

EFFECT OF APPLICATION TIME OF THREE ISOLATES OF *Trichoderma harzianum* ON THE BIOCONTROL OF SORGHUM DAMPING-OFF

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ABSTRACT

Three isolates of *Trichoderma harzianum* i.e. Isolate 1, (354.33, Netherlands), Isolate 2 (63059, Germany) and Isolate 3 (64256, Germany) were applied to the soil at different times for the control of *Drechslera* sp., three isolates of *Fusarium moniliforme* and *Phoma* sp., the causal organisms of sorghum damping-off under greenhouse conditions. The biocontrol fungi were applied to the soil either before, at the time or after sowing. The addition of *Trichoderma* isolates one week after sowing proved to be the most effective in reducing the disease except in case of *Phoma* sp. whereas the isolates application one week before sowing was better for disease control up to 8 weeks of sowing. *Trichoderma* I2 was the most effective if added before sowing as it reduced mortality percentage from 65.9% to 39.6%. However I1 was better at the time of sowing as it reduced the disease from 48.8% to 40.4%. *Trichoderma* I3 was the most effective when applied after sowing as it reduced mortality percentage from 50% to 36.3%. Maximum shoot length was achieved by using *Trichoderma* I1 before (72.7 cm) or at the time of sowing (51.8 cm) or I2 after sowing (88.8 cm). Maximum root length was achieved by using *Trichoderma* I3 before (31.4 cm) or after sowing (21.8 cm) and I1 at the time of sowing (27.4 cm). *Trichoderma* I1 gave the highest dry weight of shoot (before and at the time of sowing) and root (after and at the time of sowing), however, *Trichoderma* I2 gave the maximum dry weight of shoot (after sowing) and root (before sowing). These results indicated that although the use of the three isolates decreased the disease from 54.9% to 44.6%, but the effectiveness of the isolates of *Trichoderma harzianum* in controlling the disease and promoting growth depends on the used isolate and the time of application.

Keywords: *T. harzianum*, sorghum, damping-off, *F. moniliforme*, *Drechslera* sp., *Phoma* sp.

INTRODUCTION

Sorghum (*Sorghum bicolor* 'L' Moench) is one of the world's major food crops. Forage sorghums are valuable warm-season annual forage crops. Many fungi were recorded as seed mycoflora of sorghum which cause seed rot and seedlings damping-off (Solanke, et al., (1997). Randhawa, et al., (1998). and Shehata et al., 2002). Biological control of damping-off diseases by using *Trichoderma harzianum* was investigated in several crops (Marshall, (1982). Lifshitz, et al., (1986). Papavizas and Lewis, 1989; Turhan and Turhan, (1989). Lewis and Papavizas, (1991). Abdel-Mageed, (1997). Nazim, et al., (1997). and Shahda, 2000). Little work has been carried out on the proper time of soil infestation with *Trichoderma* for the control of the disease. In this paper, we are concerned with: The effectiveness of three isolates of *T. harzianum* in reducing sorghum damping-off severity and the proper time for *T.harzianum* application to the soil for the control of sorghum damping-off.

MATERIALS AND METHODS

I. Pathogens and Pathogenicity tests :

The fungi used during this work except *Trichoderma* isolates were previously isolated and identified by the authors.

Pathogenicity tests were carried out under greenhouse conditions in clay pots 20 cm diameter. The tested fungi were grown on sterilized barley grains in 250 ml conical flask (50 g each) for 10 days at 25°C. Two table spoon full (10g.) infested barley grains with each of the tested fungi were added to autoclaved aerated potted soil, mixed and watered daily for 7 days. Surface sterilized sorghum grains (cv. Giza 15) with 1% sodium hypochlorite for 5 min. were sown at the rate of 10 seed/pot. Uninoculated pots were served as control. Four replicates (pots)/ treatment were used. Pots were arranged in a complete randomized block design (CRBD). Percentage of pre- and post-emergence damping-off was recorded after 15, 30 and 60 days of sowing. Data were statistically analyzed using SAS Program (SAS, 1980).

II. Biocontrol experiments:

a) In vitro tests :-

The three isolates of *Trichoderma harzianum* Rifai used in these studies were: Isolate 1 from Centraalbureau voor Schimmel cultures (CBS), Netherlands, 354. 33, Isolate 2 from Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSM), Braunschweig, Germany, 630.59, and Isolate 3 from Biologische Bundesanstalt (BBA), Berlin, Germany, 64256. Inocula 7mm discs, taken from the growing margin of the tested fungus i.e., *Fusarium moniliforme* (3 isolates), *Drechslera sp.* and *Phoma sp.* were placed at the centers on malt extract agar medium in 9 cm plastic petri dishes and incubated at 25°C for 48h. Inocula (7mm discs) taken from the growing margin of each of the three isolates *T. harzianum* cultures were transferred to the same plates at the surface edge. Such prepared plates were incubated for 8 days. Inhibition zones were measured. Four replicates were used for each treatment.

b) In vivo tests :-

Biological control experiment was carried out in greenhouse using *Drechslera sp.*, *F. moniliforme* (three isolates) and *Phoma sp.* as well as the antagonists i.e., three isolates of *T. harzianum*. Soil infestation with the tested fungi was made by growing the fungus on sterilized barley grains for 10 days at 25±2°C. Twenty cm clay pots, were filled with autoclaved aerated sandy loam soil (1kg./pot), and 10g of infested grains were added to each pot. These pots were irrigated daily to allow inculum establishment. After ten days, surface sterilized sorghum grains (with 1% sodium hypochlorite for 5 min.) were sown in each pot at the rate of 10 seeds/pot. The antagonistic fungal suspension of the different *T. harzianum* isolates were prepared by growing each isolate on malt extract broth (MEB) for 10 days at 25°C in 250 ml conical flask. Fifty g of the mycelial mat were washed then blended with 500 ml sterilized water. Twenty five ml of the hyphal/spore suspension

(14x106/ml) were added to each pot as soil drench according to the following design. Pots were first divided into three groups according to the time of application of the antagonistic isolates. In group 1, spore suspension was added one week before sowing, however, in group 2, it was added at the time of sowing, and one week after sowing in the group 3. Three check treatments were used in this experiment, the first was untreated, non-infested soil, while the second contained untreated pathogen infested soil, and the third contained treated non-infested soil. Treatments were arranged in a complete randomized block design (CRBD). Survivors of sorghum plants were recorded after 2, 4 and 8 weeks of sowing. Length of shoots, roots and fresh, dry weight were measured after 8 weeks. Data were statistically analyzed using factorial design according to SAS programe.

RESULTS AND DISCUSSION

I. Pathogenicity tests :

Data in Table 1 showed that the tested fungi (*Drechslera* sp., three isolates of *Fusarium moniliforme* and *Phoma* sp.) were pathogenic to sorghum seedlings (cv. Giza 15). *Drechslera* and *Phoma* were equal in their degree of virulence as they caused the same mortality percentage (60%). However, the three isolates of *Fusarium* were slightly different in their severity which ranged from 55-60%. The tested fungi exhibited their severity in the pre- emergence stage. Similar results were reported in Egypt by Amin, *et al.*, (1965). Fahim, *et al.*, (1982). and Shehata *et al.*, (2002). Anuja, *et al.*, (1994). reported the presence of *F. moniliforme*, *Drechslera sorghicola* and *Phoma* sp. on sorghum grains. Similar findings were reported by several inestigators (Alfredo, *et al.* (1995). Melake-Berhan, *et al.* (1996). Gonzalez, *et al.* (1997). Solanke *et al.* (1997) and Randhawa *et al.* (1998).

Table (1): Effect of soil infestation with *Drechslera* sp., three isolates of *Fusarium moniliforme* and *Phoma* sp. on sorghum surviving plants up to 8 weeks.

Treatment	Pre-emergence		Post-emergence		Survivors		% of Total Mortality	Mean
	No. of Seed-lings	% of mortality	No. of Seed-lings	% of mortality	No. of plants	% of mortality		
Control	6.5	35	6.3	2.0	6.3	0.0	37	6.33 ^a
<i>Drechslera</i> sp.	4.5	55	4.3	2.0	4.0	3.0	60	4.25 ^b
<i>Fusarium moniliforme</i> I ₁	5.5	45	4.5	10.	4.5	0.0	55	4.83 ^{ab}
<i>Fusarium moniliforme</i> I ₂	5.0	50	4.5	5.0	4.2	3.0	58	4.60 ^b
<i>Fusarium moniliforme</i> I ₃	4.3	57	4.3	0.0	4.0	3.0	60	4.17 ^b
<i>Phoma</i> sp.	4.3	57	4.0	3.0	4.0	0.0	60	4.18 ^b
Mean	5.0 ^a		4.8 ^a		4.2 ^a			

* Mean of four replicates (pots), 10 seeds each.

values within a column followed by same letter (s) are not significantly different according to Duncan's multiple range test (p ? 0.05)

II. Biocontrol experiments:

a) In vitro tests : -

The three isolates of *Trichoderma harzianum* were evaluated for their antagonistic effect against *Drechslera* sp., three isolates of *Fusarium moniliforme* and *Phoma* sp. Isolates 1 and 2 showed strong antagonistic activity where the inhibition zones ranged from 2-5mm and 1-7mm respectively. However, inhibition zones ranged from 1-2mm for isolate 3 against the tested fungi (Table 2). Sas-Piotrowska and Dorszewski, 1996 found that *T. harzianum* exhibited antagonistic activity against *Phoma* sp. and *Phoma eupyrena* in vitro. However, Leonardi, et al., 1990 showed that *Trichoderma* spp. did not inhibit *Phoma tracheiphila* growth, but completely colonized the pathogen.

Table (2): Antagonistic effect of three tested isolates of *Trichoderma harzianum* on the growth of sorghum damping-off pathogens grown on MEA medium for 8 days at 25 C°.

Pathogen	Inhibition zone (mm)*		
	<i>T. harzianum</i> I ₁	<i>T. harzianum</i> I ₂	<i>T. harzianum</i> I ₃
<i>Drechslera</i> sp.	5.0	7.0	1.0
<i>Fusarium moniliforme</i> I ₁	3.0	4.0	0.0
<i>Fusarium moniliforme</i> I ₂	2.0	2.0	0.0
<i>Fusarium moniliforme</i> I ₃	3.0	3.0	2.0
<i>Phoma</i> sp.	3.0	1.0	0.0

* Mean of four replicates (plates).

b) In vivo tests :

Data in Tables (3-6) indicate that the three isolates of *Trichoderma harzianum* have significantly decreased the percentage of sorghum damping-off disease. The effectiveness of any isolate in controlling damping-off depends on the pathogen, and the time of adding the biocontrol agents. Data in Table 3 where *Trichoderma* isolates were applied one week before sowing show that *Trichoderma* I₁, and I₃ were the most effective in controlling *Fusarium* I₁ (47.5% mortality) and *Phoma* sp. (40%), however *Trichoderma* I₁ only was more effective against *Fusarium* I₂ (55%) and F. I₃ (37.5%). *Trichoderma* I₃ was the best in controlling *Drechslera* sp. (40%). Data in Table 4 where antagonists application was at the time of sowing, indicated that *Trichoderma* I₁ and I₂ were promising for the control of *Fusarium* I₂, F. I₃ and *Drechslera* where the percentage of mortality was 35 and 35% for *Fusarium* I₂, 50 and 47.5% for both *Fusarium* I₃ and *Drechslera*. However, *Trichoderma* I₁ and I₃ were much better for the control of *Fusarium* I₁ (32.5, 37.5%) and *Phoma* (47.5, 40%). Data in Table 5 where antagonists application was carried out after sowing show that *Trichoderma* I₂ & I₃ were very effective for the control of *Fusarium* I₁ (22.5, 45%) and F. I₂ inhibited the severity of both *Drechslera* and *Phoma* as the percentage of mortality was 25% and 45% respectively.

Table (3): Effect of soil infestation with *T. harzianum* isolates one week before sowing on percentage of sorghum plants survivors grown in previously infested soil with damping-off pathogens after 2, 4 and 8 weeks of sowing.

Pathogen	Treatment	Survivors %						% of Total mortality
		After 2 weeks		After 4 weeks		After 8 weeks		
		A	B	A	B	A	B	
Control	<i>T. harzianum</i> I ₁	0.05	45.0	50.0	45.0	50.0	45.0	50.0
	<i>T. harzianum</i> I ₂	25.0	29.9	27.5	31.5	20.0	23.8	80.0
	<i>T. harzianum</i> I ₃	52.0	46.0	52.5	46.4	45.0	41.8	55.0
	Control	37.5	43.8	37.5	43.8	37.5	43.8	62.5
Drechlera sp.	<i>T. harzianum</i> I ₁	32.5	34.7	32.5	34.7	32.5	34.7	67.5
	<i>T. harzianum</i> I ₂	47.5	43.5	47.5	43.5	47.5	43.5	52.5
	<i>T. harzianum</i> I ₃	55.0	47.9	60.0	50.8	60.0	50.8	40.0
	Control	35.0	35.4	35.0	35.4	35.0	35.4	65.0
Fusarium Moniliforme I ₁	<i>T. harzianum</i> I ₁	62.5	52.3	62.5	52.3	52.5	46.4	47.5
	<i>T. harzianum</i> I ₂	50.0	45.0	50.0	45.0	50.0	45.0	50.0
	<i>T. harzianum</i> I ₃	57.5	49.4	52.5	46.4	52.5	46.4	47.5
	Control	32.5	34.7	30.0	29.9	30.0	29.9	70.0
Fusarium moniliforme I ₂	<i>T. harzianum</i> I ₁	45.0	41.8	45.0	41.8	45.0	41.8	55.0
	<i>T. harzianum</i> I ₂	25.0	29.9	25.0	29.9	25.0	29.9	75.0
	<i>T. harzianum</i> I ₃	40.0	38.4	35.0	35.4	35.0	35.4	65.0
	Control	42.5	37.3	42.5	37.3	42.5	37.3	57.5
Fusarium moniliforme I ₃	<i>T. harzianum</i> I ₁	62.5	52.3	62.5	52.3	62.5	52.3	37.5
	<i>T. harzianum</i> I ₂	57.5	49.4	57.5	49.4	57.5	49.4	42.5
	<i>T. harzianum</i> I ₃	52.5	46.4	52.5	46.4	52.5	46.4	47.5
	Control	30.0	29.9	30.0	29.9	30.0	29.9	70.0
Phoma sp.	<i>T. harzianum</i> I ₁	60.0	50.8	60.0	50.8	60.0	50.8	40.0
	<i>T. harzianum</i> I ₂	37.5	43.8	37.5	43.8	37.5	43.8	62.5
	<i>T. harzianum</i> I ₃	60.0	50.8	60.0	50.8	60.0	50.8	40.0
	Control	30.0	29.9	30.0	29.9	30.0	29.9	70.0

A = % of seedlings.

B = The angular transformation of percentage to degrees.

L.S.D. at p ? 0.05 = 7.93 (After 2 weeks, between treatments)

= 7.84 (After 4 weeks, between treatments)

= 9.64 (After 8 weeks, between pathogens)

The percentage of maximum reduction of the disease by applying *T. harzianum* isolates either before, at the time or after sowing (Table 6) reached up to 38.5, 24 and 52.4%, respectively for *Drechslera* and 32.1, 35 and 57.1% for *Fusarium* I1 & 4.3, 17.6 and 41.2% for *Fusarium* I2 and 46.4, 24.0 and 50% for *Fusarium* I3 and 42.9, 11.1 and 30.8% for *Phoma* respectively. These results indicate that addition of *Trichoderma* I3 one week after sowing gave the highest diseases reduction in case of *Drechslera* (25% mortality), *Fusarium* I2 (40%) and *Fusarium* I3 (25%), however, it should be added (or *Trichoderma* I1) before sowing for the control of *Phoma* (40%). For the best control of *Fusarium* I1 *Trichoderma* I2 should be applied after sowing as the disease was at its minimum level (22.5%).

In general, *Trichoderma* I2 was the most effective if applied before sowing (39.6% mortality), while *Trichoderma* I1 was the most effective when applied at the time of sowing (40.4 %). *Trichoderma* I3, however, was the best when applied after sowing (36.3%) (Table 6). Nazim, et al., 1997 found that application of *T. harzianum* at sowing time was more effective than that at 14 or 7 days before sowing in reducing soybean damping-off.

Data in Table 7 showed that shoot and root length were significantly affected by the time of application and the type of isolate. The highest shoot length was achieved when *Trichoderma* was applied after sowing (III – 79.9 cm). Maximum length of shoot was achieved when *Trichoderma* I1 was applied either before (72.7 cm) or at the time of sowing, (51.8 cm) or I2 when added after sowing (88.8 cm).

The highest root length was obtained when *Trichoderma* was applied one week before sowing (I-29.1 cm). For achieving maximum root length, *Trichoderma* I3 applied before (31.4 cm) or after sowing (21.8 cm) and *Trichoderma* I1 applied at the time of sowing (27.4 cm).

Table (4): Effect of soil infestation with *T. harzianum* isolates at the time of sowing on percentage of sorghum plants survivors grown in previously infested soil with damping-off pathogens grown 2, 4 and 8 weeks of sowing.

Pathogen	Treatment	Survivors %						% of Total mortality
		After 2 weeks		After 4 weeks		After 8 weeks		
		A	B	A	B	A	B	
Control	<i>T. harzianum</i> I ₁	70.0	57.7	72.5	59.1	72.5	59.1	27.5
	<i>T. harzianum</i> I ₂	47.5	43.5	47.5	43.5	45.0	41.8	55.0
	<i>T. harzianum</i> I ₃	67.5	55.3	67.5	55.3	65.0	53.8	35.0
	Control	77.5	62.1	72.5	58.9	70.0	57.7	30.0
Drechslera sp.	<i>T. harzianum</i> I ₁	52.5	46.4	52.5	46.4	50.0	45.0	50.0
	<i>T. harzianum</i> I ₂	70.0	57.7	67.5	55.3	52.5	56.4	47.5
	<i>T. harzianum</i> I ₃	75.0	60.5	72.5	58.9	37.5	43.8	62.5
	Control	52.5	46.4	50.0	45.3	37.5	43.8	62.5
Fusarium Moniliforme I ₁	<i>T. harzianum</i> I ₁	67.5	55.3	67.5	55.3	67.5	55.3	32.5
	<i>T. harzianum</i> I ₂	57.5	49.4	55.0	47.9	55.0	47.9	45.0
	<i>T. harzianum</i> I ₃	62.5	52.3	62.5	52.3	62.5	52.3	37.5
	Control	72.5	58.9	50.0	45.0	50.0	45.0	50.0
Fusarium Moniliforme I ₂	<i>T. harzianum</i> I ₁	67.5	55.3	65.0	53.8	65.0	53.8	35.0
	<i>T. harzianum</i> I ₂	67.5	55.3	67.5	55.3	65.0	53.8	35.0
	<i>T. harzianum</i> I ₃	67.5	55.3	60.0	50.8	62.5	52.3	37.5
	Control	62.5	52.3	62.5	52.3	57.5	49.4	42.5
Fusarium Moniliforme I ₃	<i>T. harzianum</i> I ₁	50.0	45.0	50.0	45.0	50.0	45.0	50.0
	<i>T. harzianum</i> I ₂	52.5	46.5	52.5	46.5	52.5	46.5	47.5
	<i>T. harzianum</i> I ₃	52.5	46.5	52.5	46.5	37.5	43.8	62.5
	Control	42.5	37.5	42.5	37.5	37.5	43.8	62.5
Phoma sp.	<i>T. harzianum</i> I ₁	60.0	50.8	52.5	46.4	52.5	46.4	47.5
	<i>T. harzianum</i> I ₂	50.0	45.3	47.5	43.6	47.5	43.6	52.5
	<i>T. harzianum</i> I ₃	62.5	52.3	57.5	49.6	60.0	52.3	40.0
	Control	57.5	49.4	57.5	49.6	55.0	47.9	45.0

A = % of seedlings.

B = The angular transformation of percentage to degrees.

L.S.D. at p ? 0.05 = 7.7 (After 8 weeks, between pathogens)

Table (5): Effect of soil infestation with *T. harzianum* isolates one week after sowing on percentage of sorghum plants survivors grown in previously infested soil with damping-off pathogens after 2, 4 and 8 weeks of sowing.

Pathogen	Treatment	Survivors %						% of Total mortality
		After 2 weeks		After 4 weeks		After 8 weeks		
		A	B	A	B	A	B	
Control	<i>T. harzianum</i> I ₁	67.5	55.3	67.5	55.3	67.5	55.3	32.5
	<i>T. harzianum</i> I ₂	67.5	55.3	65.0	53.8	57.5	49.4	42.5
	<i>T. harzianum</i> I ₃	67.5	55.3	65.0	53.8	62.5	52.3	37.5
	Control	77.5	62.1	77.5	62.1	77.5	62.1	22.5
Drechslera sp.	<i>T. harzianum</i> I ₁	60.0	50.8	57.5	49.4	55.0	47.9	45.0
	<i>T. harzianum</i> I ₂	55.0	47.9	47.5	42.5	47.5	43.5	52.5
	<i>T. harzianum</i> I ₃	82.0	65.5	77.5	62.1	75.0	60.9	25.0
	Control	47.0	43.5	47.5	43.5	47.0	43.5	52.0
Fusarium Moniliforme I ₁	<i>T. harzianum</i> I ₁	52.5	46.5	52.5	46.5	50.0	45.0	50.0
	<i>T. harzianum</i> I ₂	80.0	68.6	80.0	68.6	77.5	62.1	22.5
	<i>T. harzianum</i> I ₃	57.5	49.4	57.5	49.4	55.0	47.9	45.0
	Control	55.0	47.9	55.0	47.9	47.5	43.5	52.5
Fusarium Moniliforme I ₂	<i>T. harzianum</i> I ₁	27.5	30.9	27.5	30.9	27.5	30.9	72.5
	<i>T. harzianum</i> I ₂	57.5	49.4	57.5	49.4	55.0	47.9	45.0
	<i>T. harzianum</i> I ₃	72.5	59.4	62.5	52.3	60.0	50.8	40.0
	Control	42.5	37.3	42.5	37.3	42.5	37.3	57.5
Fusarium Moniliforme I ₃	<i>T. harzianum</i> I ₁	67.5	55.3	60.0	50.8	60.0	50.8	40.0
	<i>T. harzianum</i> I ₂	77.5	62.1	77.5	62.1	70.0	57.7	30.0
	<i>T. harzianum</i> I ₃	75.0	60.9	72.5	59.4	75.0	60.9	25.0
	Control	57.5	49.9	55.0	47.9	50.0	45.0	50.0
Phoma sp.	<i>T. harzianum</i> I ₁	42.5	37.3	37.5	43.8	35.0	38.5	65.0
	<i>T. harzianum</i> I ₂	37.5	43.8	37.5	43.8	35.0	38.5	65.0
	<i>T. harzianum</i> I ₃	55.0	47.9	55.0	47.9	55.0	47.9	45.0
	Control	35.0	38.5	37.5	43.8	35.0	38.5	65.0

A = % of seedlings.
L.S.D. at P ? 0.05 =

B = The angular transformation of percentage to degrees.

After 2 weeks : Between treatments (T.) = 8.52
 Between pathogens (P.) = 10.44
 Interaction of T. × P. = 20.93
 After 4 weeks : Between pathogens (P.) = 10.19
 Interaction of T. × P. = 20.68
 After 8 weeks : Between pathogens (P.) = 9.45
 Interaction of T. × P. = 16.27

Table (6): Effect of soil infestation with *T. harzianum* isolates at three application times on percentage of sorghum plants survivors grown in previously infested soil with damping-off pathogens.

Pathogen	Treatment	Plant survivors % (8 weeks after sowing)								
		one week before sowing			at the time of sowing			one week after sowing		
		A	B	C	A	B	C	A	B	C
Control	<i>T. harzianum</i> I ₁	50.0	45.0	50.0	72.5	59.1	27.5	67.5	55.3	32.5
	<i>T. harzianum</i> I ₂	20.0	23.8	30.0	45.0	41.8	55.0	57.5	49.3	42.5
	<i>T. harzianum</i> I ₃	45.0	41.8	55.0	65.0	53.8	35.0	62.5	52.3	37.5
	Control	37.5	43.8	62.5	70.0	57.7	30.0	77.5	62.1	22.5
	% Max. reduc.			52.0			8.3			-
Drechslera sp.	<i>T. harzianum</i> I ₁	32.5	34.7	67.5	50.0	45.0	50.0	55.0	47.9	45.0
	<i>T. harzianum</i> I ₂	47.5	43.5	52.5	52.5	56.4	47.5	47.5	43.5	52.5
	<i>T. harzianum</i> I ₃	60.0	50.8	40.0	37.5	43.8	62.5	75.0	60.9	25.0
	Control	35.0	35.4	65.0	37.5	43.8	62.5	47.0	43.5	52.5
	% Max. reduc.			38.5			24.0			52.4
Fusarium moniliforme I ₁	<i>T. harzianum</i> I ₁	52.5	52.3	47.5	67.5	55.3	32.5	50.0	45.0	50.0
	<i>T. harzianum</i> I ₂	50.0	45.0	50.0	55.0	47.9	45.0	77.5	62.1	22.5
	<i>T. harzianum</i> I ₃	52.5	46.5	47.5	62.5	52.3	37.5	55.0	47.9	45.0
	Control	30.0	29.9	70.0	50.0	45.0	50.0	47.5	43.5	52.5
	% Max. reduc.			32.1			35.0			57.1
Fusarium moniliforme I ₂	<i>T. harzianum</i> I ₁	45.0	41.8	55.0	65.0	53.8	35.0	27.5	30.9	72.5
	<i>T. harzianum</i> I ₂	25.0	29.4	75.0	65.0	53.8	35.0	55.0	47.9	45.0
	<i>T. harzianum</i> I ₃	35.0	35.4	65.0	62.5	52.3	37.5	60.0	50.8	40.0
	Control	42.5	37.3	57.5	57.5	49.4	42.5	42.5	37.3	57.5
	% Max. reduc.			4.3			17.6			41.2
Fusarium moniliforme I ₃	<i>T. harzianum</i> I ₁	62.5	52.3	37.5	50.0	45.0	50.0	60.0	50.8	40.0
	<i>T. harzianum</i> I ₂	57.5	49.4	42.5	52.5	46.5	47.5	70.0	57.1	30.0
	<i>T. harzianum</i> I ₃	52.5	46.4	47.5	37.5	43.8	62.5	75.0	60.9	25.0
	Control	30.0	29.9	70.0	37.5	43.8	62.5	50.0	45.0	50.0
	% Max. reduc.			46.4			24.0			50.0
Phoma sp.	<i>T. harzianum</i> I ₁	60.0	50.8	40.0	52.5	46.4	47.5	35.0	38.5	65.0
	<i>T. harzianum</i> I ₂	37.5	43.8	62.5	47.5	43.6	52.5	35.0	38.5	65.0
	<i>T. harzianum</i> I ₃	60.0	50.8	40.0	60.0	52.3	40.0	55.0	47.9	45.0
	Control	30.0	29.9	70.0	55.0	47.9	45.0	35.0	38.5	65.0
	% Max. reduc.			42.9			11.1			30.8
Mean	<i>T. harzianum</i> I ₁			49.2			40.4			50.8
	<i>T. harzianum</i> I ₂			39.6			47.1			42.9
	<i>T. harzianum</i> I ₃			49.1			45.8			36.3
	Control			65.8			48.8			50.0

A = % of seedlings.

B = Transformed data.

C = % of Total mortality

L.S.D at p ? 0.05 :

Times (M.) = 3.57

Treatments (T.) = 4.13

(M. x pathogen (P.) = 8.76

T x P = 10.11

T x M = 7.15

Table (7): Effect of soil infestation with *T. harzianum* isolates on shoot and root length of sorghum (cv. Giza 15) grown in previously infested soil with damping-off pathogens 8 weeks after sowing.

Pathogen	Treatment	Length (cm)*					
		Shoot			Root		
		I	II	III	I	II	III
Control	<i>T. harzianum</i> I ₁	80.0	42.3	83.3	23.8	25.5	18.5
	<i>T. harzianum</i> I ₂	51.3	44.3	82.5	21.3	21.3	19.8
	<i>T. harzianum</i> I ₃	65.8	36.5	81.3	25.0	23.5	20.5
	Control	64.8	34.5	67.0	39.8	28.3	18.8
	Mean	65.5	39.4	78.5	27.3	24.7	19.4
Drechslera sp.	<i>T. harzianum</i> I ₁	78.8	57.0	84.8	21.8	24.0	24.3
	<i>T. harzianum</i> I ₂	79.5	54.8	92.5	35.0	21.0	20.3
	<i>T. harzianum</i> I ₃	61.5	49.3	88.8	25.0	21.0	27.5
	Control	70.5	56.0	65.0	26.3	25.0	23.5
	Mean	72.6	54.3	82.8	27.0	22.8	23.9
Fusarium moniliforme I ₁	<i>T. harzianum</i> I ₁	56.0	60.3	80.5	28.8	37.5	21.5
	<i>T. harzianum</i> I ₂	81.3	64.3	88.8	29.5	37.5	20.5
	<i>T. harzianum</i> I ₃	59.8	53.5	83.3	36.8	27.0	18.0
	Control	48.0	51.3	73.8	15.3	22.2	23.3
	Mean	61.3	57.4	81.6	27.6	31.1	20.8
Fusarium moniliforme I ₂	<i>T. harzianum</i> I ₁	64.3	48.5	66.3	28.3	25.3	22.8
	<i>T. harzianum</i> I ₂	69.8	46.5	91.5	37.5	26.0	17.8
	<i>T. harzianum</i> I ₃	62.0	39.0	85.0	35.0	15.2	19.8
	Control	72.0	46.0	53.8	32.5	23.5	20.0
	Mean	67.0	45.0	74.2	33.3	17.5	20.1
Fusarium moniliforme I ₃	<i>T. harzianum</i> I ₁	79.8	60.0	77.5	24.8	21.8	23.5
	<i>T. harzianum</i> I ₂	66.3	49.5	88.8	21.5	23.5	23.0
	<i>T. harzianum</i> I ₃	67.5	51.0	85.0	33.8	25.3	22.3
	Control	63.0	37.5	71.3	33.0	16.3	20.8
	Mean	69.2	49.5	80.6	28.3	21.7	22.9
Phoma sp.	<i>T. harzianum</i> I ₁	77.5	42.5	75.3	30.0	30.0	16.8
	<i>T. harzianum</i> I ₂	66.3	50.0	88.8	28.0	29.0	19.0
	<i>T. harzianum</i> I ₃	70.8	58.8	93.8	33.0	27.5	22.5
	Control	69.8	46.5	58.8	32.3	22.0	22.5
	Mean	71.1	49.5	81.6	31.0	27.1	20.2
Mean	Mean	67.8 ^b	49.2 ^c	79.9 ^a	29.1 ^a	24.2 ^b	21.2 ^c
	<i>T. harzianum</i> I ₁	72.7	51.8	77.9	26.3	27.4	21.2
	<i>T. harzianum</i> I ₂	69.1	51.6	88.8	28.8	26.4	20.1
	<i>T. harzianum</i> I ₃	64.6	48.0	86.2	31.4	23.3	21.8
	Control	64.7	45.3	64.9	29.8	22.9	21.5
L.S.D. at p ? 0.05							
Times (M.) = 3.4				1.92			
Treatments (T.) = 4.15				-			
T. x M. = 7.19				3.8			
Pathogen (P.) x M. = 8.81				4.7			
P. x T. = -				5.4			
T. x P. x M. = -				9.4			

* Mean of four replicates (pots), 10 seed each.

I. One week before sowing. II. At the time of sowing. III. One week after sowing.

In general, *Trichoderma* isolates increased fresh and dry weight of sorghum shoot and root (Figs 1-3). The application of the isolates before sowing was the best treatment for the increase of both shoot and root dry weights (Fig 1). *Trichoderma* I1 gave the maximum dry weight of shoot (before and at the time of sowing) and root (after and at the time of sowing), however, I2 gave the highest dry weight of shoot (after sowing) and root (before sowing).

Soil infestation with the three isolates of *T. harzianum* in the presence of damping-off pathogens afforded good protection to sorghum seedlings from pre- and post- emergence damping-off. These results support the findings of Raju *et al.*, 1999 who reported the improvement of sorghum seed quality and field emergence by using *T. harzianum* in seed treatment. The different isolates were partially different in reducing the percentage of mortality according to the pathogen species or isolate and to the time of application. *T. harzianum* isolate 3 was more effective against *Drechslera*, *Fusarium* (isolate 2, 3) and *Phoma*, but not as much against *Fusarium* isolate 1. The effectiveness of one isolate of *Trichoderma* against specific fungal isolate was previously mentioned by Bellet, *et al.*, 1982 who reported that a single isolate of *Trichoderma* can be highly effective against an isolate of pathogen species, but may have only minimal effects on other isolates of the same species.

The present results showed that the best time of soil infestation with *T. harzianum* for the disease control was one week after sowing where the maximum of disease reduction was found in all the tested pathogens except in case of *Phoma* sp. However the effectiveness of using any isolate depends on the time of application it. *Trichoderma* isolate 2 (I2) is very effective when added simultaneously with the pathogens (one week before sowing), however, *T.* isolate 1 (I1) is more effective when added at the time of sowing and isolate 3 (I3) is the best if added one week after sowing. These results may reflect a possible difference between the three tested isolates in the antagonistic mechanism or in growth rate.

The use of *T. harzianum* isolates in the control of sorghum damping-off has increased the seedlings shoots and roots length and fresh and dry weight. Similar results were reported by Osman, 1986 and Change, *et al.*, 1986 who found that *T. harzianum* increased shoot fresh weight of lupin and plant elongation of chrysanthemum. Increase of shoot and root length as well as dry weight by using *T. harzianum* in soil treatment was reported by Hemeda, 1992 in potato; Ashour, 1996 in alfalfa; Abdel Mageed, 1997 in lemon and Shahda, 2000 in Tomato.



Fig. (1) : Effect of soil infestation with *T. harzianum* isolates one week before sowing on fresh and dry weight of shoot and root of sorghum plants grown in previously infested soil with damping-off pathogens after 8 weeks of sowing.

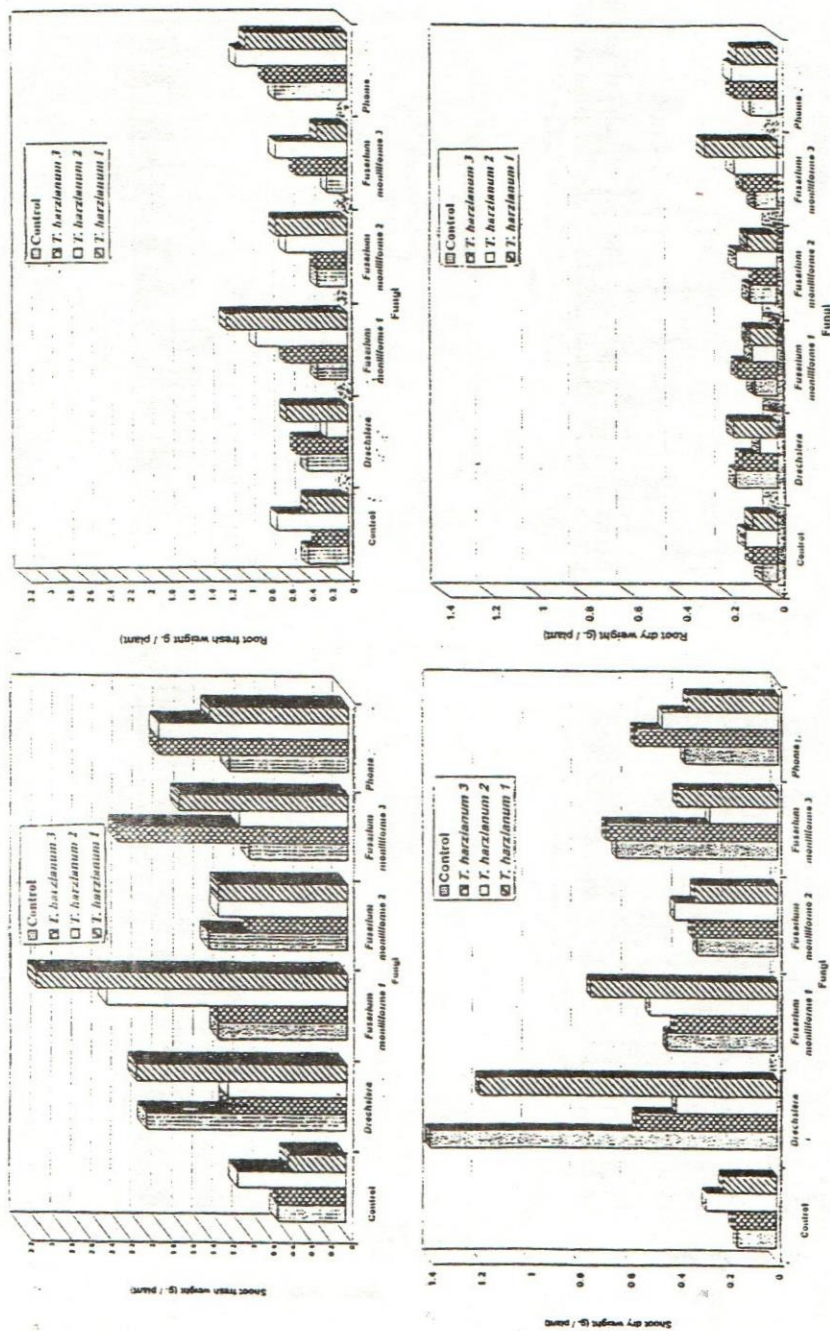


Fig. (2) : Effect of soil infestation with *T. harzianum* isolates at the time of sowing on fresh and dry weight of shoot and root of sorghum plants grown in previously infested soil with damping-off pathogens after 8 weeks of sowing.

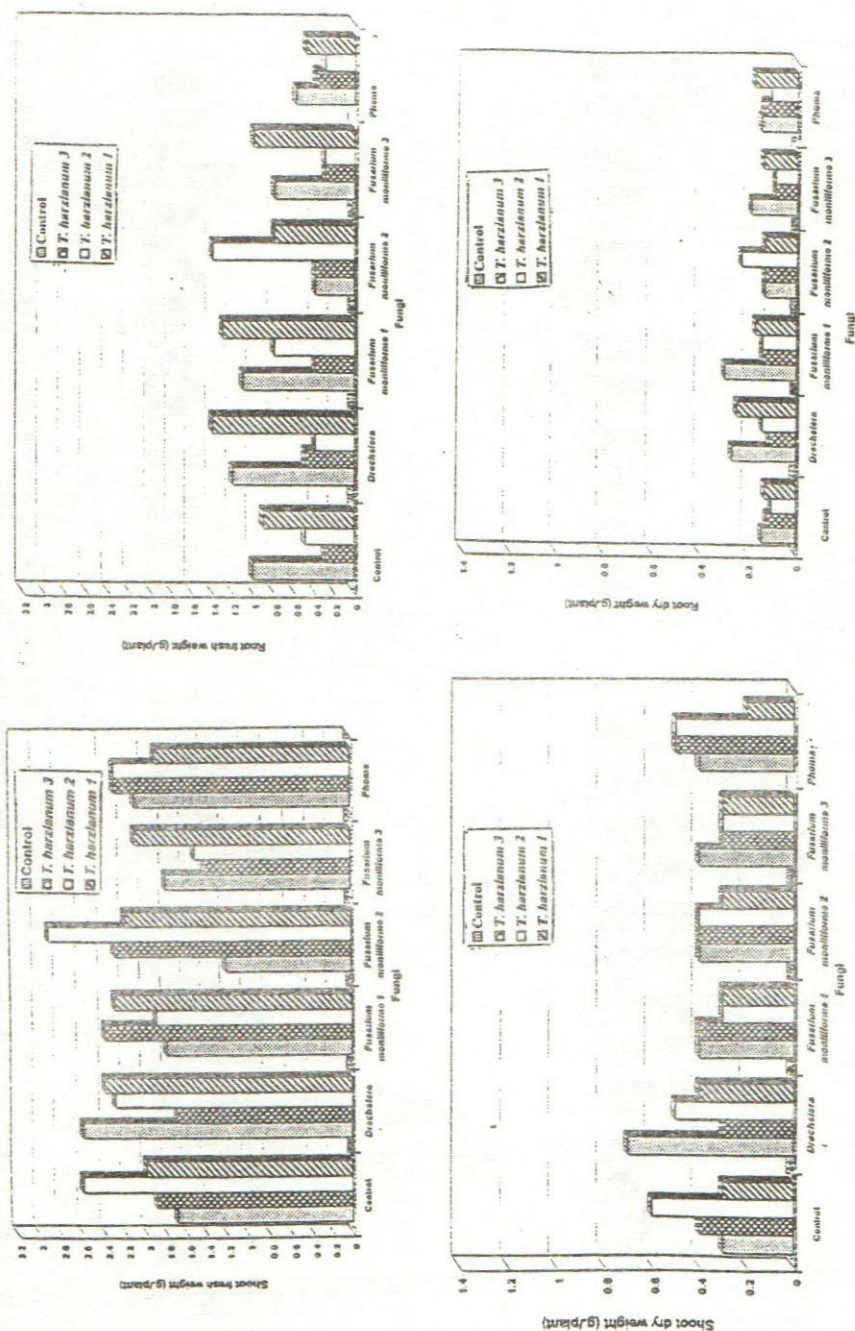


Fig. (3) : Effect of soil infestation with *T. harzianum* isolates one week after sowing on fresh and dry weight of shoot and root of sorghum plants grown in previously infested soil with damping-off pathogens after 8 weeks of sowing.

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تأثير ميعاد إضافة ثلاثة عزلات من فطر *Trichoderma harzianum* علي المقاومة الحيوية لمرض الذبول الطري في نباتات الذرة الرفيعة وفاء طاهر شهدة ، أمال حسن حميدة ، محمد ربيع عبد المعز قسم أمراض النبات - كلية الزراعة (الشاطبي) - جامعة الإسكندرية - مصر

استخدمت ثلاث عزلات من فطر *T. harzianum* وهي العزلة ١ من هولندا والعزلة ٢ ،
٣ من ألمانيا في مقاومة مرض الذبول الطري في نباتات الذرة الرفيعة والذي تسببه الفطريات
Drechslera sp. وثلاث عزلات من الفطر *Fusarium moniliforme* والفطر *Phoma sp* وذلك
تحت ظروف الصوبة الزجاجية. أضيفت الثلاث عزلات إلى التربة في ثلاثة مواعيد مختلفة وهي قبل
الزراعة بأسبوع ووقت الزراعة وبعد الزراعة بأسبوع وأخذت النتائج بعد أسبوعين ، ٤ أسابيع ، ٨ أسابيع من
الزراعة.

أوضحت النتائج أن إضافة عزلات الفطر *T. harzianum* بعد الزراعة بأسبوع كان أفضل
المواعيد حيث أدى ذلك إلى مقاومة المرض وذلك عند العدوى الصناعية بجميع الفطريات ماعدا الفطر *sp. Phoma*
والذي كانت مقاومته أفضل عند إضافة عزلات الفطر قبل الزراعة بأسبوع.
بينت النتائج أيضاً العزلة ٢ كانت أفضل العزلات في مقاومة المرض إذا أضيفت قبل الزراعة
حيث أنخفض حدوث المرض من ٦٥,٩% إلى ٣٩,٦% ، بينما كان للعزلة ١ تأثيراً كبيراً إذا أضيفت وقت
الزراعة حيث أنخفض المرض من ٤٨,٨% إلى ٤٠,٤% . أما العزلة ٣ فقد أدت إضافتها بعد الزراعة إلى
خفض نسبة المرض من ٥٠% إلى ٣٦,٣% .

أظهرت النتائج أيضاً أن إضافة العزلات الثلاثة كان له تأثيراً إيجابياً علي المجموع الخضري
والجذري فقد بلغ طول للساق باستخدام العزلة ١ ٧٢,٧ سم (عندما أضيفت قبل الزراعة) ، ٥١,٨ سم
(عندما أضيفت وقت الزراعة) أو العزلة ٢ ٨٨,٨ سم (عندما أضيفت بعد الزراعة). كما وصل طول
الجذر إلى أعلى قيمة له باستخدام العزلة ٣ فكان ٣١,٤ سم (إذا أضيفت قبل الزراعة) ، ٢١,٨ (بالإضافة بعد
الزراعة) أو العزلة ١ (٢٧,٤ سم إذا أضيفت وقت الزراعة). كذلك أعطت إضافة العزلة ١ (إذا أضيفت
قبل أو وقت الزراعة) أعلى وزن جاف للساق وللجذر (إذا أضيفت عند الزراعة أو بعده) بينما أعطت إضافة
العزلة ٢ أعلى وزن جاف للساق (بعد الزراعة) والجذر (قبل الزراعة).

مما سبق يتضح أنه على الرغم من أن العزلات الثلاثة لها تأثيراً كبيراً على مقاومة مرض الذبول
الطري في الذرة الرفيعة حيث أنخفض من ٥٤,٩% إلى ٤٤,٦% فإن فاعلية العزلات الثلاثة للفطر *T. harzianum*
في مقاومة المرض أو زيادة قوة نمو النباتات توقف على العزلة المستخدمة وكذلك على
ميعاد إضافتها إلى التربة.