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Fungi Involved in Damping-off of Cotton Seedlings and Their Differential Pathogenicity on Two Cotton Cultivars

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FOR THIS STUDY, samples were collected from seedlings infected with damping-off or root rot in adult cotton plants from different locations in the Giza governorate. The samples yielded 25 fungal isolates. The isolates were identified as *Rhizoctonia solani* (44%), *Fusarium* spp. (44%) and *Macrophomena phasolina* (12%). In the pre-emergence stage of the cotton seedlings, 24 fungal isolates were pathogenic on cultivar Giza 90, while all fungal isolates were pathogenic on Giza94 compared to the control. On Giza90, *F. fujikuroi* F10 (80%), *R. solani* RS9 (100%), *M. phasolina* M4, and M12 (34%) were the most pathogenic isolates. On Giza 94, *F. fujikuroi* F10 (100%), *R. solani* RS9 (100%), *M. phasolina* M4 (64%) were the most pathogenic isolates. In the post-emergence stage of Giza 90, *Fusarium* isolate F1 (22%), *R. solani* RS11 (18%), *M. phasolina* M4, and M12 (48%) were the most pathogenic isolates. On Giza 94, *Fusarium* isolate F1 (16%), *R. solani* RS4 (14%), and *M. phasolina*M4 (24%) were the most pathogenic isolates. All fungal isolates were pathogenic and decreased the survival percentage of the two cultivars. On Giza 90, *F. fujikuroi* F10 (0%), *R. solani* RS9(AG2-2) (0%), and *M. phasolina* M4 (18%) were the most effective isolates in decreasing survival. On Giza 94, *F. fujikuroi* F10 (0%), *R. solani* RS9(AG2-2) (0%) group, and *M. phasolina* M4 (16%) were the most effective isolates in decreasing survival. The most effective fungal isolates that decreased plant height and dry weight for both cultivars were *F. fujikuroi* F10, RS9(AG2-2), and M4.

Keywords: Cotton seedling damping-off, *Fusarium* sp., *Macrophomena phasolina,* Pathogenicity test, *Rhizoctonia solani*.

Introduction

Egyptian cotton (*Gossypium barbadense* L.), which belongs to the Malvaceae family, is considered one of Egypt's main plants. Diseases that affect cotton seedlings are a global problem in cotton farming, and often result from a complex of soil-borne organisms, such as *Rhizoctonia solani* Kühn (Teleomorph: *Thanatephorus cucumeris* (Frank) Donk). *R. solani* is one of the most primitive members of class Basidiomycetes, and exists in its vegetative form in almost all agricultural soils (Zaki et al., 2021). In this non-spore producing stage, the fungus lives saprophytically on plant residues, but can become vigorously parasitic when roots or other parts of a suitable host enter the infested zone (Watkins, 1981). The current categorization of *R. solani* is mainly based on the gathering of isolates into anastomosis groups (AGs). Anastomosis, or the fusion of hyphae between individuals, may result in the sharing of genetic material without sexual reproduction, but additionally serves to isolate individuals that do not share the alleles for somatic compatibility (Agrios, 2005).

R. solani's pathogenicity in Egyptian cotton, has been well reported in the literature. In the preponderance of Egypt's cotton-growing zones, *R. solani* has been discovered to be the

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greatest cause of cotton damping-off (Moustafa-Mahmoud et al., 1995; Asran-Amal et al., 2005). Pathogenicity tests performed under greenhouse conditions on 39 *R. solani* AG-4 isolates and 1 AG-2-2 isolate obtained from the cotton cultivar Giza 75 showed that the majority of the virulent isolates caused pre-emergence damping-off (El-Akkad, 1997). In addition, 20 *R. solani* isolates obtained from infected cotton seedlings were found to belong to AG-5, with an additional two isolates belonging to an undetermined group. Similarly,17 *R. solani* AG-4 isolates and1 binucleate *Rhizoctonia* isolate taken from Giza 83 seedlings were found to be extremely pathogenic, causing 100% death in the pre-emergence stage (El-Samawaty, 1999).

Furthermore, of 18 *R. solani* AG-4 isolates taken from Giza 83 and Giza 86 seedlings, 10, were found to be extremely pathogenic, with more than 95% mortality in the pre-emergence stage (Asran, 2001).

Various studies have also been conducted to determine which *R. solani* isolates most commonly cause disease in cotton seedlings. In one study, a total of 52 *R. solani* isolates were obtained from cotton seedlings afflicted with post-emergence damping-off disease. Of these, AG-2-2 accounted for 13%–46%, AG-4-AGI accounted for 25%, and AG-4-4-HGII accounted for 61.54% (El-Samawaty et al., 2008). In addition, pathogenicity tests of 51 *R. solani* isolates from Giza 86 under greenhouse conditions revealed that 19 of the isolates significantly caused pre- and post-emergence damping-off, as well as reduced survival, dry weight, and plant height (Kasem, 2009). Of these 19 isolates, AG-2-2 was found to be the most common pathogenic strain, accounting for 19.61% of all the pathogenic isolates (52.63%).

In another study, 3 of the 82 *R. solani* isolates obtained from infected cotton seedling roots belonged to AG-7, while the others belonged to AG-5, AG-4, and AG-2-1(Abd-Elsalam et al., 2010). Under greenhouse conditions, pathogenicity tests revealed that AG-7 caused damping-off disease, such as seed rot, hypocotyle lesions, and root rot. When 12 *R. solani* isolates were evaluated for pathogenicity from five cotton cultivars (Giza 80, Giza 87, Giza 90, Giza 92, and Giza 93), it was found that all of the isolates were able to infect cotton seedlings and cause damping-off with varying degrees of severity (Heflish, 2020). Specifically, isolates No. R8 and R9 showed the significantly highest damping-off percentage, whereas the lowest damping-off percentage were obtained from isolates No. R11 and R7 (Heflish, 2020).

Fusarium species have also frequently been isolated from diseased cotton roots and are pathogenic (Roy & Bourland, 1982; Colyer, 1988). *Fusarium* species obtained from infected cotton roots include *F. solani*, *F. oxysporum*, *F. moniliforme*, *F. equiseti*, *F. semitectum*, and *F. graminearum*. *F. solani* has been reported to be the most virulent to cotton seedlings in Louisiana (Colyer, 1988). In Egypt, *Fusarium* spp. are often obtained from cotton seedlings infected with damping-off. For example, 97 *Fusarium* spp. were isolated from cotton seedlings naturally infected with damping-off (Jakob, 1969). These isolates were classified as *F. oxysporum* (56.7%), *F. moniliforme* (23.7%), *F. solani* (9.3%), *F. orthoceras* (5.2%), *F. scirpi* (4.1%), and other *Fusarium* spp. (1 percent). The only species capable of invading and killing cotton seedlings were *F. oxysporum* and *F. moniliforme*; the rest were categorized as wound parasites or saprophytes.

Aly et al. (1996) found *Fusarium* spp. in 97.7% of 88 tested samples taken from naturally infected cotton roots in various Egyptian governorates, and also documented that *F. oxysporum* and *F. moniliforme* were significant pathogens in the etiology of cotton damping-off in Egypt. *F. oxysporum* was significant due to its high frequency of isolation, whereas *F. moniliforme* was significant due to its high virulence.

Fusarium spp. were recovered from cotton seedlings naturally infected with post-emergence damping-off or signs of rotting roots signs in adult plants in cotton producing locations in the upper Egypt governorates (El-Samawaty, 1999), with *F. oxysporum* isolated the most (15.5%). Pathogenicity tests of 46 *Fusarium* spp. isolates obtained from the Nile Delta governorates showed that 38 of the isolates (82.4%) were pathogenic to Giza 89 cotton seedlings. Of these, there were 23 *F. oxysporum* isolates, 7 *F. moniliforme* isolates, 5 *F. solani* isolates, 2 *F. avenaceum* isolates, and 1 *F. chlamydosporum* isolate. In the pre-emergence stage, just one *F. moniliforme* isolate was not pathogenic (Abd-Elsalam, 2004). When the

pathogenic actions of eight *Fusarium* spp. were evaluated on Giza 86 and Giza90 cotton cultivars under greenhouse conditions, *F. moniliforme* and *F. oxysporum* showed high pathogenicity against Giza90 and Giza 86 cultivars, respectively (Dawoud et al., 2021).

Macrophomina phaseolina (Tassi) Goid., which causes charcoal rot (ashy stem) in cotton, is a seed-borne and soil-borne pathogen with an extensive distribution and a huge host range. Although early *M. phaseolina* infections arise at the seedlings phase in cotton, they commonly remain latent until the cotton plant reaches maturity (Dhingra & Sinclare, 1978). When *M. Phaseolina* penetrates roots or cotton stems, the colonization of internal tissues occurs quickly, causing the plant to die. Examination of the infected parts show dry rot, with some small black sclerotia dispersed all over the wood and softer tissues (Watkins, 1981).

M. phaseolina has an extensive dispersion in Egyptian soil, and is easily and commonly obtained from cotton roots, mainly through the late stage of the growing season. For example, *M. phaseolina* was obtained from 37.5% of 88 examined samples from 12 different governorates (Aly et al., 1996). A negative correlation ($r= -0.85$, $P< 0.01$) has been observed between disease incidence and yield (Turini et al., 2000). *M. phaseolina* seems to affect some cotton cultivars less seriously than others, proposing the presence of some degree of resistance to *M. phaseolina* (Watkins, 1981; Lee et al., 1985; Monga & Sheo, 1994, 2000; Turini et al., 2001).

The objective of this study was to evaluate the pathogenicity of *R. solani*, *Fusarium* spp., and *M. phaseolina* isolates collected from cotton roots to cotton seedlings in the Giza governorate.

Materials and Methods

Isolation, purification, and identification of the isolated fungi

The pathogenic fungus isolates obtained in this study were acquired from cotton seedlings with damping-off symptoms that had been collected from various places throughout the Giza governorate(Middle Egypt region). To remove any clinging soil, infected seedling roots were rinsed with tap water for 2min. First, small pieces of necrotic tissues were surface-sterilized in sodium hypochlorite (10%) for 2min, then washed four times with sterilized distilled water. The surface-sterilized pieces were then dried on sterilized filter paper. Next, small pieces (approximately 0.5cm long) of the roots were cut from the infected tissues, and five pieces from each infected seedling were placed on a potato-dextrose agar (PDA),composed of 250g potato extract; 2 0.0g dextrose; 20g agar, and 1000mL distilled water, pH 6.5–7.0. Once mixed, the medium was poured into petri dishes and amended with 0.02% streptomycin sulfate or rose bengal to suppress bacterial growth. The plates were incubated for 7 days at 28°C (Sneh et al., 1991). From these cultures, the developing fungi were recovered and purified using the single spore method for *Fusarium* spp. and the hyphal tip method for *R. solani* and *M. phasolina.* The obtained isolates were cultured and maintained on PDA slants for further study. The isolates were subjected to morphological and microscopical examination at the Cotton and the Fiber Crops Diseases Research Section, Plant Pathology Research Institute, Agriculture Research Center, Giza, Egypt, and were identified as *Rhizoctonia* sp., *Fusarium* sp. and *M. phasolina* (Gilmen, 1966; Booth 1977; Barnett & Hunter, 1979). The anastomosis RS(9) group was identified according to Windels & Nabben (1989), while F10 was identified according to Booth (1977).

Preparation of fungal inoculate

The substrate to grow the tested isolates was made in 500mL glass bottles containing 50g of sorghum grains (cv. 'Balady') and 40mL of tap water. Once mixed, the bottles were autoclaved for 2h. The secluded inoculum was aseptically placed in the bottle and allowed to colonize on the sorghum for 2 weeks after being obtained from a one-week-old culture on PDA (Aly et al., 1996).

Pathogenicity test

The pathogenicity test utilized autoclaved clay loam soil. Batches of soil were inoculated individually with each isolate's inoculum at a rate of 1g/kg of soil for *R. solani*, and 50g/kg of soil for both *Fusarium* sp. and *M. phasolina*. For all Giza 90 and Giza 94 tested cultivars, infested soil was placed into 15cm-diameter clay pots, which were subsequently sown with 10 seeds per pot. In the control treatment, sorghum grains without the inoculum (cv. Balady) were thoroughly mixed with soil at a rate of 1g/kg of soil for *R. solani*

and 50g/kg of soil for both *Fusarium* sp. and *M. phasolina*. The pots were distributed randomly on a greenhouse bench at a temperature of 28–35°C. Pre-emergence damping-off was measured 15 days after planting, and post-emergence damping-off, survival, plant height, and dry weight were measured 45 days after planting.

Statistical analysis

Data were subjected to analysis of variance (ANOVA) using MSTAT-C software. The mean differences were compared by the least significant difference (LSD) test at P≤ 0.05. Percentage data were transformed into arcsin angles or $\sqrt{x + 0.5}$ where x is the percentage data, before using ANOVA to produce the approximately constant variance.

Results and Discussion

Isolation, purification, and identification of the isolated fungi

The damping-off samples yielded 25 isolates identified as *R. solani* (44%), *Fusarium* spp. (44%), and *M. phasolina* (12%). Table 1 shows the isolation frequency of fungi from the cotton seedlings showing typical damping-off symptoms collected from the Giza governorate.

TABLE 1. Frequency percentage of fungi isolated from Giza governorate

Fungal isolates	Number	Isolation frequency (%)
Fusarium sp.	11	44
Rhizoctonia solani	11	44
Macrophomena phasolina	3	12
Total	25	100

The ANOVA tests (Table 2) showed that in post-emergence damping-off, the cultivar was a very highly significant source of variation (P= 0.00), whereas in pre-emergence damping-off and survival, the cultivar was a non-significant source of variation. The fungal isolate was a very highly significant source of variation (P= 0.00) compared to the other tested variables. The cultivar x isolates interaction was a significant source of variation (P= 0.01).

Table 3 shows the effect of some fungal isolates, cultivars, and their interactions on the pre-emergence damping-off percentage of cotton seedlings grown under greenhouse conditions. Because of the significant effect of the cultivar× treatment interaction on pre-emergence damping-off, an LSD interaction was calculated to compare the fungal isolate means within each cultivar. These studies revealed that the variations in pathogenicity between the isolates and controls differed by cultivar, indicating that the cultivars reacted differentially to the isolates. Twenty-four fungal isolates were pathogenic on Giza 90, while all fungal isolates were pathogenic on Giza 94 compared to the control. On Giza 90, *Fusarium* isolate F10 (80.00%), *R. solani* RS9 (AG2-2; 100.00%), *M. phasolina* M4, and M12 (34.00%) were the most pathogenic isolates. On Giza 94, *F. fujikuroi* F10 (100.00%), *R. solani* (AG2-2) RS9 (100.00%), and *M. phasolina* M4 (64.00%) were the most pathogenic isolates.

The observed interactions also imply that the difference between isolates may vary, depending on the cultivar tested. For instance, on Giza 90, the difference between F10 and F6 was not significant; but, on Giza 94, it was. On Giza 90, the difference between F11 and F3 was significant; however, on Giza 94, the difference was not significant. The difference between RS8 and RS4 was not significant on Giza 90, whereas it was significant on Giza 94. The difference between RS11 and RS8 was significant on Giza 90; however, the difference was non-significant on Giza 94. The difference between the M4 and M12 isolates was not significant on Giza 90, but it was significant within Giza 94.

The LSD was calculated to compare the fungal isolate means on each cultivar, given the significant effect that the cultivar treatment interactions had on post-emergence damping-off (Table 4). The variations in the post-emergence damping-off percentage between isolates and the control were not the same for each cultivar in this study, indicating that the cultivars behaved differently to the isolates. On Giza 90, the *Fusarium* isolate F1 (22.00%), *R. solani* RS11 (18.00%), *M. phasolina* M4, and M12 (48.00%) were the most pathogenic isolates. On Giza 94, *Fusarium* isolate F1 (16.00%), *R. solani* RS4 (14.00%), and *M. phasolina* M4 (24.00%) were the most pathogenic isolates.

TABLE 2. Analysis of variance of some fungal isolates, cultivars, and their interaction on certain growth variables of cotton seedlings grown under greenhouse conditions

Growth variables and sources of variation	Degrees of freedom	Mean square	*f*. value	P≥ *f*
Pre-emergence damping-off				
Replicates	4	78.03	0.44	0.78
Cultivars(V)	1	0.93	0.01	0.94
Isolates(I)	25	2397.56	13.59	0.00
V × I	25	411.60	2.33	0.01
Error	204	176.42		
Post-emergence damping-off				
Replicates	4	0.79	0.30	0.90
Cultivars(V)	1	25.60	9.62	0.00
Isolates(I)	25	10.50	3.95	0.00
V × I	25	4.85	1.82	0.01
Error	204	2.66		
Survival				
Replicates	4	78.57	0.38	0.82
Cultivars(V)	1	753.71	3.65	0.06
Isolates(I)	25	2811.76	13.61	0.00
V × I	25	413.69	2.00	0.01
Error	204	206.62		

TABLE 3. Effect of some fungal isolates, cultivars, and their interaction on pre-emergence damping-off percentages of cotton seedlings grown under greenhouse conditions

Isolates	Pre-emergence damping-off (%)					
	Giza 90		Giza 94		Mean	
	%	Transformed [a]	%	Transformed[a]	%	Transformed [a]
F1	46.00	42.47	50.00	45.00	48.00	43.73
F2	68.00	55.15	36.00	36.13	52.00	45.46
F3	64.00	56.53	46.00	45.23	55.00	50.88
F4	72.00	57.69	40.00	39.00	56.00	48.35
F5	38.00	37.62	30.00	30.18	34.00	33.90
F6	64.00	53.40	48.00	44.02	56.00	48.71
F7	40.00	39.18	64.00	53.40	52.00	46.29
F8	68.00	55.15	60.00	50.26	64.00	52.71
F9	44.00	41.54	48.00	46.98	46.00	44.26
F10	80.00	65.31	100.00	90.00	90.00	77.65
F11	34.00	35.92	56.00	48.64	45.00	42.02
RS1	68.00	55.89	44.00	41.31	56.00	48.60
RS2	80.00	65.36	84.00	74.53	82.00	69.95
RS3	72.00	57.56	60.00	51.22	66.00	54.39
RS4	54.00	47.31	30.00	32.49	42.00	39.90
RS5	66.00	53.82	44.00	38.53	55.00	46.18
RS6	52.00	46.20	56.00	48.69	54.00	47.45
RS7	66.00	54.73	62.00	52.15	64.00	53.44
RS8	70.00	56.48	50.00	45.05	60.00	50.77
RS9	100.00	90.00	100.00	90.00	100.00	90.00
RS10	40.00	36.00	62.00	52.20	51.00	44.10
RS11	36.00	36.00	64.00	52.49	50.00	44.25
M4	34.00	35.02	64.00	52.62	49.00	43.82
M6	20.00	23.78	42.00	39.69	31.00	31.74
M12	34.00	35.44	28.00	31.75	31.00	33.60
Control	4.00	5.31	0.00	0.00	2.00	2.66
Mean	54.39	47.49	52.62	47.37	53.50	47.42

LSD (P≤ 0.05) (transformed data) for isolates x cultivars= 16.63.

[a] Before performing the analysis of variance, the percentage values were transformed into arcsine angles to achieve approximately constant variance.

TABLE 4. Effect of some fungal isolates, cultivars, and their interaction on post-emergence damping-off percentages of cotton seedlings grown under greenhouse conditions

Isolates	Post -emergence damping-off (%)					
	Giza 90		Giza 94		Mean	
	%	Transformed [a]	%	Transformed [a]	%	Transformed [a]
F1	22.00	4.60	16.00	3.70	19.00	4.15
F2	18.00	3.48	8.00	2.49	13.00	2.98
F3	4.00	1.48	12.00	2.85	8.00	2.16
F4	12.00	2.94	2.00	1.22	7.00	2.08
F5	4.00	1.73	16.00	3.70	10.00	2.71
F6	2.00	1.23	6.00	2.23	4.00	1.72
F7	16.00	3.70	2.00	1.22	9.00	2.46
F8	4.00	1.72	8.00	2.49	6.00	2.10
F9	6.00	1.98	12.00	2.85	9.00	2.42
F10	20.00	4.24	0.00	0.71	11.00	2.48
F11	8.00	2.49	12.00	2.85	10.00	2.67
RS1	8.00	2.47	0.00	0.71	4.00	1.60
RS2	2.00	1.23	4.00	1.72	3.00	1.47
RS3	12.00	3.19	2.00	1.22	7.00	2.20
RS4	10.00	2.99	14.00	3.51	12.00	3.25
RS5	10.00	2.99	10.00	2.74	10.00	2.87
RS6	4.00	1.72	10.00	1.99	7.00	2.86
RS7	4.00	1.72	4.00	1.47	4.00	1.60
RS8	14.00	3.44	6.00	1.98	10.00	2.71
RS9	0.00	0.71	0.00	0.71	0.00	0.71
RS10	16.00	3.70	4.00	1.72	10.00	2.71
RS11	18.00	4.21	8.00	2.49	13.00	3.35
M4	48.00	6.85	24.00	4.83	36.00	5.84
M6	14.00	3.13	0.00	0.71	7.00	1.92
M12	4.00	1.72	4.00	1.72	4.00	1.72
Control	0.00	0.71	0.00	0.71	0.00	0.71
Mean	10.92	2.73	7.08	2.10	9.00	2.41

LSD ($P \leq 0.05$) (transformed data) for isolates × cultivars= 2.04.

[a]Before performing the analysis of variance, the percentage values were transformed into arcsine angles to achieve approximately constant variance.

These interactions also imply that the difference between isolates may vary depending on the cultivars tested. For example, on Giza 90, the difference between F1 and F4 was not significant, but, it was on Giza 94. On Giza 90, the difference between F2 and F6 was significant; however, the difference was not significant on Giza 94. On Giza 90, the difference between the RS4 and RS1 isolates was not significant, but it was on Giza 94. On Giza 90, the difference between RS11 and RS7 was significant; however, on Giza 94, it was not.

To compare the fungal isolate means on each cultivar, an interaction LSD was performed due to the significant influence of cultivar × treatment interactions on survival (Table 5). It was found that the variations in pathogenicity between the isolates and controls differed by cultivar, indicating that the cultivars reacted differentially to the isolates. All fungal isolates were pathogenic and decreased the survival percentage on both cultivars. On Giza 90, F10 (0.00%), RS9 (0.00%), and M4 (18.00%) were the most effective isolates in decreasing survival. On Giza 94, F10 (0.00%), the RS9 (0.00%) group, and M4 (16.00%) were the most effective isolates in decreasing survival. These interactions imply that the difference between isolates may vary depending on cultivar. For instance, the difference between F11 and F5 was not significant on Giza 90, but it was on Giza 94. On Giza 90, the difference

between F9 and F4 was significant; however, the difference was not significant on Giza 94. On Giza 90, the difference between RS1 and RS2 was not significant, but it was on Giza 94. On Giza 90, the difference between isolates RS10 and RS8 was significant, but it was not significant on Giza 94.

ANOVA (Table 6) showed that cultivar was a non-significant source of variation in plant height and dry weight. Fungal isolates were a very highly significant source of variation (P= 0.00) of both tested variables. Cultivar x isolate interactions were a non- significant source of variation of both tested variables.

Table 7 shows the effect of the most pathogenic fungal isolates, cultivars, and their interactions on dry weight andplant height of cotton seedlings grown under greenhouse conditions. There was no cultivar × treatment interaction on plant height and dry weight, so the general means were used to compare the isolate means. The isolates M4, F9, and Rs9 significantly reduced plant height. Rs10 increased plant height. The other isolates did not significantly affect plant height, regardless of cultivar. The difference between the general means of plant height of Giza 90 and Giza 94 was not significant, regardless of isolate. The most effective fungal isolates that decreased dry weight regardless ofcultivar were F10 (0.00g) in the *Fusarium* group, RS9 (0.00g) in the *R. solani* group, and M4 (0.20g) in the *M. phasolina* group, regardless of cultivar. The difference between the general means of the two cultivars was not significant, regardless of isolate.

TABLE 5. Effect of some fungal isolates, cultivars, and their interactions on the survival percentage of cotton seedlings grown under greenhouse conditions

	Survival (%)					
Isolates	**Giza 90**		**Giza 94**		**Mean**	
	%	**Transformed [a]**	**%**	**Transformed [a]**	**%**	**Transformed [a]**
F1	32.00	34.11	34.00	35.62	33.00	34.87
F2	16.00	20.95	58.00	49.15	37.00	35.06
F3	32.00	31.14	42.00	37.15	37.00	34.13
F4	18.00	19.15	58.00	49.84	38.00	34.50
F5	58.00	49.84	54.00	50.49	56.00	50.17
F6	34.00	35.44	46.00	42.05	40.00	38.74
F7	34.00	32.49	34.00	35.27	34.00	33.87
F8	30.00	32.48	24.00	23.49	27.00	27.99
F9	50.00	45.00	40.00	35.95	45.00	40.48
F10	0.00	0.00	0.00	0.00	0.00	0.00
F11	58.0	49.84	24.00	28.80	41.00	39.32
RS1	24.00	28.93	56.00	48.69	40.00	38.81
RS2	20.00	20.95	12.00	12.69	16.00	16.82
RS3	18.00	21.69	38.00	37.45	28.00	29.57
RS4	36.00	36.77	56.00	48.69	46.00	42.73
RS5	26.00	27.13	46.00	42.69	36.00	34.91
RS6	44.00	41.44	34.00	35.32	39.00	38.35
RS7	30.00	32.49	34.00	35.32	32.00	33.90
RS8	18.00	18.73	44.00	41.44	31.00	30.09
RS9	0.00	0.00	0.00	0.00	0.00	0.00
RS10	44.00	44.267	34.00	35.27	39.00	39.77
RS11	46.00	42.52	30.00	29.53	38.00	36.03
M4	18.00	19.38	14.00	16.85	16.00	18.11
M6	66.00	57.69	60.00	50.31	63.00	54.00
M12	62.00	52.20	68.00	55.84	65.00	54.02
Control	96.00	84.69	100.00	90.00	98.00	87.34
Mean	35.00	33.82	40.00	37.22	37.50	35.52

LSD (P≤0.05) (transformed data) for isolates × cultivars= 18.00.

[a]Before performing the analysis of variance, the percentage values were transformed into arcsine angles to achieve approximately constant variance.

TABLE 6. Analysis of variance of the most pathogenic fungal isolates, cultivars, and their interaction on plant height and dry weight of cotton seedlings grown under greenhouse conditions

Growth variables and sources of variation	D.F	Mean square	*f*. value	P≥*f*
Plant height				
Replicates	4	28.2	0.92	0.46
Cultivars(V)	1	87.61	2.83	0.10
Isolates(I)	9	819.63	26.48	0.00
V × I	9	23.44	0.76	0.66
Error	76	30.95		
Dry weight				
Replicates	4	0.29	1.46	0.22
Cultivars(V)	1	0.32	1.59	0.21
Isolates(I)	9	2.49	12.59	0.00
V× I	9	0.23	1.18	0.32
Error	76	0.20		

TABLE 7. Effect of the most pathogenic fungal isolates, cultivars, and their interactions on plant height and dry weight of cotton seedlings grown under greenhouse conditions

Isolates	Plant height (cm)			Dry weight (g)		
	Giza 90	Giza 94	Mean	Giza 90	Giza 94	Mean
M4	8.86	11.64	10.25	0.20	0.20	0.20
M6	18.42	19.46	18.94	1.15	1.18	1.17
M12	21.34	20.64	21.01	1.01	1.19	1.10
F9	16.86	12.16	14.51	0.73	0.80	0.76
F10	0.00	0.00	0.00	0.00	0.00	0.00
F11	22.04	16.14	19.09	1.33	0.44	0.89
RS9	0.00	0.00	0.00	0.00	0.00	0.00
RS10	28.38	25.86	27.12	1.10	0.93	1.02
RS11	23.68	17.20	20.44	0.80	0.76	0.78
Control	20.58	18.38	19.48	1.56	1.24	1.40
Mean	16.02	14.15	15.08	0.79	0.68	0.73

For plant height, LSD (P≤ 0.05) for isolates =4.93 and for cultivars was non- significant.
For dry weight,LSD (P≤ 0.05) for isolates =0.39 and for cultivars was non- significant.

Discussion

This fungi survey involved in cotton seedling damping-off revealed that *Fusarium* spp. (44%), *R. solani* (44%), and *M. phasolina* (12%) were the most predominant fungi involved in the disease. *Fusarium* spp. and *R. solani* were more virulent than *M. phasolina* as causal agents of the disease. Similar results were also reported by Jakob (1969), Omar (1999), Asran (2001), Mikhail et al. (2009) and Abd-Elsalam et al. (2010). In other countries where commercial cotton is *Gossypium hirsatum*, *Fusarium* spp., *R. solani*, and *M. phasolina* were also among the most commonly isolated fungi from infected seedlings (Monga & Sheo, 1994; Colyer, 1988; Aqil & Batson, 1999; Baird et al., 2004).

All commercial cotton in Egypt are *Gossypium barbadense*. Of these cottons, Giza 90 and Giza 94 were chosen for this study, since Giza 90 is the predominant cultivar in upper Egypt and Giza 94 is the predominant cotton cultivar in lower Egypt (Nile Delta). Giza 94 was slightly more susceptible to infection than Giza 90, as demonstrated in this study.

The fungi involved in cotton damping-off are non-specialized with a wide host range; therefore, rotation of cotton with other crops

is a questionable practice for controlling the disease. Commercial cotton cultivars in Egypt, as this work has demonstrated, are susceptible to damping-off because selection for damping-off resistance has not been emphasized in their development. Consequently, seed-dressing fungicides are currently the only commercially available management practice (Mohamed & Akladious, 2017; Mohamed et al., 2018). Thus, the widespread use of seed-dressing fungicides treated with effective and eco-friendly fungicidal materials for controlling cotton damping-off has become indispensable under Egyptian conditions. While effective fungicides are available (Eisa et al., 1983; Aly et al., 1992; Abdel-Aziz et al., 1996; Osman et al., 2009), it is becoming increasingly clear that their extensive use is related to a number of issues, including the latent harmful effect on non-target organisms, the development of pathogen resistant races, and the potential for carcinogenicity. Other issues include the progressive removal and phase-out of certain chemicals (Zaki et al., 1998). These issues could be solved by using cotton cultivars that are resistant to damping-off. Thus, resistance genes must be introduced into cotton cultivars in order to improve resistance. Future cotton improving strategies should include control of damping-off; however, the involvement of several pathogenic fungi from different genera may complicate efforts to develop such strategies.

Conclusion

In this study, 25 fungal isolates were obtained from infected cotton seedlings from different cotton-growing areas in the Giza governorate. The isolates were identified as *R. solani* (44%), *Fusarium* spp. (44%), and *M. phasolina* (12%). The most pathogenic fungal isolates on Giza 90 and Giza 94 were *F. fujikuroi* F10, RS9 (AG2-2), and M4.

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الفطريات المسببة لمرض موت البادرات والتباين في قدرتها المرضية على صنفين من القطن

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في هذه الدراسة جمعت من بادرات القطن المصابة بمرض موت البادرات أو نباتات بالغة مصابة بأعفان الجذور من مواقع مختلفة في محافظة الجيزة. اظهرت العينات عن وجود 25 عزلة فطرية وتم تعريف العزلات فود أنها ريزوكتونيا سولاني (44%)، فيوزاريوم (44%)، ماكروفومينا فاسولينا (12%). في مرحلة ما قبل ظهور البادرات قوق سطح التربة، كانت هناك 24 عزلة ممرضة علي الصنف جيزة 90، بينما كانت جميع العزلات الفطرية ممرضة على جيزة 94 مقارنة بالمعاملة الغير معدية. كانت أكثر الفطرية هي فطر فيوزاريوم فيوجيوكوري (ف10) بنسبة 80%، وريزوكتونيا سولاني (رس9)) بنسبة 100%، ماكروفومينا فاسولينا (م4 و م12) بنسبة 34% على الصنف جيزة 90. أما على الصنف جيزة 94، فكانت أكثر الفطريات الممرضة فطر فيوزاريوم فيوجيوكوري (ف10) بنسبة 100%، وريزوكتونيا سولاني (رس9)) بنسبة 100%، ماكروفومينا فاسولينا (م4) بنسبة 34%. في مرحلة ما بعد ظهور البادرات فوق سطح التربة، كان أكثر الفطريات الممرضة للصنف جيزة 90 فطر فيوزاريوم (ف1) بنسبة 22%، وريزوكتونيا سولاني (رس1)) بنسبة 18%، ماكروفومينا فاسولينا (م4 و م12) بنسبة 48%. وبالنسبة لجيزة 94 كانت أكثر الفطريات الممرضة فطر فيوزاريوم (ف1) بنسبة 16%، وريزوكتونيا سولاني (رس9)) بنسبة 18%، ماكروفومينا فاسولينا (م4) بنسبة 24%. كانت جميع العزلات الفطرية ممرضة و قللت من نسبة البارات الباقية على قيد الحياة، وكانت كانت أكثر العزلات الفطرية التي تقلل من نسبة بقاء الصنف جيزة 90 هي فطر فيوزاريوم فيوجيوكوري (ف10) بنسبة 0%، وريزوكتونيا سولاني (رس9)) بنسبة 0%، ماكروفومينا فاسولينا (م4) بنسبة 14%. كانت العزلات الفطرية ف10,رس9, م4 هي الأكثر فاعلية في التقليل من طول النبات ووزنها الجاف لكلا الصنفين.