestation with T. harzianum:

Mycelial plugs of three old cultures of *T. harzianum* grown on PDA medium were put into sterilized maize meal sand medium (maize meal 10g, sand 90g and water 16ml) in 250 ml conical flask and incubated at room temperature for nine days. Before application into soil the *Trichoderma* biomass was mixed with sterilized field soil.

Observation was recorded and data were analyzed statistically using one way ANOVA followed by Duncan's Multiple range test (Duncan, 1955).

Results and Discussion

Observation in *vitro* dual culture showed that the high antagonistic effect (over growth 100, 100 and 95%) was found against *F. oxysporum* f.sp. *lycopersici* in case of *T. hamatum*, *T. harzianum* and *T. longiconis*. Only 27 and 3% overgrowth were found in case of *T. koningi* and *T. viride* respectively (Table 1). The growth reduction activity of the pathogen by *T. hamatum* and *T. harzianum* started earlier after four days of contact but the reduction effect of the latter was more (55%) than the former (28%). No growth reduction activity was observed by *T. longiconis* and *T. viride* even after six days of contact. The different percentage of over growth records of *T. hamatum*, *T. harzianum* and *T. longiconis* after four days was insignificant compared with *T. koningi* and *T. viride* at the same period (Table 1). Therefore, the antagonist *T. harzianum* is chosen to be the most promising bio-control agent for *F. oxysporum* f.sp. *lycopersici*.

In pot culture under glasshouse condition biomass formulation of *T. harzianum* applied into pot soil caused highly significant reduction in wilt incidence as compared to control treatment (Table 2). However, the same antagonist when applied as seed coating could not reduce the disease significantly. Similarly, seed treatment with carbendazim @ 2 g/kg of seed could not cause disease reduction. When the biomass of *T. harzianum* @ 50 g/kg applied in natural soil the percentage of wilt incidence has been recorded as 13.8, which is significant as compared to control (Table 2). The inhibitory effect of these bioagents against tested pathogen was probably due to competition and/or antibiosis.

Demands for *in vitro* effectiveness of *Trichoderma* against species of *Fusarium* have been reported (Calvet *et al.*, 1990; Jee & Kim, 1987; Morsy *et al.*, 2009; Padmadaya & Reddy, 1996; Ramezani, 2008, 2009; Sabalpara *et al.*, 2009). The antagonist *Trichoderma harzianum*, *T. coningi* and *T. viride* were reported to be equally antagonistic to *F. udum in vitro* (Bahatnagar, 1986). Results of this evaluation were similar to the finding of Biswas & Das (1999), Cal *et al.* (2004), Morsy *et al.* (2009), Ramezani (2009), Siven & Chet (1987) and Somasekhar *et*

Fusarium wilt in many crops with application of different species of *Trichoderma* have been found (Bell *et al*., 1982; Biswas, 1992; Biswas & Das, 1999; Elad & Kapat, 1999; Morsy *et al.*, 2009; Papavizas, 1985; Ramezani, 2008; Ramezani, 2009; Sabalpara *et al*., 2009; Siven & Chet, 1986; Siven & Chet, 1987). However, it is also reported that all the isolates of *Trichoderma* spp. are not equally effective in control of pathogen *in vitro* (Bell *et al.*, 1982; Biswas, 1992; Biswas & Das, 1999; Elad & Kapat, 1999; Morsy *et al.*, 2009; Papavizas, 1985; Ramezani, 2008, 2009; Sabalpara *et al.*, 2009; Siven & Chet, 1986; Siven & Chet, 1987) and *in vivo* conditions to control diseases (Lewis & Papavizas, 1987; Ramezani, 2008, 2009). Therefore, specific isolates are needed for successful control of a particular pathogen. The objective of the present work is concerned with an attempt to evaluate the relative bio-control efficiency of five isolates of *Trichoderma* spp. in dual culture to inhibit the growth of *F. oxysporum* f.sp. *lycopersici* and control of wilt of tomato caused by this pathogen using the most effective *Trichoderma* sp. under glasshouse conditions.

Materials and Methods

In vitro assay:

The antagonistic properties of five isolates of *Trichoderma*, viz., *T. hamatum*, *T. harzianum*, *T. koningi*, *T. longiconis* and *T. viride* obtained from Institute of Agricultural Research, Shiraz, Iran, were tested for their efficacy to inhibit growth of *F. oxysporum* f.sp. *lycopersici* (obtained from I.A.R., Shiraz, Iran) in dual culture on PDA medium. The mycelial plug of 5 mm diam. from the margin of five days old culture of *Trichoderma* and pathogen were used. Three plugs of pathogen were placed into Petri-plate at three equally distant places of the periphery region and one for *Trichoderma* at the centre of the plate. The *Trichoderma* was placed after three days because the growth rate of the pathogen was slower than *Trichoderma*. Dual plates were incubated at 26 ± 2°C in Biological Oxygen Demand (B.O.D.) incubator and the extent of interaction was observed and measured on the base of radial growth of antagonists at time intervals.

Greenhouse experiment:

Soil infestation with the pathogen:

Efficacy of *Trichoderma* to control the wilt disease was tested after application and sowing of tomato seeds into artificially made *Fusarium* infested soil described by Bell *et al.*, 1982 with suitable modification. The earthen pots (25 cm diam.) were filled with naturally infested field soil where *Fusarium* wilt was found for several years. The pathogen culture was isolated from field infected tomato stem, subculture and inoculated into autoclaved tomato flour sand medium (tomato flour 10g, sand 90 g and water 20 ml) in 250 ml conical flask and incubated at room temperature for 15 days. Green tomato aerial parts were chopped into small pieces, autoclaved and mixed with *Fusarium* infested medium by 1:1 ratio. This mixture was incorporated into pot soil down to 10-20 cm depth. Then 20 seeds of susceptible tomato var. Cherry 9812 were sown into this soil and after 45 days all the infected and healthy plants were removed. Wilted plants were chopped into small pieces and incorporated into the same pot soil. This method of

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Antagonistic effects of *Trichoderma* spp. against *Fusarium oxysporum* f.sp. *lycopersici* causal agent of tomato wilt

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Abstract

In this study, the mycoparasitism inhibitory effects of five Trichoderma species (*T. harzianum*, *T. koningi*, *T. longiconis*, *T. hamatum* and *T. viride*) on the growth of the causal agent of tomato Fusarium wilt (*Fusarium oxysporum* f.sp. *lycopersici*) were investigated by dual culture in laboratory condition. In this step, the maximum and minimum inhibitory effect was caused by *T. harzianum* and *T. viride*. In the greenhouse, the comparison of the efficacy of disease decrease was carried out between soil and seed treatments affected by *T. harzianum* spores. Results showed that seed treatment did not cause disease decrease but soil treatment caused disease decrease by 92%.

Key words: *Trichoderma* spp., Mycoparasitism, Fusarium wilt, Tomato, Biological control

Introduction

Tomato (*Lycopersicon esculentum* Mill.) is one of the most important of the popular vegetables in the word (Rick, 1979). It is affected by several diseases, reflecting negatively on plant growth and the produced yield. Out of these, pathogenic fungi especially, the wilt caused by species of *Fusarium* remain to be a challenging task in terms of management (Agrios, 2000; Rick, 1976; Srinon *et al.*, 2006). Wilt of tomato caused by *Fusarium oxysporum* f.sp. *lycopersici* (Sacc.) W., is one of the most economically important diseases world-wide (Alexander & Tucker, 1945; Cal *et al.*, 2004; Rick, 1979; Srinon *et al.*, 2006). As *Fusarium* wilt is soil-borne in nature, application of fungicides to control this disease is not practical. Besides, chemicals pose serious health hazards to an applicator as well as to a consumer of the treated material. In addition to target organism, pesticides also kill various beneficial organisms. Their toxic forms persist in soil and contaminate the whole environment (Hayes & Laws, 1991). Prospects of biological control of soil-borne plant pathogens using most promising bio-control agent, the genus *Trichoderma* has been described (Elad & Kapat, 1999; Morsy *et al.*, 2009; Papavizas, 1985; Sabalpara *et al.*, 2009). Successful reductions of

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