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Pathological Assessment of Seed Borne Fungi Involved in Cotton Seedlings Damping-off

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ABSTRACT

To determine the involvement of seed born fungi in cotton seedlings damping-off, 39 isolates representing 13 species belonging to seven genera were isolated from seven cotton cultivars and tested on the same cultivars under greenhouse conditions. Obtained results showed that cotton seedling damping off was varied among cultivars as well as within the same cultivar. The occurrence of damping off was dependent upon cultivars responsibility and the virulence of isolate used. It was also found that some tested isolates i.e., *Fusarium moniliforme* isolate No. 11, *Fusarium semitectum* isolate No. 20, *Macrophomina phaseolina* isolate No. 25, *Penicillium* isolate No. 30 and all tested isolates of *Rhizoctonia* were capable of infecting all tested cultivars. Tested isolates of *Macrophomina phaseolina* were highly virulent against different cotton cultivars.

Key words: Cotton, seedlings, *Macrophomina* spp., *Rhizoctonia* spp., seed-borne fungi

INTRODUCTION

Cotton (*Gossypium hirsutum* L.) is the main source of natural fiber and one of the most important oil crops grown under a wide variety of conditions worldwide. It is affected by various biotic diseases that affect plant growth, causing stunting, defoliation and sometimes seedlings death. Cotton seedlings are vulnerable to disease injuries up to one month after sowing. Of these, fungal diseases are the most widespread and devastating diseases that affect crop yield quantitatively and qualitatively (Aly *et al*., 2000; Nehl *et al*., 2004).

Cotton seedling diseases are a worldwide problem; they are caused by a complex of microorganisms. Fungi are the widest pathogens affect cotton crop especially at the seedling stage causing pre or post emergence damping off (Aly *et al*., 2008). Cotton seedling diseases may lead to stand losses when the disease is not managed or environmental conditions are highly conducive for disease occurrence and development (Blasingame and Mukund, 2001; Rothrock *et al*., 2007).

Cotton seedlings damping-off, the serious problem in most of cotton producing regions often attributes to *Rhizoctonia, Pythium, Fusarium* (Disfani and Zangi, 2006; Omar *et al*., 2007). *Alternaria, Fusarium, Macrophomina, Rhizoctonia* and several other fungi were frequently isolated from cotton seeds and seedlings (Colyer and Vernon, 2005; Asran-Amal, 2007; Mikhail *et al*., 2009; Fard and Mojeni, 2011).

In addition to diseased seedlings, *Alternaria, Aspergillus* and *Diplodia* were also associated with the seed hairs and the actual seed during boll development. Palmateer *et al.* (2004) isolated 58 species of fungi belonging to 37 genera, including 9 species of *Fusarium. Fusarium oxysporum, F. solani* and *F. equiseti* were the most common members of this genus occurring at seedling stage. The effect of cotton seed borne fungi on the incidence of seedling diseases have extensively been studied (Aly *et al.*, 2004). Moreover, response of commercial cotton cultivars to some seed-borne fungi was evaluated (Abd-Elsalam *et al.*, 2007; Aly *et al.*, 2006).

The objectives of this study were to: (1) Isolate and identify seed borne fungi associated with commercial cotton seeds. (2) Evaluate the effect of isolated fungi on the incidence of cotton seedlings damping off.

MATERIALS AND METHODS

Isolation of cotton seed borne fungi: Cotton seed samples of seven cultivars; Giza-45, Giza-70, Giza-85, Giza-86, Giza-88, Giza-89 and Giza-90 obtained from Cotton Research Institute, Agri. Rec. Center, Giza Egypt in April 2011, were investigated from April to July 2011. Subsamples of 100 cotton seeds/cultivar were randomly selected for fungal isolation. Such cotton seeds were surface sterilized using 5.2% sodium hypochlorite solution for 3 minutes then washed several times in sterilized water and blotted between filter paper. Seed-borne fungi of cotton seeds was counted according to the standard blotter method (ISTA, 1999). Ten seeds of surface sterilized or non-sterilized for each treatment and cultivars were blotted on five layers of filter paper in Petri dishes and each one was replicated for each cultivar. Blotted seeds were incubated at 20±2°C for 7 days after which growing fungal colonies were examined and purified. The isolated fungi was identified according to Barnett and Hunter (1972) and then fungi occurrence percentage was calculated for each cultivars.

Inoculums preparation and soil infestation: Inoculums were raised in glass bottles (500 g in capacity), containing about 50 g wet sorghum grains per each. The bottles were autoclaved for 30 min, aseptically inoculated with the cotton seed borne fungi and incubated at 30°C until sufficient growth of the fungus was obtained after about 3-4 weeks.

Interaction between seed-borne fungi and cotton cultivars under greenhouse conditions: A total of 39 fungal isolates obtained from cotton seeds of seven cultivars (Table 1) were used in this study. Pathogenicity tests were carried out under greenhouse conditions following the soil infestation technique. Autoclaved clay loam soil was dispensed in 10 cm diameter sterilized pots, infested with the inoculums of each isolate separately at the rate of 1-50 g/pot and planted with 10 non sterilized seeds per pot for each cultivar. Pots (3 for each treatment) were randomly distributed on a greenhouse bench under temperature ranged from 23 to 37±5°C. Pre-emergence damping-off was recorded 15 days after planting, post-emergence damping-off and plant survivals were recorded 45 days after planting.

Statistical analysis of the data: Percentage data of seedlings damping-off were transformed into arc sin angles before carrying out analysis of variance (ANOVA) to normalize and stabilize variance. The Least Significant Difference (LSD) was used to identify differences. ANOVA of the data was performed with MSTAT-C statistical package (A microcomputer Program for the Design, Management and Analysis of Agronomic Research Experiments, Michigan State Univ., USA).

Table 1: Seed borne fungi used in the pathogenicity test

Fungi	Source
Alternaria alternata	Giza 45
Alternaria alternata	Giza 85
Alternaria alternata	Giza 86
Alternaria alternata	Giza 89
Alternaria alternata	Giza 90
Aspergillus flavus	Giza 86
Aspergillus flavus	Giza 88
Aspergillus flavus	Giza 89
Aspergillus fumigatus	Giza 85
Aspergillus niger	Giza 70
Fusarium moniliforme	Giza 45
Fusarium moniliforme	Giza 88
Fusarium oxysporum	Giza 45
Fusarium oxysporum	Giza 70
Fusarium oxysporum	Giza 85
Fusarium oxysporum	Giza 86
Fusarium oxysporum	Giza 89
Fusarium semitectum	Giza 86
Fusarium semitectum	Giza 86
Fusarium semitectum	Giza 89
Fusarium solani	Giza 90
Fusarium solani	Giza 90
Macrophomina phaseolina	Giza 70
Macrophomina phaseolina	Giza 86
Macrophomina phaseolina	Giza 88
Macrophomina phaseolina	Giza 90
Penicillium sp.	Giza 45
Penicillium sp.	Giza 86
Penicillium sp.	Giza 89
Penicillium sp.	Giza 90
Rhizoctonia solani	Giza 85
Rhizoctonia solani	Giza 70
Rhizoctonia solani	Giza 88
Rhizoctonia solani	Giza 89
Trichoderma harzianum	Giza 86
Trichoderma sp.	Giza 85
Trichoderma sp.	Giza 88
Trichoderma sp.	Giza 89
Trichoderma sp.	Giza 90

RESULTS AND DISCUSSION

Cotton seed borne fungi: The mean percentage of isolated fungi (Table 2) showed that *A. alternata* (19.22%), *A. flavus* (19.00%) and *A. niger* (17.43%) were the most dominant fungi associated with cotton seeds. Other fungi occurred at frequencies ranged from 1.36 to 11.86%. The predominance of *A. alternata*, *A. flavus* and *A. niger* relative to the other fungi isolated from cotton seeds, is consistent with the findings of Aly *et al.* (2011) in that *A. flavus* and *A. niger* were among the predominant fungi isolated from cotton seeds. Aly *et al.* (2004) demonstrated that *A. alternata* and *A. niger* was dominant and isolated from cotton seeds of all tested cultivars.

Table 2: Frequencies of fungi isolated from cotton seeds

Fungi	Frequencies (%)
Alternaria alternata	19.22
Aspergillus flavus	19.00
Aspergillus fumigatus	10.14
Aspergillus niger	17.43
Cladosporium spp.	11.86
Fusarium moniliforme	05.57
Fusarium oxysporum	10.93
Fusarium semitectum	03.07
Fusarium solani	02.97
Macrophomina phaseolina	01.72
Penicillium spp.	10.79
Rhizoctonia solani	06.64
Trichoderma harzianum	01.36
Trichoderma spp.	04.93
Trichothecium spp.	05.86

Table 3: Analysis of variance of the interaction between cotton cultivars and fungal isolates under greenhouse conditions

Source of variation	df	MS	F-value	p>F
Replication	2	369.098	2.1283	0.1200
Fungus (F)	39	5293.573	30.4865	0.0000
Cultivars (C)	6	4910.318	28.2793	0.0000
F×C	234	859.811	4.9518	0.0000
Error	558	173.636		

Replication is random, while each of cultivar and isolate is fixed

Interaction between seed-borne fungi and cotton cultivars: ANOVA (Table 3) showed that Fungus, cultivar and fungus x cultivar interactions were all very highly significant source of variation in cotton seedlings damping off. The highly significant F×C interaction indicates that cultivar responsibilities are different as the testing fungal species and isolates. Relative contribution (Fig. 1) indicated that fungus was the most important source of variation in cotton seedling damping off, while cultivar was the least importance. Effect of the interaction between cotton cultivars and seed borne fungi on cotton seedlings damping-off (Table 4) showed that the occurrence of damping off was dependent upon cultivar responsibilities to fungal isolates. For example while, Giza 86, Giza 89 and Giza 90 cultivars were susceptible to *F. oxysporum* isolate No. 13; Giza 45, Giza 85, Giza 88 cultivars were susceptible to *F. oxysporum* isolate No. 17. Other cultivars were infected by some isolates and non-infected by the others even within the same fungal genus. Although both of cultivars Giza 45 and Giza 70 infected by 15 from 39 of the tested fungal isolates, they exhibited the same responsibility to 10 isolates (isolates No. 7, 11, 20, 25, 26, 30, 31, 32, 33 and 34) but different responsibility to the other five isolates. While Giza 45 cultivar responds to isolates No. 2, 8, 17, 29 and 39; Giza 70 cultivar responds to isolates No. 3, 23, 24, 28 and 35. Differences in cultivar responsibilities to the causal agents of damping off disease had previously been documented and might be due to several factors (Howell *et al*., 2000; Howell, 2002; Disfani and Zangi, 2006; Abd-Elsalam *et al*., 2007).

ANOVA (Table 4) showed that isolate and isolate x cultivar interactions were highly significant as source of variation in cotton seedlings damping off caused by all tested fungi. Cultivar was

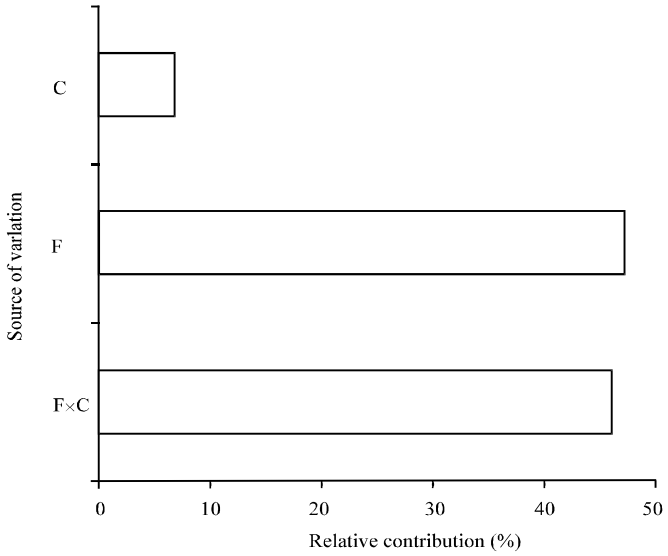

Fig. 1: Relative contribution (%) of cotton cultivars (C), fungi (F) and their interaction (F×C) to variation in infection of cotton seedlings

Table 4: Analysis of variance of the effect of the interaction between cotton cultivars and isolates of seed borne fungi on damping-off of cotton seedlings under greenhouse conditions

Fungus	Source of variation	df	MS	F-value	p>F
Alternaria	Replication	2	9.932	0.0517	-
	I	5	4982.395	25.9486	0.0000
	C	6	299.436	1.5595	0.1695
	I×C	30	1013.609	5.2789	0.0000
	Error	82	192.010		
Aspergillus	Replication	2	90.370	0.4658	-
	I	5	8728.949	44.9898	0.0000
	C	6	847.811	4.3697	0.0007
	I×C	30	1244.834	6.4160	0.0000
	Error	82	194.021		
Fusarium	Replication	2	70.760	0.3737	-
	I	12	2976.706	15.7228	0.0000
	C	6	3050.859	16.1144	0.0000
	I×C	72	822.446	4.3441	0.0000
	Error	180	189.395		
Macrophomina	Replication	2	167.110	0.7874	-
	I	4	8277.315	39.0015	0.0000
	C	6	462.916	2.1812	0.0553
	I×C	24	1086.718	5.1205	0.0000
	Error	68	212.231		
Penicillium	Replication	2	186.888	1.0932	0.3409
	I	4	6499.993	38.0228	0.0000
	C	6	458.738	2.6835	0.0213
	I×C	24	889.203	5.2015	0.0000
	Error	68	170.950		

Table 4: Continue

Fungus	Source of variation	df	MS	F-value	p>F
Rhizoctonia	Replication	2	2.492	0.1297	-
	I	4	21865.923	1137.8712	0.0000
	C	6	37.556	1.9544	0.0845
	I×C	24	37.556	1.9544	0.0165
	Error	68	19.217		
Trichoderma	Replication	2	226.102	1.3986	0.2528
	I	5	2534.870	15.6803	0.0000
	C	6	1942.425	12.0156	0.0000
	I×C	30	772.464	4.7783	0.0000
	Error	82	161.659		

I: Isolate, C: Cultivar

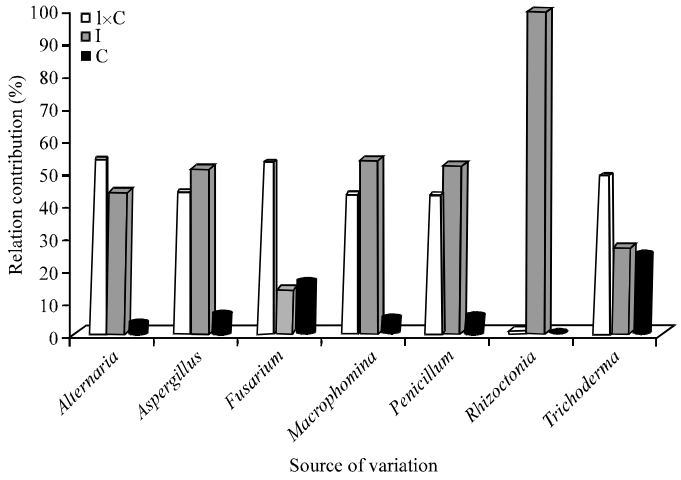

Fig. 2: Relative contribution (%) of isolates of seed-borne fungi (I), cotton cultivars (C) and their interaction (I×C) to variation in damping-off

highly significant as a source of variation in seedling damping-off resulted by *Aspergillus*, *Fusarium* and *Trichoderma* only. The highly significance of isolate x cultivar interactions indicates differences in virulence of fungal isolates or species according to tested cultivar. Relative contribution of seed borne fungi, cotton cultivars and their interaction to variation in damping-off (Fig. 2) exhibited that isolate was the most important source of variation in cotton seedling damping off caused by *Aspergillus*, *Macrophomina*, *Penicillium* and *Rhizoctonia*. On the other hand isolate x cultivar interaction was the most important source of variation in cotton seedling damping off caused by *Alternaria*, *Fusarium* and *Trichoderma*.

Cotton seedlings damping off caused by *Alternaria alternata* isolates (Table 5) varied among cultivars as well as within the same cultivar. For example; *Alternaria alternata* isolate No. 2 was pathogenic on Giza 45, Giza 68, Giza 88, Giza 89 and Giza 90; while, *Alternaria alternata* isolate No. 3 was pathogenic on Giza 70, Giza 89 and Giza 90 only. Pathogenicity of *Aspergillus* fungi was also varied from isolate to isolate among and within the same species. While *A. flavus* isolate No. 7 was pathogenic on all tested cultivars except Giza 88, *A. flavus* isolate No. 6 was pathogenic on Giza 85, Giza 89 and Giza 90 only (Table 5). It was also showed that *Penicillium* isolate No. 30 was pathogenic on all cultivars, *Penicillium* isolate No. 27 was pathogenic on Giza 85, Giza 86, Giza

Table 5: Effect of the interaction between cotton cultivars and isolates of seed-borne *Alternaria* and *Aspergillus* on damping-off of cotton seedlings under greenhouse conditions

	Giza 45		Giza 70		Giza 85		Giza 86		Giza 88		Giza 89		Giza 90	
Cotton cultivars														
Fungal isolates	%	Trans[a]	%	Trans[a]	%	Trans[a]	%	Trans[a]	%	Trans[a]	%	Trans[a]	%	Trans[a]
A. alternata	20.00	26.07	43.33	40.78	96.67	83.85**	69.67	83.85**	20.00	22.14	60.00	56.15**	56.67	48.93**
A. alternata	80.00	73.08**	23.33	28.78	40.00	38.85	50.00	45.00**	83.33	70.78**	43.33	40.78**	66.67	55.08**
A. alternata	40.00	38.85	63.33	53.36*	30.00	33.00	33.33	34.63	26.67	30.29	36.67	36.15*	36.67	37.22**
A. alternata	46.67	42.99	46.67	42.99	83.33	70.08**	50.00	45.00**	80.00	67.86**	83.33	75.00**	43.33	40.78**
A. alternata	50.00	45.00	40.00	39.23	40.00	39.15	43.33	40.78*	100.00	90.00**	90.00	78.93**	96.67	83.85**
Control	16.67	23.85	23.33	28.78	16.67	23.85	10.00	15.00	6.67	12.26	10.00	15.00	3.33	6.15
	Giza 45		Giza 70		Giza 85		Giza 86		Giza 88		Giza 89		Giza 90	
Cotton cultivars														
Fungal isolates	%	Trans[b]	%	Trans[b]	%	Trans[b]	%	Trans[b]	%	Trans[b]	%	Trans[b]	%	Trans[b]
A. flavus	30.00	31.92	23.33	28.78	56.67	49.22*	30.00	32.22	36.67	37.22	56.67	48.93**	50.00	45.08**
A. flavus	83.33	75.00**	100.00	90.00**	100.00	90.00**	93.33	60.00**	13.33	32.71	50.00	45.00**	100.00	90.00**
A. flavus	100.00	90.00**	56.67	48.85	93.33	81.15**	83.33	75.00**	60.00	51.15**	96.67	81.15**	40.00	39.15**
A. fumigatus	43.33	41.15	16.67	19.22	40.00	38.85	33.33	34.22	66.67	55.78**	43.33	41.15**	100.00	90.00**
A. niger	46.67	42.70	36.67	37.22	50.00	45.00*	63.33	53.07**	100.00	90.00**	100.00	90.00**	100.00	90.00**
Control	16.67	23.85	23.33	28.78	16.67	23.85	10.00	15.00	6.67	12.29	10.00	15.00	3.33	6.15

Values followed by (*) were significantly different at p≤0.05, [a](Trans formed data for cultivar×fungal isolate interaction = 22.51 (p≤0.05), or 29.84 (p≤0.01) throughout the data range, [b](Transformed data) for cultivar×fungal isolate interaction = 22.62 (p≤0.05) or 29.99 (p≤0.01) while they followed by (**) were significantly different at p≤0.01

Table 6: Effect of the interaction between cotton cultivars and isolates of seed-borne *Macrophomina* and *Penicillium* on damping-off of cotton seedlings under greenhouse conditions

	Giza 45		Giza 70		Giza 85		Giza 86		Giza 88		Giza 89		Giza 90	
Cotton cultivars														
Fungal isolates	%	Trans[a]	%	Trans[a]	%	Trans[a]	%	Trans[a]	%	Trans[a]	%	Trans[a]	%	Trans[a]
M. phaseolina	46.67	43.08	76.67	66.93**	93.33	81.15**	80.00	73.08**	26.67	30.99	43.33	41.07**	50.00	45.00**
M. phaseolina	23.33	23.85	80.00	73.08**	60.00	50.85*	53.33	46.92**	50.00	45.08**	46.67	42.70**	90.00	78.93**
M. phaseolina	100.00	90.00**	76.67	62.71**	86.67	72.29**	70.00	58.08**	96.67	90.00**	100.00	90.00**	46.67	42.29**
M. phaseolina	60.00	51.15*	60.00	51.15*	83.33	70.78**	33.33	35.22	100.00	90.00**	100.00	90.00**	86.67	72.78**
	Giza 45		Giza 70		Giza 85		Giza 86		Giza 88		Giza 89		Giza 90	
Cotton cultivars														
Fungal isolates	%	Trans[b]	%	Trans[b]	%	Trans[b]	%	Trans[a]	%	Trans[b]	%	Trans[b]	%	Trans[b]
Penicillium spp.	36.67	36.86	30.00	32.22	76.67	61.92**	40.00	39.15**	30.00	33.00	33.33	34.92*	53.33	47.01**
Penicillium spp.	46.67	42.99	86.67	76.92**	73.33	60.00**	60.00	50.94**	40.00	37.15**	30.00	32.71*	60.00	50.85**
Penicillium spp.	76.67	71.07**	26.69	30.78	20.00	26.07	30.00	32.22	73.33	77.71**	36.67	36.85*	73.33	59.71**
Penicillium spp.	53.33	46.92*	70.00	62.01**	50.00	45.00*	46.67	43.08**	100.00	90.00**	100.00	90.00**	100.00	90.00**
Control	16.67	23.85	23.33	28.78	16.67	23.85	10.00	15.00	6.67	12.29	10.00	15.00	3.33	6.15

Values followed by (*) were significantly different at p≤0.05, [a](Transformed data) for cultivar X fungal isolate interaction = 23.74 (p≤0.05) or 31.52 (p≤0.01), [b](Transformed data) for cultivar X fungal isolate interaction = 21.30 (p≤0.05) or 28.29 (p≤0.01) while they followed by (**) were significantly different at p≤0.01

89 and Giza 90 only (Table 6). The involvement of these fungi in the cotton seedlings damping off was discussed by Aly *et al.* (2000), who stated that these fungi are of minor importance in the etiology of cotton seedling disease. They can become pathogenic to the weakened hosts instead of living as saprophytes in the soil. Differences in virulence of such fungal isolates or species may attribute to the fungal colonization ability and competitions on the released compounds of seedling roots that stimulate germination of pathogen propagules (Howell *et al.*, 2000).

Table 7: Effect of the interaction between cotton cultivars and isolates of seed-borne *Fusarium* on damping-off of cotton seedlings under greenhouse conditions

Cotton cultivars	Giza 45		Giza 70		Giza 85		Giza 86		Giza 88		Giza 89		Giza 90	
Fungal isolates	%	Trans[a]	%	Trans[a]	%	Trans[a]	%	Trans[a]	%	Trans[a]	%	Trans[a]	%	Trans[a]
F. moniliforme	56.67	48.93*	83.33	66.15**	86.67	72.29**	73.33	59.22**	50.00	45.00**	80.00	68.85**	73.33	59.22**
F. moniliforme	20.00	26.07	40.00	39.15	40.00	39.15	16.67	19.92	73.33	59.22**	46.67	43.08**	70.00	55.78**
F. oxysporum	33.33	35.22	46.67	42.99	26.67	30.99	80.00	63.93**	30.00	33.00	40.00	38.85**	53.33	47.01**
F. oxysporum	30.00	32.71	30.00	33.00	30.00	32.71	23.33	28.78	73.33	64.92**	90.00	78.93**	80.00	68.85**
F. oxysporum	33.33	34.22	30.00	32.71	43.33	41.07	13.33	17.22	70.00	62.01**	46.67	43.08**	26.00	76.92**
F. oxysporum	20.00	21.15	46.67	42.78	76.67	66.15**	63.33	52.86**	60.00	50.85**	36.67	36.93**	63.33	53.15**
F. oxysporum	83.33	75.00**	43.33	41.07	53.33	47.22*	16.67	19.92	90.00	75.00**	76.67	68.85**	100.00	90.00**
F. semitectum	33.33	34.14	23.33	28.29	40.00	38.85	30.00	33.00	76.67	66.15**	66.67	55.08**	50.00	45.08**
F. semitectum	10.00	11.07	30.00	32.22	73.33	60.00**	13.33	17.22	56.67	49.92**	100.00	90.00**	80.00	67.86**
F. semitectum	100.00	90.00**	73.33	63.93**	96.67	83.85**	73.33	63.93**	66.67	55.37**	60.00	51.15**	43.33	41.15**
F. solani	30.00	33.00	20.00	26.07	40.00	39.15	83.33	66.64**	76.67	65.85**	86.67	72.78**	53.33	46.92**
F. solani	56.67	49.14	36.67	36.15	53.33	46.92*	26.67	30.99	100.00	90.00**	86.67	76.92**	86.67	76.92**
Control	16.67	23.85	23.33	28.78	16.67	23.85	10.00	15.00	6.67	12.29	10.00	15.00	3.33	6.15

Values followed by (*) were significantly different at $p \leq 0.05$, [a](Transformed date) for cultivar×Fungal isolate interaction = 22.17 ($p \leq 0.05$) or 29.25 ($p \leq 0.01$) while they followed by (**) were significantly different at $p \leq 0.01$

Table 7 showed that pathogenic variability was found among *Fusarium* species and isolates. For example while, *F. semitectum* isolate No. 20 was pathogenic on all cultivars *F. moniliforme* isolate No. 12 was pathogenic on Giza 88, Giza 89 and Giza 90 only. Regarding the pathogenic variability within the same species, while, *F. oxysporum* isolate No. 15 was pathogenic on Giza 88, Giza 89 and Giza 90 only, *F. oxysporum* isolate No. 16 was pathogenic on all cultivars except Giza 45 and Giza 70. Moreover, *F. moniliforme* isolate No. 11 and *F. semitectum* isolate No. 20 were capable of infecting all tested cultivars (El-Samawaty *et al*., 2008; Abd-Elsalam *et al*., 2006). Palmateer *et al*. (2004) found that *Fusarium moniliforme, F. semitectum* and *F. solani* were the most pathogenic fungi causing mortality of cotton plants. *F. semitectum* was also found to be the major seed colonizing fungus in the commercial cotton seeds (Costa *et al*., 2005).

Macrophomina phaseolina isolates were pathogenic against all tested cotton cultivars but their virulence's were varied from cultivar to another (Table 6). *M. phaseolina* isolate No. 25 was capable of infecting all tested cultivars. *Macrophomina phaseolina*, is of widespread distribution in the Egyptian soil and it is easily and frequently isolated form cotton roots (Mahmoud *et al*., 2006; Aly *et al*., 2007; Asran-Amal, 2007). *M. phaseolina* were found to be pathogenic to 11 commercial cotton cultivars which exhibited considerable variation in their response to infection (Abd-Elsalam, 2010).

Table 8 showed that both of *Trichoderma* isolates No. 35 and 36 have the same capabilities of infecting Giza 88, Giza 89 and Giza 90, moreover, isolate No. 35 was pathogenic on Giza 70 and isolate No. 36 was pathogenic on Giza 85. *Trichoderma* are free-living fungi that are common in soil and root ecosystems. They are opportunistic, avirulent, plant symbionts but at least some strains establish robust and long-lasting colonization of root surfaces penetrating into the epidermis or a few cells below this level (Harman *et al*., 2004). *Trichoderma* were previously isolated as cotton seed borne fungi by Aly *et al*. (2004). Who stated that, *Trichoderma* tended to colonize the outer seed coat so could lead to less germination damping off.

Table 8: Effect of the interaction between cotton cultivars and isolates of seed-borne *Rhizoctonia* and *Trichoderma* on damping-off of cotton seedlings under greenhouse conditions

Cotton cultivars	Giza 45		Giza 70		Giza 85		Giza 86		Giza 88		Giza 89		Giza 90	
Fungal isolates	%	Trans[a]	%	Trans[a]	%	Trans[a]	%	Trans[a]	%	Trans[a]	%	Trans[a]	%	Trans[a]
Rhizoctonia	100.00	90.00**	100.00	90.00**	100.00	90.00**	100.00	90.00**	100.00	90.00**	100.00	90.00**	100.00	90.00**
Rhizoctonia	100.00	90.00**	100.00	90.00**	100.00	90.00**	100.00	90.00**	100.00	90.00**	100.00	90.00**	100.00	90.00**
Rhizoctonia	100.00	90.00**	100.00	90.00**	100.00	90.00**	100.00	90.00**	100.00	90.00**	100.00	90.00**	100.00	90.00**
Rhizoctonia	100.00	90.00**	100.00	90.00**	100.00	90.00**	100.00	90.00**	100.00	90.00**	100.00	90.00**	100.00	90.00**
Control	16.67	23.85	23.33	28.78	16.67	23.85	10.00	15.00	6.67	12.29	10.00	15.00	3.33	6.15
Cotton cultivars	Giza 45		Giza 70		Giza 85		Giza 86		Giza 88		Giza 89		Giza 90	
Fungal isolates	%	Trans[b]	%	Trans[b]	%	Trans[b]	%	Trans[b]	%	Trans[b]	%	Trans[b]	%	Trans[b]
T. harzianum	33.33	34.63	76.67	65.85**	43.33	40.78	40.00	38.85	53.33	46.92**	36.67	36.15*	46.67	43.00**
Trichoderma sp.	23.33	27.29	20.00	26.56	50.00	45.00*	20.00	26.07	100.00	90.00**	86.67	72.29**	33.33	34.22**
Trichoderma sp.	23.33	27.29	20.00	25.36	66.67	60.00**	46.67	42.99**	56.67	49.22**	60.00	51.15**	56.67	48.85**
Trichoderma sp.	10.00	18.07	6.67	12.29	66.67	55.37**	30.00	33.00	86.67	72.78**	83.33	75.00**	10.00	18.43
Trichoderma spp.	63.33	53.15**	36.67	36.93	36.67	36.93	30.00	33.21	83.33	70.78**	46.67	43.08**	70.00	57.29**
Control	16.67	23.85	23.33	28.78	16.67	23.85	10.00	15.00	6.67	12.29	10.00	15.00	3.33	6.15

Values followed by (*) were significantly different at $p \leq 0.05$, [a](Transformed data) for cultivar fungal isolate interaction = 7.14 ($p \leq 0.05$) or NS (p≤0.0.01), [b](Transformed data) for cultivar×fungal isolate interaction = 20.65 ($p \leq 0.05$) or 27.38 ($p \leq 0.01$) while they followed by (**) were significantly different at $p \leq 0.01$

All *Rhizoctonia* isolates were capable of infecting all tested cultivars (Table 8). *Rhizoctonia solani* is a destructive fungal pathogen with a wide host range. It was found in all cotton producing areas in Egypt (Asran-Amal *et al.*, 2005). Pathogenicity of 21 *R. solani* isolates collected from different governorate in Egypt, was previously evaluated on cotton cultivar Giza 86 under greenhouse conditions. Variable levels of pre-emergence damping off were recorded and the most pathogenic 4 isolates were significantly affected all tested parameters (Mikhail *et al.*, 2009).

CONCLUSION

Cotton seed borne fungi, isolated from tested cultivars, were found to play critical role in the earlier stages of cotton crop life. Cotton seeds should be treated with effective and eco-friendly fungicidal materials prior to sowing to prevent subsequent infection with these fungi.

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