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Biological Control of Damping Off and Root Rot of Wheat and Sugar Beet with *Trichoderma harzianum*

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Abstract: *Fusarium nivale* (Fries) Samules and Hallett, *Fusarium graminearum* (Schwabe) and *Fusarium tricinctum* (Corda) Sacc. caused damping off and root rot of wheat. *Fusarium lateritium*, (Nees) *Fusarium xylarioides* (Steyaert) and *Fusarium camptocearas* (Wollenw and Reinking.) caused damping off and root rot of sugar beet. Each of *Trichoderma harzianum* and its filtrate inhibited the growth of the entire tested *Fusarium* spp. *In vitro* treatment of either the seeds of Sads1 wheat or the seeds of Dempoloy sugar beet growing in the soil with *T. harzianum* decreased damping off and root rot severity of each wheat and sugar beet compared with untreated seeds and untreated soil with *T. harzianum* under greenhouse condition during growing seasons 2009 and 2010. *T. harzianum* increased the (shoot and root) seedling dry weight compared with seedling grown without bioagent. Disease reduced in seed coating method more than soil method.

Key words: *Fusarium nivale*, *Fusarium graminearum*, *Fusarium tricinctum*, *Fusarium lateritium*, *Fusarium xylarioides*, *Fusarium camptocearas*, *Trichoderma harzianum*

INTRODUCTION

Wheat is considered an essential crop in human nutrition. Damping off and root rot diseases caused by *Fusarium nivale*, *F. graminearum* and *F. tricinctum*, these are an important diseases of wheat (Burmeister and Plattner, 1987; Bushnel *et al*., 2003; Bai and Shaner, 2004; Schaafsma and LincieI, 2005; Tunali *et al*., 2006a, b; Kohl *et al*., 2007; Nedelink *et al*., 2007; Chibundu *et al*., 2008; Gargouri-Kammoun *et al*., 2009). Sugar beet is considered as the second crop for sugar in Egypt. Damping off and root rot caused by *F. lateritium*, *F. xylarioides* and *F. camptocearas* is an important disease of sugar beet (El-Kazzaz *et al*., 2008). Herbicides and fungicides are environmental pollutants, therefore, using biological control such as *T. harzianum* which is one the efficient biocontrol agents that commercially produced to prevent development of several soil pathogenic fungi (Shalini *et al*., 2006; Ozbay and Newan, 2004; Osman *et al*., 2011; Yadav *et al*., 2011). Different mechanisms have been suggested as being responsible for their bio-control activity which includes mycoparasitism, antibiosis, competition for nutrients and space and secretion of chitinolytic enzymes (Harman, 2000). Chitinase 42 kDa produced by *Trichoderma harzianum* has been proven as a prime compound to be excreted into the hyphae of the pathogen causing localized cell wall lyses at the point of interaction (Muskhazli *et al*., 2008). The most effective biocontrol agent was *Trichoderma harzianum*. Hyphal interactions between *T. harzianum* and *R. solani* were observed by scanning electron microscopy. *T. harzianum* attached to the host by hyphal coils (Tarek and Moussa, 2002). *Trichoderma harzianum* not only prolonged the metabolic activity of the entrapped organism but also it promotes slow release of microbial spores into the medium for successful enzyme production (Attitalla and Salleh, 2010). The objective of the study was to show that disease reduced more in seed coating method than soil method.

MATERIALS AND METHODS

Pathogens: *F. nivale*, *F. graminearum* and *F. tricinctum* were isolated from naturally infected wheat plants as well as *F. Lateritium*, *F. xylarioides* and *F. camptocearas* were isolated from naturally infected sugar beet plants and showing damping off and root rot symptoms on cultivated plants in Assiut Governorate, Egypt. Isolation technique was carried out according to Galal and Hefnawy (2002). Isolated fungi were purified using single spore and hyphal tip protein patterns technique and identified according to descriptions in the manual of the isolated fungi were identified on the basis of morphological and culture characteristic according to Nelson *et al*. (1983), Booth (1971), Moubasher (1993) and Leslie and Summerell (2006) and then confirmed by Assiut University Mycological Center (AUMC).

***Trichoderma* isolation:** The microorganism was isolated from rhizosphere and bulk soil of wheat fields according to Elad and Chet (1983).

Inoculum preparation of antagonistic *Trichoderma*: To produce inoculum 80 g wheat seeds, 80 g sand and 80 mL distilled water for *Trichoderma harzianum* were separately added to 500 mL Erlenmeyer flask and sterilized twice at 121°C for 20 min, with a 3 day interval between autoclaving. Flasks containing autoclaved medium were inoculated with *Trichoderma* young colonies grown on 1/4-strength PDA, respectively and incubated in light at 21-25°C, until completely colonized 10 days for soil treatment application the prepared inoculum was aired, milled and incubated until application time in ice house (Duffy *et al*., 1997) to supply suspension of *Trichoderma* spore for seed coating method 20 mL distilled water was added to flasks containing *Trichoderma* inoculums (Not milled sporulated. Stock suspension was collected into 20 mL tubes and homogenized by vibrator. Quality of the inoculum the number of propagules per gram or milliliter inoculums was set 10^6 propagules per gram of inoculums for soil treatment and 10^7 conidia on seed surface for seed coating method using colony forming unit and haemocytometer laboratorial process, respectively.

Greenhouse tests: The effect of *T. harzianum* on the incidence of damping off of wheat and sugar beet disease was carried out under the greenhouse conditions at the Assiut University during growing seasons 2010 and 2011 Salty loam soil with 1% organic matters was used. Completely randomize of designs of seed coating and soil treatment methods were accomplished in greenhouse. Sterilized pots (25 cm in diameter) were filled with sterilized clay loam soil. The soil was artificially infested with tested fungi in individually by adding infested barley grains medium (Fahim *et al*., 1981) at the rate of 5% of soil weight and mixed well the pots were maintained under greenhouse. The surface sterilized Sads1 wheat seeds and Dempoloy sugar beet seeds were coated with (*T. harzianum*) at a rate of 10^7 conidia/averagely on seed surface for seed coating method.

In soil treatment method, contaminated soil was treated with inoculums of *Trichoderma* isolates averagely 10^6 propagules per gram at the rate of 10 g kg^{-}. Five wheat seeds or five sugar beet seeds were planted in each pot at 2 cm depth.

The percentage of pre and post emergence damping off, survival seedlings and disease index for seedlings were recorded after 21 and 45 days, respectively from planting. Disease index were recorded at the end of the experiment by using the scale of 0-4 used by (Achenbach and Jeunifer, 1996). At the end of the experiment the dry weight of shoot and root was.

Evaluation of antagonistic activity of *Trichoderma* species

In dual culture technique (*in vitro*): *T. harzianum* was carried out according to Coskuntuna and Ozer (2008) by using dual culture technique. *F. nivale*, *F. graminearum*, *F. tricinctum*, *F. lateritium*, *F. xylarioides* and *F. camptocearas*, separately, on PDA medium for 7 days at 25°C. Disc (5 mm-diameter) from each bio-control fungus was inoculated on surface of PDA medium in side of petri dish. A disc (5 mm-diameter) of *F. nivale, F. graminearum*, *F. tricinctum*, *F. lateritium*, *F. xylarioides* and *F. camptocearas*, separately was inoculated at equal distance of the opposite side of petri dish. Petri dishes were inoculated with each pathogenic fungus only as control. Three Petri dishes for each bio-control-pathogenic fungus treatment as well as the control were used as replicates. The inoculated Petri dishes were incubated at 25°C at 7 days when the pathogen fungi covered the plate surface of the control treatment, then *T. harzianum* and pathogens were evaluated based on radial growth of colony of pathogen, over growth of *Trichoderma.*

Antagonistic effect of *T. harzianum* as decrease of the mycelia growth of pathogenic fungi was determined using the following formula:

$$\text{Antagonistic effect} = \frac{\text{A-B}}{\text{A}} \times 100$$

Where:

A = The diameter of mycelia growth of pathogenic fungus in control

B = The diameter of mycelia growth of pathogenic fungus with *Trichoderma* fungus

Culture filtrate (nonvolatile metabolites) and early volatile metabolites tests: Mycelia disks of each *Trichoderma* isolate grew on 1/4-strength PDA was separately inoculated into 100 mL flasks containing potato dextrose liquid and incubated at 20-29°C and 120 RPM in rotary shaker incubator for 10 days. The cultures were then filtered through 0.22 mm Millipore filters and 15 mL of these filtrates were added into sterile Erlenmeyer flasks containing 50 mL 1/4-strength PDA with 25% further agar at 45°C. After medium solidifying, mycelia disks of *F. nivale*, *F. graminearum*, *F. tricinctum*, *F. lateritium*, *F. xylarioides* and *F. camptocearas* individually agent derived from actively growing colonies were placed on one edge of medium plates and were incubated at 25±3°C (Dennis, 1971; Kucuk and Kivanc, 2003, 2004). For early volatile metabolites test, pathogen and *Trichoderma*

actively growing colonies were subculture on PDA and incubated in dark condition at 25°C. Then, opened Petri dishes containing 48 h old colony agent *F. nivale*, *F. graminearum*, *F. tricinctum*, *F. lateritium*, *F. xylarioides* and *F. camptocearas* placed on 24 h old colony of *Trichoderma* and were airtight using parafilm. Control was Petri dishes containing PDA medium. The Petri dishes were incubated in the same temperature and dark conditions (Dennis, 1971; Fiddaman and Rossall, 1993). Radial growth on pathogen was measured daily in both tests. Inhibitory percentages were calculated by above formula.

Statistical analysis: Data were subjected to statistical analysis and means were compared using LSD at test (Gomez and Gomez, 1984).

RESULTS

***In vitro* antagonistic effect of *T. harzianum* against the cause of wheat and sugar beet damping off disease:** Dual culture assays provided evidence that *T. harzianum* substantially reduced the growth of *F. nivale*, *F. graminearum* and *F. tricinctum* the causal pathogens of wheat as well as *F. lateritium*, *F. xylarioides* and *F. camptoceras* the causal pathogen of sugar beet compared with the control. The *Trichoderma harzianum* grew over and sporulated of the different *Fusarium* spp. resulting of complete degradation (Table 1-2).

Data also indicate that the *T. harzianum* and its filtrate inhibited the growth of the pathogens (*F. nivale*, *F. graminearum*, *F. tricinctum*, *F. lateritium*, *F. xylarioides* and *F. camptoceras*) (Table 3-4).

F. xylarioides and *F. graminearum* showed the highest percentage of growth reduction while the *F. lateritium* and *F. camptoceras* showed the lowest percentage of growth reduction.

Data also indicate that filtrate of *T. harzianum* significant different inhibited between (*F. nivale*, *F. graminearum*, *F. tricinctum*) the causal pathogen of damping off wheat. Data also indicate that filtrate of *T. harzianum* significant different inhibited between *F. lateritium*, *F. xylarioides* and *F. camptoceras*) the causal pathogen of damping off sugar beet.

Antagonistic effect of *T. harzianum* against the causative pathogen of wheat and sugar-beet damping off and root rot disease under greenhouse conditions: Coating wheat seeds with fungal bioagent or soil treatment with fungal bioagent resulted in different degree of protection against the causal pathogen at the seedling stage. Minimal amount of disease were observed on plants inoculated with pathogen and bioagent compared with untreated control. Science *T. harzianum* reduced the percentages of disease incidence and disease severity of *Fusarium* spp. the causal pathogen of wheat and sugar beet compared with control.

The highest percentage of seedling survival, shoot dry weight, root rot weight and the least disease severity were associated with *F. graminearum* and *F. lateritium* in two growing seasons but the lowest percentage of seedling survival, shoot dry weight, root rot weight and highest disease severity were associated with *F. tricinctum* and *F. camptoceras* in tested seeds treatment. Seed coating method was more effective than soil treatment method. Seed and soil treatment decreased seedling damping off and increased the average of shoot dry weight and root dry weight of wheat and sugar beet compared with the untreated and control). Data also indicate that *T. harzianum* reduce the percentages of pre not significantly caused by *Fusarium nivale* *F. graminearum* and *F. tricinctum* during growing two seasons. Data also indicate that *T. harzianum* reduce the percentages of disease severity significantly caused by *Fusarium nivale*, *F. graminearum* and *F. tricinctum*.

Table 1: Reaction and antifungal of *Trichoderma harzianum* on the causative agent of wheat damping off and root rot *in vitro*

Fungi	Inhibition zone
Fusarium nivale	-
Fusarium graminearum	-
Fusarium tricinctum	-

-: Over growth

Table 2: Reaction and antifungal of *Trichoderma harzianum* on the causative agent of sugar beet damping and root rot *in vitro*

Fungi	Inhibition zone
Fusarium lateritium	-
Fusarium xylarioides	-
Fusarium camptocearas	-

-: Over growth

Table 3: Effect of culture filtrates of *T. harzianum* on growth on the causative agent of wheat damping-off and root rot

Fungi	Growth reduction (%)
Fusarium nivale	29.6
Fusarium graminearum	33.3
Fusarium tricinctum	19.5

LSD 5% = 4.519

Table 4: Effect of culture filtrates of *T. harzianum* on growth on the causative agent of sugar beet damping-off and root rot

Fungi	Growth reduction (%)
Fusarium lateritium	44.4
Fusarium xylarioides	40.0
Fusarium camptoceras	33.3

LSD 5% = 0.176

Table 5: Effect of *T. harzianum* on disease incidence and disease severity of damping off and root rot of wheat and dry weight of shoot and root under green house condition during growing season 2010

	Disease incidence (%)											
	Pre		Post		Survival		Disease severity (%)		Shoot dry weigh (g)		Root dry weight (g)	
Treatment	Soil	Seed	Soil	Seed	Soil	Seed	Soil	Seed	Soil	Seed	Soil	Seed
Fusarium nivale	12	8	24	20	64	72	43	33	2.62	2.80	2.14	2.32
Fusarium graminearum	8	4	12	8	80	88	25	27	2.04	2.12	3.28	3.32
Fusarium tricinctum	20	16	20	16	60	68	43	37	0.80	1.00	1.33	1.50
Fusarium nivale+Trichoderma harzianum	12	8	12	8	76	84	20	21	4.91	4.94	3.00	3.10
Fusarium graminearum+Trichoderma harzianum	4	8	12	8	84	92	17.2	13	5.28	5.50	5.20	5.92
Fusarium tricinctum+Trichoderma harzianum	16	12	16	16	68	72	25	27	4.00	4.04	2.42	2.25
Control	0	0	0	0	100	100	0	0	8.00	8.00	7.00	7.00
LSD 5%	21.24	19.653	16.314	16.804	28.909	31.782	22.802	23.124	0.41	0.58	0.612	0.234

Table 6: Effect of *T. harzianum* on disease incidence and disease severity of damping off and root rot of wheat and dry weight of shoot and root under green house condition during growing season 2011

	Disease incidence (%)											
	Pre		Post		Survival		Disease severity (%)		Shoot dry weigh (g)		Root dry weight (g)	
Treatment	Soil	Seed	Soil	Seed	Soil	Seed	Soil	Seed	Soil	Seed	Soil	Seed
Fusarium nivale	24	16	20	16	56	68	43.0	37	2.68	2.8	2.14	2.32
Fusarium graminearum	8	4	12	8	80	88	25.0	29	2.04	2.12	3.28	3.32
Fusarium tricinctum	20	16	24	20	56	64	43.0	41	0.70	1.00	1.32	1.50
Fusarium nivale+Trichoderma harzianum	12	12	16	8	72	80	20.0	21	4.11	4.94	3.00	3.70
Fusarium graminearum Trichoderma harzianum	16	8	12	8	72	84	17.2	17	5.28	5.50	5.20	5.42
Fusarium tricinctum+Trichoderma harzianum	20	16	20	16	60	68	20.0	29	3.60	4.04	2.42	2.52
Control	0	0	0	0	100	100	0.00	0	8.00	8.00	7.00	7.00
LSD 5%	22.028	19.653	16.013	16.804	24.24	26.843	22.802	27.203	0.175	0.58	0.612	0.234

Table 7: Effect of *T. harzianum* on disease incidence and disease severity of damping off and root rot of sugar beet and dry weight of shoot and root under green house condition during growing season 2010

	Disease incidence (%)											
	Pre		Post		Survival		Disease severity (%)		Shoot dry weigh (g)		Root dry weight (g)	
Treatment	Soil	Seed	Soil	Seed	Soil	Seed	Soil	Seed	Soil	Seed	Soil	Seed
Fusarium lateritium	40	36	12	8	48	56	57	49	11	11	19	20
Fusarium xylarioides	60	56	16	12	24	32	77	68	3.6	37	9.2	9.18
Fusarium camptocearas	60	56	16	16	24	28	77	73	2.6	2.6	8.6	8.7
Fusarium lateritium+Trichoderma harzianum	28	24	8	4	64	72	41	33	14	15	39.32	39.8
Fusarium xylarioides+Trichoderma harzianum	36	32	12	8	52	60	53	45	6.14	6.2	24.8	24.9
Fusarium camptocearas+Trichoderma harzianum	40	36	8	8	52	56	53	49	3.5	3.6	9.4	9.5
Control	0	0	0	0	100	100	0	0	20	20	60	60
LSD 5%	7.631	11.814	9.785	11.032	10.349	16.314	11.128	15.401	0.32	0.345	0.33	0.289

Table 8: Effect of *T. harzianum* on disease incidence and disease severity of damping off and root rot of sugar beet and dry weight of shoot and root under green house condition during growing season 2011

	Disease incidence (%)											
	Pre		Post		Survival		Disease severity (%)		Shoot dry weigh (g)		Root dry weight (g)	
Treatment	Soil	Seed	Soil	Seed	Soil	Seed	Soil	Seed	Soil	Seed	Soil	Seed
Fusarium lateritium	36	32	12	4	52	64	51	45	12	12	20	21
Fusarium xylarioides	56	48	12	8	32	44	70	60	3.8	3.9	9.58	9.6
Fusarium camptocearas	52	48	12	12	36	40	68.2	63	2.1 8	2.8	8.8	8.8
Fusarium+lateritium Trichoderma harzianum	24	20	8	4	68	76	39	37	15	16	39.34	39.8
Fusarium xylarioides+Trichoderma harzianum	28	24	8	4	64	72	43	41	6.54	6.66	24.9	25.1
Fusarium+camptocearas Trichoderma harzianum	32	28	16	4	52	68	47	53	3.74	3.9	9.74	9.7
Control	0	0	0	0	100	100	0	0	22	22	62	63
LSD 5%	13.177	11.251	10.958	8.157	12.866	15.757	8.12	9.93	0.345	0.237	0.298	1.939

Control untreated during growing two seasons Data also indicate that *T. harzianum* reduce the percentages of pre and the percentages of disease severity significantly caused by *F. lateritium, F. xylarioides* and *F. camptoceras*. Data also indicate that *T. harzianum* increase shoot dry weight, root rot weight significantly of wheat and sugar beet compared with the untreated and control during growing two seasons (Table 5-8).

DISCUSSION

T. harzianum has been reported as the best antagonists for damping off disease caused by *Fusarium* spp. under laboratory conditions. *T. harzianum* completely overgrew on the colony of the pathogens fungi. *Trichoderma* sp. have been previously demonstrated (Howell, 2003; Harman *et al*., 2004; Harman, 2006). *T. harzianum* treatment reduced the mycelia growth of the pathogenic fungi due to the rapid growth of *T. harzianum* which colonized medium surface and substrate. These observations are similar to Kucuk and Kivan (2004) and Mir *et al.* (2011).

Results also indicated that *Trichoderma* sp. reduced the disease incidence at pre and post emergence stage in pots. These results agree with those recorded by Kohl *et al.* (2007), El-Meleigi *et al.* (2007), Hajieghrari *et al.* (2008) and Siameto *et al.* (2010).

Results based on soil treatment with the *T. harzianum* demonstrated reduction to incidence of damping off and root rot disease in wheat and sugar beet under pot conditions (Riunge *et al*., 2007; Kohl *et al*., 2007; El-Meleigi *et al*., 2007; Hajieghrari *et al*., 2008; Siameto *et al*., 2010).

Treatment with *Trichoderma* sp. gave the highly protection to wheat and sugar beet seedlings against damping of disease at post emergence stage compared with pre emergence. It is may be related to the ability of *Trichoderma* strains is mycoparasitism mediated by the production of chitinases and other cell wall-degrading enzymes (Chet, 1987; Lorito *et al*., 1996).

Treating seeds and soil with *T. harzianum* greatly increased the average of shoot dry weight and root dry weight of wheat and sugar beet as compared with the control (Zafari *et al*., 2008).

Trichoderma harzianum may be very useful fungi in biological control against cereal aggressive and toxigenic *F.* species preventing *Fusarium* mycotoxin accumulation in plant tissues my results indicated that the potential of using of *Trichoderma* competitors for the control of mycotoxin production in grain as well as to reduce the toxigenic *Fusarium* inoculums levels in cereal debris it is considered a biocontrol strategy to reduce DON levels in crops residues as previously recorded by Busko *et al.* (2007).

It is concluded that *Trichoderma* species may be very useful fungi in biological control against wheat and toxigenic *Fusarium* species to reduce their inoculums and to prevent *Fusarium* mycotoxin accumulation in plant tissues.

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