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Biological control of Fusarium wilt of potato (*Fusarium oxysporum* f. sp. *tuberosi*) by *Trichoderma* isolates under field condition and their effect on yield

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Abstract: Application of *Trichoderma* species is a harmless method in controlling soil borne plant diseases thus reducing hazardous pesticide use and environmental pollution. Some *Trichoderma* isolates performing proper inhibitory effect against *Fusarium oxysporum* f. sp. *tuberosi* under laboratory and green house conditions were screened against wilt disease of potato caused by this pathogen under field condition in Shahrood Agricultural Research Center, Iran, during 2009-2010. Results of two years field studies indicated that *T. virens* (T7) followed by *T. asperellum* (T2) were superior to others in reducing the disease in comparison to infested control. In comparison to Fusarium infested plots, yield was higher in all plots treated with *Trichoderma*, but highest yields were obtained from plots in which *T. virens* (T7) and *T. asperellum* (T2) were involved respectively. It might be stated that isolates of *T. virens* and *T. asperellum* are among the effective biocontrol agents against Fusarium wilt disease of potato and can be used as formulated biofungicides in reducing this disease.

Keywords: Biocontrol, *Trichoderma*, Fusarium wilt, potato, yield

Introduction

Potato (*Solanum tuberosum* L.) is the world's fourth-largest food crop, following rice, wheat and maize (Harris, 1992). This crop is cultivated in about 4500 ha of agricultural lands of Semnan province (Anonymous, 2010). Fusarium wilt is a world wide important disease of this crop and can be transmitted by seed cuts (Powelson and Rowe, 1993). A survey indicated that between 15 to 70% of potato fields of Semnan province are contaminated with Fusarium wilt causal agents mainly *Fusarium oxysporum* Schlecht. emend. Snyd. & Hans. (Ommati and Sharifi, 2008).

In view of difficulties and problems associated with chemical control of soil borne plant pathogens and environmental pollution, employment of biocontrol agents for plant disease management is considered as a good alternative. The potential of *Trichoderma* species as biocontrol agents was recognized for the first time in early 1930s (Weindling, 1932). During recent decades, attention has been paid to this group of fungi and subsequently they have been applied successfully as biocontrol agents against several plant diseases in commercial agriculture (Howell, 2003). Papavizas (1985) in his studies approved the efficacy of *Trichoderma* species in controlling several plant pathogens and reported that *Trichoderma* (*Gliocladium*) *virens* has been one of the most widely studied species of fungal biocontrol agents, proven effective as a suppressant of several soil-borne plant pathogens

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due to production of gliotoxin and gliovirin. Mao *et al*., (1997) in a study treated maize seeds with some antagonistic bacteria and fungi including *T. virens* and *T. viride* and found suitable control of maize damping-off (*Pythium* sp.) and Fusarium wilt diseases by them. Larkin and Fravel (1998) used commercial formulations of *T. virens* (Soilgard) and *T. harzianum* (Rootshield) against Fusarium wilt of tomato under field condition and observed 62 and 68% disease control by these formulations respectively. Soltani *et al*., (2005) reported that under green house condition Fusarium wilt of potato (*Fusarium oxysporum*) was better controlled by *T. harzianum* in comparison to field condition yet under field condition higher yield was achieved. Ashrafizadeh *et al*., (2005) studied different mechanisms of *Trichoderma* isolates in controlling Fusarium wilt of melon under labaratory, green house and field conditions and found *T. virens* (DAR-7429) as the most effective isolate in controlling the disease. In another study, the disease severity of yellow disease of *Brassica campestris* caused by *F. oxysporum* in the first, second, and third crops in green house were 33%, 48% and 35% in contrl plots, while they were 1.4%, 11.5% and 3.0% in *T. viride* amended plots respectively (Kataoka *et al*., 2010). Sallam *et al*., (2008) reported that isolates of *T. harzianum, T. viride* and *T. spirale* showed different inhibitory effects against mycelial growth of *Rhizoctonia solani* and *F. oxysporum* f. sp. *phaseoli* of bean in lab. tests and under greenhouse and field conditions, soil treatment with a powder formulation of *Trichoderma* spp. two weeks before planting or at the time of planting not only significantly reduced the incidence of both diseases but also enhanced the bean yield in comparison to infested control. In Plots treated with *T. harzianum* formulation, a yield equal to that of healthy control was observed. In Hungary, Boureghda and Bouznad (2009) reported that among several isolates of *T. atroviride, T. harzianum,* and *T. longibrachiatum*, three strains of *T. atroviride* as well as *T. harzianum* (Th. 16) were found to be the most effective isolates in protecting chickpea seedlings against Fusarium wilt and observed that the reduction of disease severity was associated with an increase in vegetative growth of tested plants. Howell (2006) stated that among *Trichoderma* species, *T. virens* appears to be one of the most versatile and effective biocontrol agents that have been studied and one of the salient features of this species is that it is an aggressive mycoparasite of other fungi, many of which are plant pathogens. Akrami *et al*., (2011) evaluated biocontrol efficacy of *T. harzianum* (T1), *T. asperellum* (T2) and *T. virens* (T3) against Fusarium rot of lentil (*F. oxysporum*) and reported that T1 and T2 and their combination were more effective than other treatments in controlling the disease with 20 to 44% disease reduction as well as dry weight improvement of 23 to 52%. In a study the antagonistic potential of *Aspergillus niger, Penicillium citrinum*, *T. harzianum,* and *T. viride* for management of Fusarium wilt (*F. oxysporum* f sp. *Phaseoli*) of common bean was assessed and maximum disease control (71.4%) was observed in plants treated with *T. harzianum* followed by *T. viride* (67.8%), *P. citrinum* (53.5%) and *A. niger* (35.7%) (Alwathnani *et al*., 2010). Tsai *et al*., (2008) reported that among five *Trichoderma* strains isolated from *Anoectochilus* rhizospheres, a conidial formulation of a strain of *T. asperellum* mixed with carboxymethyl cellulose (CoCMC) had an excellent ability in controlling stem rot disease (*F. oxysporum*) of Taiwan *Anoectochilus* and could completely protect tested plants against this disease for 9 weeks. They also found that the protective effect of CoCMC appeared to be further strengthened by the addition of wheat bran as a food base. Schubert *et al*., (2008) examined the potential of *Trichoderma* spp. as a wound treatment for controlling wood decay fungi in urban trees and reported that *T. atroviride* (T-15603.1) could be successfully applied as biological wound treatment against wood decay fungi.

Materials and Methods

Preparation of pathogenic isolate of *F. oxysporum*

Potato plants showing wilt symptoms were collected from different fields at flowering stage. Small pieces of diseased specimens (stem

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vascular tissue) were grown on Fusarium selective medium (Nash and Snyder, 1965). After purification, isolates were identified according to their morphological characteristics with the help of standard key (Nelson *et al*., 1983) and five isolates of *F. oxysporum* were selected for pathogenicity tests. Pathogenicity tests were conducted on potted potato plants (Variety Agria). Seven days old *F. oxysporum* isolates grown on potato dextrose agar (PDA) at 25 ± 1 °C were transferred to 500 ml Erlenmayer flasks containing 250 ml PDB (potato dextrose liquid broth) and the flasks were incubated in an orbital shaker at 25 ± 1 °C for 5 days. Cultures were passed through cheesecloth to separate the mycelia from the spores and the final concentration of the spore suspension was adjusted to10^8 spores/ml. Twenty ml of these suspensions was added to each pot in which potato seeds were grown. Four pots were used for each isolate and plants were observed for appearance of disease symptoms (Abeysinghe, 2006). Pathogens were re-isolated from randomly selected diseased plants, the Koch's postulates were followed in order to confirm the pathogenicity of *F. oxysporum* isolate (s).

Selection of *F. oxysporum* f. sp. *tuberosi* isolate (s)

In order to determine the special form of the pathogen as "*F. oxysporum* f. sp. *tuberosi*", healthy seedlings of three solanaceous crops namely tomato, pepper and brinjal were grown in pots containing pasteurized soil along with healthy small potato seeds (Agria variety). The procedure of pathogenicity tests deserибed above was followed (Abeysinghe, 2006) and isolate (s) pathogenic to potato and non-pathogenic to other solanaceous plants were selected.

Preparation and identification of *Trichoderma* isolates

Obtaining *Trichoderma* isolates from soil

Soil samples were collected from potato rhizosphere (20 cm deep) of different fields. Twenty g of each soil sample was thoroughly mixed with 500 ml distilled water containing 0.02% citric acid. Five ml of prepared solution were then added to plates containing 15 ml water agar at 50 °C and shacked gently to mix properly. Five mm plugs of solidified water agar containing soil mixtures were then transferred to plates containing *Trichoderma*-selective medium (Davet, 1979) and were incubated at 25 °C. After proper growth, isolates were purified and identified on the basis of their morphological characteristics (Rifai, 1969).

Obtaining *Trichoderma* isolates from other sources

Several *Trichoderma* isolates were obtained from Mashhad and Abu-Ali-Sina University fungal collections, from among which three isolates having proper antagonistic ability against *F. oxysporum* under in vitro condition, were used for field trails (Table 1).

Mass production of *Trichoderma* isolates

Sufficient amount of wheat kernels were sosked in water and boiled for two hours. Twenty four g of limestone powder and 6 g of gypsum powder were added to 1 kg of wheat grain to prevent adhesiveness of wheat grains and were mixed properly. Five hundred g of boiled grain were kept in proper cellophane bags and were autoclaved at 121 °C for one hr on three successive days. After cooling, 5 ml spore suspension of young *Trichoderma* isolates (10^8 spores/ml) were added into cellophane pockets, mixed gently and were incubated at 25-27 °C. After 1 month colonized grains were air dried and ground (Ordentlich *et al*., 1990).

Mass production of *F. oxysporum* f. sp. *tuberosi* inocula

Five hundred ml Erlenmeyers containing 100 g of Durum wheat cultivar and 100 ml distilled water were autoclaved at 121 °C for 1 hr on three successive days. After cooling, about 5-7 small plugs of young culture of *F. oxysporum* f. sp. *tuberosi* were dropped into each Erlenmeyer. These were kept at 25 °C for 4 weeks. Colonized wheat grains were then transferred into paper pockets, and were

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dried and ground. Fourteen g of prepared powder was used to infest 1 Kg of soil (Frommel *et al*., 1991).

Preparation of plots

Furrows were prepared and irrigated a week before sowing. Three days later ground with kernels colonized by *F. oxysporym* f. sp. *tuberosi* were placed in seed beds at the rate of 25 g/m^2 of plot, and after that the same amount of ground with colonized by *Trichoderma* isolates were also added (Ordentlich *et al*., 1990). In the case of healthy control only sterilized wheat powder was added to seed beds. Healthy small potato tubers of Agria variety (50-60 g) were then sown in seed beds. Each plot contained four rows of 5 m length with 75 cm distance between rows and 25 cm between plants. After initial growth, plants were inspected carefully and if necessary protected with systemic insecticides. Cultural practices of all treatments were done equally. Data on disease severity were collected after flowering and at the time of tuber formation following Chandra *et al*., (1983) disease assessment key (Table 2). Tubers were collected and weighted at the end of growing season.

Statistical method

A completely randomized block design with eight treatments and three replications was used in this study. Data were analyzed in MSTAT-C and means were compared by Duncan's multiple range test.

Results

Six *Trichoderma* isolates showing acceptable antagonistic ability against *F. oxysporum* of potato under laboratory and green house conditions, were evaluated in field studies (Table 1). Results of two years field studies indicated significant disease reduction in Fusarium infested plots treated with *T. virens* (T7) and *T. asperellum* (T2) compared to Fusarium infested control (P = 0.05). In this connection *T. virens* (T7) demonstrated superiority over *T. asperellum* (T2) while these two treatments were placed in the same statistical group with healthy control (Table 3). In comparison to plots of infested control, harvested yields were higher owing to *Trichoderma* applications specially in the two mentioned treatments which were at par with healthy control (p = 0.01). Yield achieved from plots treated with T7 and T2 were 40.26 and 38.64 ton/ha respectively while the infested control yielded 28.30 tons/ha (Table 3). On the basis of results, a regression model was prepared as: y = -0.6697x + 38.954, which indicates the corelation between percentage of disease severity (PDS) and yield (X is percentage of disease severity and is calculated according to the formula: S = Σ (Xi Ni / 5N), where: S is disease severity, Xi is degree of disease severity, Ni is the number of infected plants and N is total number of plants. According to regression model a 10 % increase in disease severity resulted in a yield reduction of 6.5 ton/ha in the present study (Fig. 1)

Table 1 Sources of *Trichoderma* isolates used.

Isolate	Source
T. virens (T7)	Mashhad University, East Iran
T. atroviride (T3)	Abu-Ali-Sina University, West Iran
T. longibrachiatum (T5)	Abu-Ali-Sina University, West Iran
T. asperellum (T2)	Potato fields, North Iran
T. brevicompactum (T10)	Potato fields, North Iran
T. harzianum (T21)	Potato fields, North Iran

Table 2 Disease assessment key (Chandra *et al.,* 1983).

Symptom	disease seveity (%)	grade
Healthy plants	0	0
Yellowing of older leaves	10	1
Yellowing and wilting of older leaves	25	2
Wilting of two or some branches	50	3
Wilting of all branches except apical shoot	75	4
Wilting and dying of whole plant	100	5

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Table 3 Mean comparisons of effect of different treatments on potato Fusarium wilt and potato yield under field condition.

Treatment	disease severity (%)	Yield (ton/ ha)
Fusarium infested Control	24.66 a	28.30 c
T. longibrachiatum (T5) + *F. oxysporum*	20.78 a	33.81 abc
T. brevicompactum (T10) + *F. oxysporum*	18.83 ab	31.80 bc
T. harzianum (T21) + *F. oxysporum*	18.16 ab	31.27 bc
T. atroviride (T3) + *F. oxysporum*	17.50 ab	33.08 abc
T. asperellum (T2) + *F. oxysporum*	10.33 bc	38.64 ab
T. virens (T7) + *F. oxysporum*	9.00 bc	40.26 ab
Healthy Control	7.83 c	41.85 a

According to Duncan's multiple rang test, values followed by different letters are significantly different [disease incidence ($p = 0.05$), yield ($p = 0.01$)].

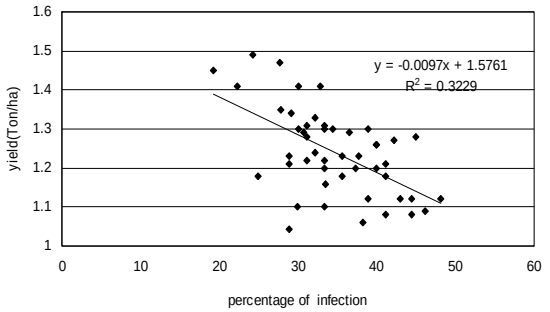

Figure 1 Regression model showing correlation between PDI and yield.

Discussion

Reviewing of several reports shows that *Trichoderma* spp. can be used as biocontrol agents against Fusarium wilt disease of many field crops including potato but their efficacy may be variable in different agro-climatical conditions. Laboratory and green house evaluations of *Trichoderma* isolates are useful preliminary tests but not enough for approval of their antagonistic ability, therefore field evaluations in which *Trichoderma* isolates are influenced by several physicochemical and biological factors of the soil seem to be necessary for their final approval as effective biocontrol agents. During this research out of 6 different *Trichoderma* treatments, *T. virens* (T7) and *T. asperellum* (T2) demonstrated promising ability in reducing the disease, thus reducing yield loss as well. Formulation of *T. virens* is available under trade name of SoilGard (Larkin and Fravel 1998) and is commercially used against Fusarium wilts of several crops. But in the case of *T. asperellum* isolates, although several research articles including the present study have approved its effectiveness as suitable for reducing Fusarium wilt, no work has been done on its formulation. Our findings can be compared with several reports showing efficiency of these species in controlling several Fusarium wilts. In this connection, Papavizas (1985) in his studies focused on biological strength of *T. virens* and believed that due to production of two important inhibitors it stands among the most effective species for suppressing several soil-borne pathogens. Howell (2006) also believed that among *Trichoderma* species, *T. virens* is one of the most versatile and effective biocontrol agents that have been studied. Others like Mao *et al*., (1997), Ashrafizadeh *et al*., (2005) and Larkin and Fravel (1998) also found this species as one of the most efficient antagonists against Fusarium wilts. On the basis of Larkin and Fravel,s report its effectiveness in controlling *F. oxysporum* of tomato has been equal to that of *T. harzianum*, the well known species formulated as RootShield. According to our results, *T. asperellum* (T2) was the second with respect to superiority in controlling the disease. Findings of Akrami *et al*., (2011) and Tsai *et al.,* (2008) are in agreement with our results because they reported that isolates of *T. asperellum* had best antifungal potential in controlling Fusarium rot (*F. oxysporum*) of lentil and stem rot (*F. oxysporum*) of Taiwan *Anoectochilus* respectively when they compared it with other *Trichoderma* species. In this study *T. virens* (T7) performed best against Fusarium wilt of potato and *T. asperellum* (T2) was also as efficient as the former species. In a

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study conducted by Ommati and Zaker (2012), among several *Trichoderma* isolates, *T. asperellum* (T2) demonstrated best performance against *F. oxysporum* under lab. and green house conditions. Therefore it can be stated that isolate of this species may be incorporated in biofungicide formulations and along with *T. virens* formulations can be used in controlling this disease under field condition.

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کنترل بیولوژیکی پژمردگی فوزاریومی سیب‌زمینی (*Fusarium oxysporum*) با جدایه‌های *Trichoderma* در شرایط مزرعه و تأثیر آنها در میزان محصول

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چکیده: استفاده از گونه‌های تریکودرما از جمله روش‌های بی‌خطر برای کنترل بیماری‌های گیاهی و همچنین مفید در کاهش مصرف سموم و آلودگی‌های زیست‌محیطی می‌باشد. در این تحقیق تعدادی از جدایه‌های تریکودرما که توانایی خوبی در کنترل عامل پژمردگی فوزاریومی سیب‌زمینی در شرایط آزمایشگاه و گلخانه از خود نشان داده بودند، در شرایط طبیعی مزرعه علیه این بیماری مورد ارزیابی قرار گرفتند. این بررسی‌ها در طی سال‌های ۸۸ و ۸۹ در مرکز تحقیقات کشاورزی شاهرود صورت گرفت. براساس نتایج دو سال بررسی مزرعه‌ای جدایه *T. virens* (T7) و بعد از آن *T. asperellum* (T2) در مقایسه با دیگر تیمارها و شاهد آلوده بهترین کارآیی را در کاهش میزان بیماری از خود نشان دادند. همچنین در مقایسه با شاهد آلوده در تمامی کرت‌های تیمار شده با تریکودرما افزایش محصول محسوس بود ولی در تیمارهایی که جدایه‌های *T. virens* (T7) و *T. asperellum* (T2) در آنها دخیل بودند در مقایسه با شاهد آلوده اختلاف معنی‌دار مشاهده گردید. بنابراین می‌توان اذعان نمود که جدایه‌های *T. virens* و *T. asperellum* می‌توانند کارآیی لازم برای کنترل بیماری پژمردگی فوزاریومی سیب‌زمینی را ازخود بروز داده و می‌توان به‌عنوان قارچ‌کش‌های بیولوژیکی در این ارتباط از آنها سود جست.

واژگان کلیدی: بیوکنترل، *Trichoderma*، سیب‌زمینی، پژمردگی فوزاریومی، عملکرد.

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