SORGHUM DAMPING-OFF BY BIOCONTROL OF Gliocladium ISOLATES

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## **ABSTRACT**

Biocontrol treatment were conducted under greenhouse conditions to test the efficacy of two isolates of Gliocladium roseum [G-17, G-18] and two isolates of G. penicilloides [G-1, G-19] on controlling sorghum grain rot and seedlings damping-off [caused by Drechslera sp., Fusarium moniliforme and Phoma sp.]. Soil infestation with such biocontrol fungal isolates were applied either one week before, at the time, or one week after planting. The applications of Gliocladium isolates one week before planting significantly (p≤0.05) increased seedlings stand from 34.2% to 50.4% and reduced mortality percentage from 65.8 to 49.6%. G. roseum isolates were more effective at this time. When soil infestation was applied at the same time of planting, G-17 and G-19 gave the highest seedlings stand, however, G-18 was the best in increasing shoot and root fresh and dry weight. The application of the antagonists one week after planting, significantly (p≤0.05) increased seedlings stand from 50% to 58.8%, and decreased the percentage of mortality from 50% to 41.3%. 17 gave the maximum shoot length and 25% increase in shoot dry weight, however, isolate G-1 gave the highest seedlings stand. The results revealed that the proper time for soil treatment was one week before planting.

Keywords: Gliocladium, Sorghum, Damping-off, Drechslera, Fusarium, Phoma,

Biocontrol.

## INTRODUCTION

Fungal antagonists have been used with some success for controlling damping-off disease of plants (Shu-yen and Vaughan, 1965; Kelly, 1976; Lewis and Papavizas, 1991; Abdel-Mageed, 1997 and Shahda, 2000). Gliocladium spp. considered as effective biocontrol agents against some pathogenic fungi (Tu and Vaartaja, 1981; Howell, 1982; Lewis and Papavizas, 1985; Howell, 1986; Cipriano et al., 1990; Hemeda, 1992; Lewis et al., 1993; Hodges et al., 1994; Burgess and Hepowrth, 1996; Burgess and Keane, 1997; Lacicowa and Pieta, 1997 and Granada et al., 1999).

The antagonistic activity of Gliocladium roesum and G. penicilloides has been exploited successfully for the biological control of potato wilt disease Therefore, the attempts for testing these two species for (Hemeda, 1992). controlling sorghum damping-off were tried. The objectives of this study were : (1) Estimating the efficiency of two isolates of each of G. roseum and G. penicilloides as biocontrol agents for controlling sorghum damping-off. (2) Determination of the proper time for soil infestation with the isolates under greenhouse conditions.

### MATERIALS AND METHODS

Fungi:

Two isolates of each of *Gliocladium roseum* and *G. penicilloides* (which were previously isolated by Hemeda, 1992 from roots and rhizosphere of wilted potato plants) were used in this study. Sorghum damping-off pathogens i.e., *Drechslera* sp., three isolates of *Fusarium moniliforme* (1, 2, 3) and *Phoma* sp. were previously isolated (Shehata *et al.*, 2002) and tested for their pathogenecity (Shahda *et al.*, 2002).

#### In vitro tests:

Inocula (7mm diam discs) taken from the growing margin of each of the four isolates of *Gliocladium* cultures were transferred onto malt extract agar medium at the surface edge in 9 cm plastic petri dishes and incubated at 25°C for two days. Inocula (7mm diam discs) taken from the growing margin of the pathogenic fungal cultures i.e., *Fusarium moniliforme* (3 isolates), *Drechslera* sp. and *Phoma* sp. were placed at the centers of the same plates which containing the antagonistic fungal growth of *Gliocladium* isolates. Such prepared cultures were reincubated for 8 days. Inhibition zones between each of the pathogenic fungi and the antagonistic isolates were measured. Four replicates were used for each treatment.

#### In vivo tests:

Biological control experiment was carried out in greenhouse using Drechslera sp., Fusarium moniliforme (Three isolates) and Phoma sp. as well as the antagonists i.e. two isolates of each of Gliocladium roseum (G-17, G-18) and G. penicilloides (G-1 & G-19). Soil infestation with the pathogenic fungi was made by growing the fungus on sterilized barley grains for 10 days 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 inoculum 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 isolates of Gliocladium were prepared by growing each isolate on Malt Extract Broth (MEB) for 10 days at 25°C in 250 ml conical flask. Fifty q. of the mycelial mat were washed then blended in 500 ml sterilized water. Twenty five ml of hyphal / spore suspension (14 x 10<sup>6</sup> 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 applied one week before sowing, however in group 2, it was added at the time of sowing, and one week after sowing in group 3. Three check treatments were used in this experiment, the first contained 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 and roots, as well as fresh and dry weight were measured after 8 weeks. Data were statistically analyzed using factorial design according to SAS program (Anonymous, 1980).

# **RESULTS AND DISCUSSION**

In vitro tests:

The two isolates of each of Gliocladium roseum Bain and Gliocladium penicilloides Corda were evaluated for their antagonistic effect against Drechsler sp., three isolates of Fusarium moniliforme and Phoma sp.. Gliocladium roseum (isolate G-17) was effective against Drechslera and Fusarium I2 where the inhibition zones were 1.2 and 3.0 cm. respectively. However isolate G-18 was moderately effective against the three isolates of Fusarium and Phoma where the inhibition zones ranged from 0.20-0.40 cm... G. penicilloides (isolate G-1) was the most effective isolate against Drechslera sp. where the inhibition zone was 2.0 cm. (Table 1).

Table (1): Antagonistic effect of Gliocladium roseum and G. penicilloides isolates on the growth of damping-off pathogens of sorghum plants grown on MEA medium for 8 days at 25: C.

Inhibition zones (cm) *						
G. ro	seum	G. penicilloides				
G-17	G-18	G-1	G-19			
	0.00	2.10	0.0			
		0.00	0.4			
		0.23	0.2			
		-	0.0			
			0.3			
	G-17 1.2 0.0 3.0 0.3	G. roseum G-17 G-18 1.2 0.00 0.0 0.40 3.0 0.35	G. roseum         G. penic           G-17         G-18         G-1           1.2         0.00         2.10           0.0         0.40         0.00           3.0         0.35         0.23           0.3         0.20         0.30			

<sup>\*</sup> Mean of four replicates (plates).

This results supports the work of Whipps, (1987) who found that Fusarium oxysporum was inhibited in vitro by G. roseum and the work of Hemeda, (1992) who reported that G. penicilloides and G. roseum isolates were effective in inhibiting the growth of F. oxysporum and Verticillium alboatrum isolates in vitro

#### In vivo tests:

Data in Fig 1-c show that, the biocontrol isolates had a beneficial effect when applied to the soil before planting. Only 34.2% of plant stand were grown in untreated pathogen-infested soil (control<sub>1</sub>) compared with 37% for untreated non- infested soil (control2). Soil treatment with Gliocladium isolates increased seedling stand from 34.2 to 50.4%. Isolates G-17 and G-18 increased seedlings stand significantly higher than the untreated infested control. The use of both isolates reduced the percentage of mortality from 65.8% to 49.6% and 54.6 respectively (Table 2). Soil treatment with the antagonistic isolates before planting did not increase the length, fresh and dry weight of shoot and root which were more or less significantly similar to the control (Table 2).

When soil treatment was applied at the time of planting, isolates G-17 (G. roseum) and G-19 (G. penicilloides) gave the highest seedlings stand, although it was not significantly different than the untreated infested control (Fig. 2-c). Same isolates significantly increased shoot and root length, isolate increasing shoot, root, fresh and dry G-18 (G. roseum) was the best in

weight as compared with the control (Table 3).

The application of the *Gliocladium* isolates one week after plants revealed that G-1 (*G. penicilloides*) and G-17 (*G. roseum*) were very effective in increasing seedlings stand where G-1 was significantly different than untreated infested control and similar to the untreated non-infested control (Fig. 3-c). The same isolates were the best in decreasing percentage of mortality from 50% to 41.3% and 42.9% respectively (Table 4). Isolate G-17 gave the maximum shoot length and 25% increase in shoot dry weight.

Table (2): Effect of soil treatment with antagonistic fungione week before planting on percentage of mortality, length, fresh and dry weight of shoot and root of sorghum (cv.Giza 15) grown in previously infested soil with damping-off pathogens 8

weeks after planting.

				% Total	Shoot			Root			
Treatment		Pathogen		Lengt F.W.		D.W.	Lengt	F.W.	D.W		
				mortality	h (cm)	g/p	g/p	h (cm)	1	g/p	
		Drechslera sp.		47.5	67.0	2.44	0.66	27.5	1.05	0.21	
		Fusarium monilifo		40.0	57.00	1.86	0.41	27.3	1.25	0.20	
	G-17	Fusarium monilifo		47.5	45.5	1.19	0.28	17.5	0.37	0.07	
		Fusarium monilifo	rme I <sub>3</sub>	45.0	59.8	1.74	0.40	26.0	0.78	0.22	
13		Phoma sp.		57.5	58.8	3.55	0.87	17.3	1.11	0.28	
en		Control		60.0	71.0	5.50	2.29	23.8	2.09	0.35	
G. roseum		Oznahalaza an		55.0	56.0	2.46	0.52	15.3	1.06	0.17	
()		Drechslera sp. Fusarium monilifoi	rma I	47.5	57.8	2.24	0.32	26.3	1.63	0.17	
-	G-18	Fusarium monilifor		45.0	61.8	2.12	0.51	27.5	1.05	0.25	
	0-10	Fusarium monilifor		67.5	625	3.32	0.81	22.8	1.94	0.43	
		Phoma sp.	1110 13	45.0	51.5	2.48	0.58	16.8	1.55	0.35	
		Control		67.5	61.3	3.86	1.22	25.00	1.82	0.35	
		Drechslera sp.		67.5	65.5	4.91	0.97	26.3	3.02	0.75	
		Fusarium monilifor		50.0	55.8	1.83	0.31	28.5	0.86	0.14	
	G-1	Fusarium monilifor		45.0	43.3	0.92	0.28	18.8	0.90	0.14	
es		Fusarium monilifor	me I <sub>3</sub>	70.0	57.5	4.03	0.84	25.5	2.50	0.47	
bic		Phoma sp.		55.0	44.5	1.91	0.42	17.8	0.94	0.24	
penicilloides		Control		77.5	44.8	1.59	0.37	9.3	0.56	0.13	
ben		Drechslera sp. Fusarium monilifor	ma l	52.5 47.5	54.5 57.0	2.29	0.62	22.3	1.01	0.18	
9	G-19	Fusarium monilifor		52.5	59.3	2.91	0.65	28.0	1.09	0.23	
	0-13	Fusarium monilifor		75.0	65.3	4.99	1.17	24.3	1.79	0.56	
		Phoma sp.	ine is	55.0	73.8	3.73	0.77	23.8	1.29	0.20	
		Control		65.0	75.8	4.23	0.61	26.0	1.46	0.31	
		Drechslera sp.		65.0	70.5	4.88	1.22	26.3	1.28	0.24	
		Fusarium monilifor		70.0	48.0	1.70	0.38	15.3	0.51	0.17	
ontrol		Fusarium monilifor		57.5	72.0	3.73	0.87	32.5		0.33	
		Fusarium monilifor	me l <sub>3</sub>	70.0	63.0	3.13	0.83	33.0		0.33	
		Phoma sp.		70.0	69.8	2.74	0.71	32.3	1.01	0.26	
		Control		62.5	64.8	3.81	1.09	39.8	1.39	0.35	
Mean		G. roseum :	G-17	49.6	59.83	2.71 ª	0.82 a	23.21 b	1.11 a	0.22 ª	
		G. roseum:	G-18	54.6	58.46	2.75 a	0.68 a	22.3°	1.51 a	0.30 ª	
Carl		G. penicilloides : G	-1	60.8	51.88°	2.53	0.53 ª	21.00	1.46 ª	0.31ª	
		G. penicilloides : G		57.9	65.92	3.45 ª	0.74 ª	25.0°	1.32 ª	0.29 a	
		Control		65.8	64.67 ac	3.33 *	0.85 ª	29.8 ª	1.09 ª		

F.W.: fresh weight D.W.: dry weight g/p: gram/plant
 Means with the same letter (s) are not significantly different from each other according to L.S.D (p? 0.05).

Table (3): Effect of soil treatment with antagonistic fungi at the time of planting on percentage of mortality, length, fresh and dry weight of shoot and root of sorghum (cv.Giza 15) grown in previously infested soil with damping-off pathogens 8

Wee	ks	after	n	ant	ing.
AACC	N 3	aitti	<b>D</b>	41116	

	-	Weeks after plants			Shoot		Succession.	Root	
Trea	atment	Pathogen	% Total	Length	F.W.	D.W.	Length	F.W.	D.W.
1100	deliterit		mortality	(cm)	g/p	g/p	(cm)	g/p	g/p
		Drechslera sp.	37.5	57.3	1.94	0.56	26.0	0.54	0.14
		Fusarium moniliforme I1	47.5	58.3	2.28	0.49	26.0	1.06	0.20
	G-17	Fusarium moniliforme I <sub>2</sub>	55.0	48.8	1.51	0.52	25.5	0.70	0.13
	0 11	Fusarium moniliforme I <sub>3</sub>	50.0	53.3	2.39	0.58	30.3	1.26	0.26
		Phoma sp.	40.0	51.3	1.33	0.38	27.5	0.44	0.12
G. roseum		Control	40.0	42.5	0.77	0.18	26.0	0.39	0.10
108		Drechslera sp.	52.5	49.3	1.45	0.90	24.3	0.61	0.24
0		Fusarium moniliforme I <sub>1</sub>	72.5	58.5	4.39	1.21	39.3	4.16	0.65
	G-18	Fusarium moniliforme I2	30.0	44.0	1.03	0.43	25.3	0.29	0.09
	0 10	Fusarium moniliforme I <sub>3</sub>	55.0	61.8	2.79	0.64	25.8	0.73	0.13
		Phoma sp.	45.0	56.3	1.63	0.46	27.0	0.95	0.22
		Control	55.0	38.8	1.10	0.29	22.5	0.30	0.14
		Drechslera sp.	65.0	56.3	2.63	0.90	25.0	0.82	0.22
		Fusarium moniliforme I1	67.5	52.0	2.34	0.56	33.8	1.19	0.19
	G-1	Fusarium moniliforme I2		52.5	1.78	0.88	22.8	0.60	0.17
		Fusarium moniliforme I3		47.5	1.89	0.40	21.8	0.49	0.13
les		Phoma sp.	42.5	50.5	1.36	0.55	24.3	0.68	0.18
penicilloides		Control	40.0	40.0	0.83	0.22	24.0	0.42	0.10
enic		Drechslera sp.	45.0	53.0	1.28	0.56	25.0	0.33	0.12
G. p		Fusarium moniliforme I1	60.0	57.5	2.16	0.47	32.5	0.95	0.16
0	G-19	Fusarium moniliforme I2		53.8	1.69	0.60	21.8	0.37	0.13
		Fusarium moniliforme 13		50.0	1.07	0.28	25.8	0.42	0.08
		Phoma sp.	57.5	54.5	1.76	0.60	31.3	0.49	0.13
		Control	40.0	41.3	0.95	0.21	30.5	0.40	0.14
		Drechslera sp.	52.5	56.0	2.01	1.36	25.0	0.43	0.17
		Fusarium moniliforme I1	30.0	51.3	1.34	0.45	22.3	0.28	0.10
Contr	ol	Fusarium moniliforme I2	42.5	46.0	1.03	0.35	23.5	0.28	0.08
		Fusarium moniliforme 13		37.5	1.03	0.65	10.25	0.19	0.09
		Phoma sp.	45.0	46.5	1.21	0.38	22.0	0.68	0.11
		Control	30.0	34.5	0.69	0.16	28.3	0.47	0.08
		G. roseum : G-17	45.0	51.9 a			26.9 abc	0.73 ab	016ª
		G. roseum: G-18	51.7	51.4 ab	2.07 a	0.65 a	27.3 ab	1.17 a	0.24
Mean	1	G. penicilloides: G-1	52.9	49.8 ab	1.80 ab	0.58 ª	25.3 abc	0.70 b	0.16
		G. penicilloides: G-19	46.2	51.7 ab	1.49 b	0.45 b	27.8ª	0.49 b	0.12
		Contro	43.7	45.3 b	1.22 b	0.56 b	22.9°	0.39 b	0.10

F.W.: fresh weight D.W.: dry weight g/p: gram/plant Means with the same letter (s) are not significantly different from each other according to L.S.D (P? 0.05).

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Table (4): Effect of soil treatment with antagonistic fungi one week after of planting on percentage of mortality, length, fresh and dry weight of shoot and root of sorghum (cv.Giza 15) grown in previously infested soil with damping-off pathogens 8

weeks after planting.

					Shoot			Root	
Trea	atment	Pathogen	% Total	Length	F.W.	D.W.	Length	F.W.	D.W.
110001110111		, attroger.	mortality	(cm)	g/p	g/p	(cm)	g/p	g/p
		Drechslera sp.	27.5	90.0	2.64	0.62	26.3	0.47	0.18
		Fusarium moniliforme I <sub>1</sub>	42.5	88.8	0.98	0.47	23.5	0.48	0.12
	G-17	Fusarium moniliforme I <sub>2</sub>	42.5	96.8	2.83	0.52	21.3	0.50	0.11
	0-17	Fusarium moniliforme I <sub>3</sub>	35.0	105.0	2.95	0.55	23.5	0.51	0.17
		Phoma sp.	57.5	96.3	3.11	0.70	21.3	0.38	0.18
une		Control	52.5	83.8	1.77	0.44	21.0	0.39	0.16
G. roseum		Descholors on	42.5	112.5	2.23	0.46	21.8	0.52	0.68
(5)		Drechslera sp.	52.5	80.0	2.12	0.40	20.5	0.64	0.12
	0.40	Fusarium moniliforme I <sub>1</sub>		81.3	2.86	0.50	27.5	0.67	0.13
	G-18	Fusarium moniliforme I <sub>2</sub>	62.5	50.5	4.77	0.91	24.8	0.74	0.23
		Fusarium moniliforme I <sub>3</sub>	72.5	90.0	5.01	0.82	23.8	0.64	0.24
		Phoma sp.	62.5	75.0	2.67	0.36	26.0	0.99	0.17
		Control	62.5	75.0	2.01	0.50	20.0		
		Drechslera sp.	45.0	71.3	2.63	0.50	30.0	0.55	0.16
		Fusarium moniliforme I1	37.5	85.0	1.67	0.32	20.5	0.34	0.11
	G-1	Fusarium moniliforme l2		76.3	1.80	0.35	18.5	0.77	0.22
		Fusarium moniliforme I <sub>3</sub>		86.3	1.62	0.39	23.8	0.23	0.13
les		Phoma sp.	52.5	77.5	1.78	0.43	18.8	0.25	0.12
G. penicilloides		Control	52.5	77.5	2.13	0.33	19.3	0.38	0.10
enic		Drechslera sp.	60.0	93.8	2.49	0.60	24.3	0.54	0.21
De		Fusarium moniliforme I <sub>1</sub>	1	97.5	4.54	0.71	24.8	2.84	0.31
0	G-19	Fusarium moniliforme I <sub>2</sub>		87.5	3.22	0.71	29.5	1.11	0.36
	G-13	Fusarium moniliforme I <sub>3</sub>		57.5	2.54	0.48	19.5	0.81	0.22
		Phoma sp.	67.5	48.8	1.72	0.47	16.5	0.55	0.21
		Control	45.0	65.0	1.21	0.36	19.3	0.29	0.11
		Drechslera sp.	52.5	65.0	2.61	0.68	23.5	1.15	0.27
		Fusarium moniliforme I <sub>1</sub>		73.8	1.81	0.44	23.3	1.08	0.30
Contro	ol	Fusarium moniliforme I <sub>2</sub>	The second second	53.8	1.15	0.35	20.0	0.38	0.13
		Fusarium moniliforme I <sub>3</sub>		71.3	1.77	0.41	20.8	0.82	0.19
		Phoma sp.	65.0	58.8	2.06	0.43	22.5	0.64	0.15
		Control	22.5	67.0	1.69	0.35	18.8	1.01	0.15
		G. roseum: G-17	42.9	93.4°	2.71 ab	0.55 ab	22.8ª	0.45 c	0.15
		G. roseum: G-18	58.8	81.5 ab		0.57°	24.0 a	0.70 abo	0.26
Mean		G. penicilloides : G-1	41.3	78.9 bc	1.94 cd	0.39 b	21.8ª	0.42°	0.14
		G. penicilloides : G-19	57.0	75 0 bcd	2.62 abc	0.55 ab	22.3ª	1.02 a	0.23
		Control	50.0	64.9 d	1.85 d	0.44 ab	21.5ª	0.85 ab	0.20

F.W.: fresh weight D.W.: dry weight g/p: gram/plant Means with the same letter (s) are not significantly different from each other according to L.S.D (p? 0.05).

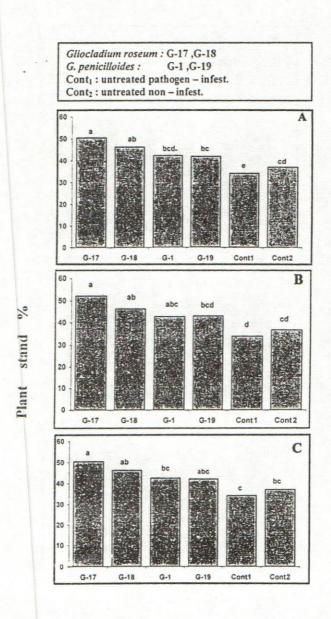


Fig. (1): Effect of soil treatment with different isolates of *Gliocladium* sp. one week before sowing on plant stand in soil previously infested with damping-off pathogens after 2 weeks (A), 4 weeks (B) and 8 weeks (C) of planting. Means with the same letter(s) are not significantly different from each other according to L.S.D ( $P \le 0.05$ ).

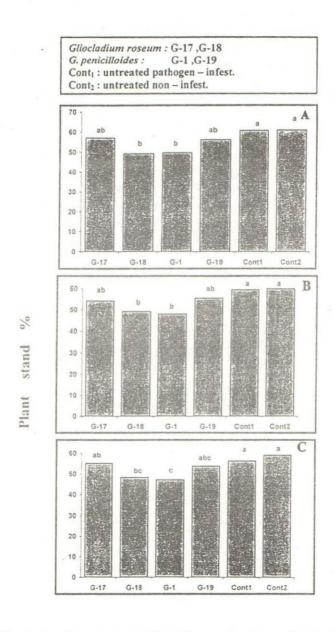


Fig. (2): Effect of soil treatment with different isolates of *Gliocladium* sp. at the time of sowing on plant stand in soil previously infested with damping-off pathogens after 2 weeks (A), 4 weeks (B) and 8 weeks (C) of planting. Means with the same letter(s) are not significantly different from each other according to L.S.D ( $P \le 0.05$ ).

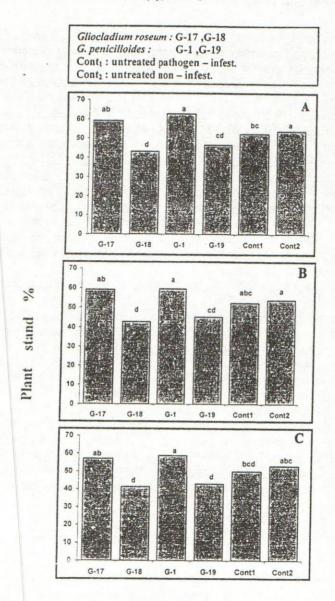


Fig. (3): Effect of soil treatment with different isolates of *Gliocladium* sp. One week after sowing on plant stand in soil previously infested with damping-off pathogens after 2 weeks (A), 4 weeks (B) and 8 weeks (C) of planting. Means with the same letter(s) are not significantly different from each other according to L.S.D (P ≤ 0.05).

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Gliocladium roseum (isolates G-17, G-18) were effective in the first time of application, however same isolates and G-19 were superior when the application was at the time of planting, while G-17, G-1 were the best when applied after planting. This result shows that G-17 was the most effective isolate followed by isolate G-18. The antagonistic effect tests in this study (Table 1) coincide with these results where G-17 showed a clear and wide inhibition zones against *Drechslera* and *Fusarium* I<sub>2</sub>. Isolates G-17 & G-18 are belonging to *G. roseum* which proved its effectiveness in controlling sorghum damping-off pathogens under this study conditions.

Application of *Gliocladium* isolates to the soil one week before planting was the proper time for the disease control. This result might be attributed to its secretion of certain substances such as antibiotics (Brian *et al.*, 1951), enzymes (Pachenari and Dix, 1980) and toxins as gliotoxin (Papavizas, 1985) more than mycoparasitism, where the addition was simultaneously with the pathogens (one week before planting) and there was

no hyphal growth for parasitism.

The present results are in agreement with the worke of Ebben and Budge (1984) who found that soil drenches with *G. roseum* gave good control against carnation wilt caused by *Fusarium oxysporum*. In greenhouse experiments, Hemeda (1992) reported that *G. roseum* and *G. penicilloides* isolates applied to the soil 10 days before planting were effective in controlling *Fusarium* and *Verticillium* wilt of potato plants and resulted in an increase in the number of healthy plants raised in soil inoculated with *Verticillium alboatrum*. Castejon-Munoz and Oyarzum (1995) reported that *G. roseum* isolates significantly reduced pea root rot severity and prevented root weight losses caused by *Fusarium solani* f. sp. *pisi* isolate 48. Lacicowa and Pieta (1997) found that *G. roseum* gave the best control in protection of pea seeds against soil pathogenic fungi. Treatment of chickpea seed with isolates of *G. roseum* reduced seedling soft rot by seed borne *Botrytis cinerea* under controlled environmental conditions and in the field (Burgess and Kean, 1997).

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المقاومة الحيوية لمرض الذبول الطرى في نباتات الذرة الرفيعة باستخدام عزلات من فطر Gliocladium

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أجريت دراسة على المقاومة الحيوية لمرض عفن الحبوب والذبول الطري لنباتات الذرة الرفيعة (والذي يسببه الفطريات .Phoma ،Fusarium moniliforme ،Drechslera sp والذي يسببه الفطر sp باستخدام عزلتين من الفطر G-17 ، G-18 ، G-17 وعزلتين من الفطر G penicilloides و هما G-17 تحت ظروف الصوبة الزجاجية.

تم معاملة التربة (المعداه مسبقا بالفطريات المسببة للمرض كل على حددة) بالعزلات المختلفة في ثلاث مواعيد مختلفة هي قبل الزراعة أو بعدها بأسبوع وكذلك وقت الزراعة وأخذت النتائج على مدي ٨ أسابيع. دلت النتائج على أن إضافة العزلات المقاومة قبل الزراعة بأسبوع أدت إلى زيادة النسبة المئوية لظهور النباتات من ٣٤,٢% إلى ٤٠٠٥% (أى نقص النسبة المئوية للمرض من ٨,٥٥٨ الى ٤٩.٦% إلى ٥٠,٤%) وكانت أكثر العزلات تقوقا هي عزلات النوع G. roseum في هذا الوقت من المعاملة.

أدت معاملة التربة بعد الزراعة بأسبوع بالعزلات المقاومة إلى زيادة نسبة ظيور النباتات من ٥٠٠ إلى ٨,٨٥٪ (تفاقص نسبة حدوث المرض من ٥٠٠ إلى ٨,٨٤٪). وقد أعطت العزلة G. roseum) أعلى طول للساق وكذلك زيادة في الوزن الجاف للساق بمقدار ٢٥٪ بينما أعطت العزلة G-1 أعلى نسبة ظهور للنباتات.

-من النتائج السابقة يمكن استخلاص أن أفضل الأوقات لإضافة هذه العزلات للتربة هـــو قبل الزراعة بأسبوع وذلك للوصول إلى أعلى درجات مقاومة المرض وزيادة قوة نمو النباتات.