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Biological efficacy of *Trichoderma harzianum* isolate to control some fungal pathogens of wheat (*Triticum aestivum*) in Turkey

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Abstract: *Gaeumannomyces graminis* var. *tritici*, *Fusarium culmorum* and *F. moniliforme* are highly important and widespread pathogens of wheat in Turkey. *Trichoderma* isolates have been used as biocontrol agents to protect plants against soilborne diseases in several crops. The present work was carried out to evaluate the potential of *Trichoderma harzianum* isolate T1 as biocontrol agents for *G. graminis, F. culmorum* and *F. moniliforme* under field conditions in 2001 and 2002. Quantitative differences were found in microbial number in soil. *T. harzianum* T1 had considerable effect on population densities of the tested pathogens. The total number of *G. graminis, F. culmorum* and *F. moniliforme* were lower in the *T. harzianum* T1 application made to seed. *T. harzianum* T1 application to seed had increasing affect on the yield components of wheat through better control over pathogens. The greatest counts of *T. harzianum* T1 were detected on root segments. Seed application by *T. harzianum* T1 had increasing effect on yield components of wheat.

Key words: *Trichoderma harzianum*; fungi; wheat; plant pathogens; biocontrol; yield component

Introduction

Wheat grown in Asia, Australia and Europe is attacked by a complex of root rot pathogens and resulting in substantial yield losses. Take-all caused by *Gaeumannomyces graminis* var. *tritici* (Ggt) and root rot caused by *Rhizoctonia* spp. and *Fusarium* spp., are among the most destructive and widespread diseases (Cook & Veseth 1991). Take-all, *Rhizoctonia* and *Fusarium* root rots are particularly difficult to control because resistant varieties are not available and few effective chemicals are registered (Cook & Veseth 1991). Control strategies aimed at managing both diseases should be more effective in the long run (Cook & Veseth 1991). A complementary strategy within the integrated management is the possibility of biological control. Different species of *Trichoderma* have the potential to control soilborne plant pathogens more effectively than chemicals (Harman et al. 2004). Use of these fungi is not as harmful to the environment as chemical pesticides. They are present in substantial quantity in nearly all agricultural soils and in other environments and their use is only now being recognized world-wide as an alternative in plant disease control (Benitez et al. 1998; Perello et al. 2003; Brewer & Larkin 2005).

Many studies in the past have proved the potential of *Trichoderma* spp. as biocontrol agents of several soilborne plant pathogens such as *Rhizoctonia solani, Sclerotium rolfsii, Pythium* spp., *Drechslera tritici* and *Fusarium* spp. (Hadar et al. 1979; Elad et al. 1980; Sivan et al. 1984; Samuels 1986; Perello et al. 2003). A commercial preparation of *Trichoderma* species containing 2–4 compatible isolates, effectively controlled a variety of soilborne plant pathogens and also improved plant growth (Kleifeld et al. 1996; Brewer & Larkin 2005). In a few studies, wheat bran was reported as a suitable food base for introduction of *Trichoderma* spp. into soil (Hadar et al. 1984; Sivan et al. 1984; Sivan & Chet 1986; Elliott et al. 2001). The application of wheat bran colonized by *T. harzianum* to soils infested with *R. solani* and *F. culmorum* reduced the incidence of disease caused by these pathogens in beans and wheat (Hadar et al. 1979; Sivan & Chet 1989). The aim of this study was to investigate the interactions among *T. harzianum*, *F. moniliforme, F. culmorum* and *Gaeumannomyces graminis* in order to make the conclusion on application of the mycoparasitic fungus into seed bed mixed with the wheat bran or applied to seed.

Material and methods

Test organisms

Gaeumannomyces graminis var. *tritici, Fusarium culmorum* and *F. moniliforme* were used as pathogenic organisms for the experiment. Fungal species were obtained from Küçük (2000) who isolated them from the rhizosphere of wheat fields. *Trichoderma harzianum* T1 isolated from the rhizosphere of wheat seedlings kept on a medium selective for

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Table 1. Estimates of microbial numbers (cfu/g air-dried soil in field).

	Treatments	Total[a] ($\times 10^{-4}$) aerobic microorganisms	Gram-negative[b] ($\times 10^{-5}$)	Fungi [c] ($\times 10^{-5}$)	Yeast[d] ($\times 10^{-4}$)
T. harzianum applied to seed	*F. culmorum* soil mixed	75.5	6.9	8.0	14.8
	F. moniliforme soil mixed	75.3	3.0	8.5	20.7
	G. graminis soil mixed	82.7	8.5	9.8	17.9
T. harzianum wheat bran mixed	*F. culmorum* applied to seed	23	1.8	4.8	14.2
	F. moniliforme applied to seed	32	2.1	4.5	18.6
	G. graminis applied to seed	31.5	2.3	2.7	17.5

[a] total number of aerobic microorganisms estimated on 1/10 TSA; [b] number of Gram-negative bacteria estimated on 1/10 TSA + CV; [c] number of filamentous fungi estimated on RBS; [d] number of yeasts estimated on RBS.

Trichoderma spp. (Elad 1980). Fungal cultures were maintained by cryo conservation in water and glycerol (20%) at −80 °C. From these sources, the fungi were transferred onto plates of PDA and cultured at 25 °C for preparation of inoculum as described below.

Fungal inoculum preparation

G. graminis var. *tritici, F. culmorum* and *F. moniliforme* inocula were prepared using a method of Küçük (2000), and used according to two different modalities as application of suspensions into seed bed at planting or seed coating. Twenty mL of each suspension were used to coat 11.12 g of wheat seeds.

T. harzianum T1 was applied in two ways:

a) Application by hand into soil as mixed with wheat bran. Moisture of the mixture was amended with 50 mL of tap water per 100 grams of mixture. The mixture was then autoclaved in glass bottles for 1 h at 121 °C on three successive days. 20 mL of suspension containing approximately 1×10^7 conidia mL^{-1} of *T. harzianum* T1 was transferred into wheat bran preparation and incubated 7 days at 30 °C. Wheat bran colonised by mycelium and the conidia of the *T. harzianum* were air dried and stored at 4 °C until use in the experiment.

b) Seed coating; conidia were collected from cultures grown in Erlenmayer flasks each containing 200 mL of solidified potato dextrose agar (PDA, Merck). The conidial suspension adjusted to 1×10^7 conidia mL^{-1} and 20 mL of this suspension were used to coat 11.12 g of wheat seeds (previously treated with 1% sodium hypochlorite for 2 min) (Küçük 2000).

Field trials

Field experiments were conducted in two successive years. The total precipitations during two years were about 212.5 mm and 372.6 mm, respectively. The soil was alkaline as most part in the region, organic matter was low (1.7%) and soil texture was clay. Experimental design was factorial in randomized complete block with three replications. Bezostaja-1, a winter bread wheat cultivar was used as research material to inoculate seeds by pathogens and control with no application. Sowing was made by hand, into 6 rows × 4 m plots. Seeding rate was 540 seeds m^2. During planting in fall, 70 kg P_2O_5 ha^{-1} DAP (diamonium phosphate) and in spring 130 kg ha^{-1} NH_4NO_3 (ammonium nitrate) were applied to the soil.

Plants were sampled from the central four rows of each plot to measure yield components. Soil samples were taken from the root zone of plants from each replication and bulked. Density of propagules of *Trichoderma* in these samples was determined using the soil dilution method on TSM (Elad et al. 1981).

Then plants from each plot were selected for measurements of the yield components: Spike length (measured from the collar to the apex of the last spikelet as cm), number of spikelets per spike, grain weight per spike (g), thousand kernel weight (g). The reduction in values of wheat yield component by pathogen and *Trichoderma* treatment was calculated as % reduction: $100 \times (C - T)/C$ where C and T are the values of variables in control and pathogen and *Trichoderma* treatment (Küçük 2000).

Estimation of microbial numbers in field soil

Microbial numbers in the soil were estimated using the soil dilution plate method (Johnson & Curl 1972). The groups of organisms selected for enumeration and the media used were as follows; the total number of aerobic microorganisms in the soil on one-tenth strength Tryptic Soy Broth Agar (1/10 TSA); the Gram negative bacteria on 1/10 TSA containing crystal violet at a concentration of 2 μg mL^{-1} (TSA + CV); the soil fungi (filamentous fungi and yeasts) on a medium containing rose bengal and streptomycin (Johnson & Curl 1972; Küçük & Kivanç 2003).

Results and discussion

There were quantitative differences in microbial numbers in the soil. The estimated total number of aerobic microorganisms, and numbers of both gram-negative organisms and fungi were greater in the soil zone in which *T. harzianum* was applied to seed than in the treatments where *T. harzianum*-wheat bran was mixed into the soil (Table 1).

After the application of *T. harzianum* T1 as a seed treatment, *T. harzianum* T1 was recovered from rhizosphere, indicating its active proliferation along the developing roots. The greatest counts of *T. harzianum* T1 in the rhizosphere were detected on root segments. The relatively high population levels of antagonists recovery from root tips were also demonstrated with bacteria (Kleifeld et al. 1996). After a seed coating treatment, *T. harzianum* T1 effectively colonized the rhizosphere soil but failed to establish in nonrhizosphere soil.

Serial dilutions of soils were carried out to determine the colonization of wheat rhizosphere and nonrhizosphere soils that were infested with pathogens and treated with *T. harzianum* T1.

Application of *T. harzianum* T1 as a wheat bran preparation resulted in high colonization of both rhizosphere and nonrhizosphere soils. At the same time

Table 2. Effect of *T. harzianum* T1 on test pathogens population in wheat rhizosphere and nonrhizosphere soil.

Trichoderma Treatments	Colony-forming units per gram of soil × 10^3					
	Trichoderma		*Fusarium sp.*		*G. graminis*	
	Rhizosphere soil	Nonrhizosphere soil	Rhizosphere soil	Nonrhizosphere soil	Rhizosphere soil	Nonrhizosphere soil
None	3.2	0.9	1.200	150	2.100	320
Seed coating	510	12	27	98	41	21
Wheat bran	620	100	42	29	52	69

Table 3. Mean values, mean squares from variance for agronomic characters of wheat in 2001.

Components		SL (cm)	SS	SY	TH
Control		10	16.7	1.60	46
T. harzianum to seed-patho.[1] to soil		10.3	22.3	1.24	48.9
T. harzianum to soil-patho.[1] to seed		9.83	17.3	1.53	45.8
Dif[2]. (%)		−4.6	−22.4	24.2	−6.4
ANOVA					
	df				
Replication	2	0.14	0.15	0.02	0.35
Treatment	2	0.23	28.8	0.13**	12.4*
Control and others	1	0.04	20.1	0.14**	8
Others	1	0.42	37.5	0.11**	16.6*
Error	4	0.47	64.9	0.005	1.7

* Significant at the 0.05 and, ** at 0.01 levels of probability, respectively. [1] patho. – pathogen; [2] Dif. – difference; SL – spike length (cm); SS – spikelet per spike; SY – spike yield (g); TKW – thousand kernel weight (g)

Table 4. Mean values, mean squares from variance for agronomic characters of wheat in 2002.

Components		SL	SS	SY	TKW
Control		9.30	19	1.67	46
T. harzianum to seed- patho.[1] to soil		10.3	19.8	1.74	50
T. harzianum to soil-patho.[1] to seed		9.93	19.6	1.88	47
Dif[2]. (%)		−3.6	−1.1	9.8	−5.8
ANOVA					
	df				
Replication	2	0.3	0.17	0.0009	0.45
Treatment	2	0	0.47	0.02**	5.8**
Control and others	1	0	0.85	0.003	0.9*
Others	1	0	0.08	0.027**	10.6**
Error	4	0.8	0.63	0.0008	0.1

* Significant at the 0.05 and, ** at 0.01 levels of probability, respectively. [1] patho. – pathogen; [2] Dif. – difference; SL – spike length (cm); SS – spikelet per spike; SY – spike yield (g); TKW – thousand kernel weight (g)

the treatments reduced the numbers of test pathogens in the rhizosphere soil (Table 2). Recently Brewer & Larkin (2005), reported biological control of *Rhizoctonia solani.* In their study they used a different formulation that consisted of *Bacillus subtilis, Trichoderma harzianum* and *Trichoderma viriens.* Our study, however, reveals that *Trichoderma harzianum* T1 successfully controlled *G. graminis, F. culmorum* and *F. moniliforme* in natural soils.

Due to a competitive mechanism in biocontrol, *T. harzianum* T1 had considerable effect on the population densities of *G. graminis, F. culmorum* and *F. moniliforme* in rhizosphere soil. This effect may come from the ability of *Trichoderma* to compete with fungal pathogens for nutrients and for occupation of the infection area during the penetration period. This fact was also observed in the interaction among *Trichoderma harzianum* and pathogenic fungi (Kleifeld et al. 1996; Küçük 2000). Other mechanisms involved in *Trichoderma harzianum* antagonism could be antibiosis and mycoparasitism promoted by lytic enzymes (Sivan et al. 1984; Küçük 2000; Küçük & Kivanç 2003).

In this study some important yield components such as spike length, spikelet number per spike, spike yield and thousand kernel weights were recorded and evaluated under field conditions. Average values of each component with two different applications of the pathogens and *T. harzianum* were estimated and compared (Tables 3–4). In both years of experiment, except spike yield, the values of spike length, spikelet

number per spike and thousand kernel weights were reduced more when the pathogens were applied to seed and *T. harzianum* applied into soil in mixture of wheat bran than when the pathogens were applied to soil and *T. harzianum* applied to seed as seed coating. Results of ANOVA indicated that there were significant differences among treatments (control included) for spike yield, thousand kernel weight in both years. This finding is noticeable because both traits are considered as important yield components. These results showed that *T. harzianum* application to seed has more effect against the pathogens than *T. harzianum* application into soil in relation with these yield parameters.

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