Fusaria and other fungi associated with seedling blight and root rot of flax in the Nile Delta

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Fifty-five samples of blighted flax (*Linum usitatissimum*) seedlings or rotted roots of adult plants were randomly obtained from six flax producing governorates in the Nile Delta. The mean percenttages of fungal recovery from samples showed that *Fusarium* spp. (30.7%) were the most dominant fungi present. Other fungi occurred at frequencies ranging from 0.3–12.1%. Isolation frequencies of *Fusarium* spp. did not significantly differ from between governorates. The nature of the previous crop had an important bearing on the activity of flax root-colonizing *Fusarium* spp. Thus, samples preceeded by rice had the lowest isolation frequency (23.7%), while those preceeded by cotton had significantly higher isolation frequency (42.8%). Regression analysis revealed that root colonization incidence (RCI) and root colonization severity (RCS) relationship of root-colonizing fungi of flax conformed to the linear model. According to the generated model, RCI accounted for 81.7% of the total variation in RCS. *Fusarium* spp. were remotely related to *Pythium* spp. in their prevalence pattern, while they were unrelated to the other fungi. A total of 112 randomly selected isolates were tested for pathogenicity on seedlings of flax cultivar Giza 7 under greenhouse conditions. *Fusarium* spp. represented 50.0 and 51.5% of the tested isolates and demonstrated convincingly that *Fusarium* spp. are the major causal agents of flax seedling blight and root rot in the Nile Delta as they accounted for 57.0% of the pathogenic isolates. Thirty-six *Fusarium* spp. isolates were identified to species level and tested for pathogenicity on seedlings of flax cultivar Giza 7 under greenhouse conditions. *F. oxysporum* (50.0%) and *F. solani* (33.3%) were the predominant species. Other species were *F. lateritium* (5.5%), *F. semitectum* (2.7%), and unidentified *Fusarium* spp. (8.3%). Most pathogenic isolates belonged to *F. oxysporum* (47.0%) and *F. solani* (35.2%). The high frequency of *F. oxysporum* and *F. solani*, and their ability to cause considerable losses during seedling stage, strongly suggest that they are the most important pathogenic fusaria involved in the etiology of seedling blight and root rot of flax in the Nile Delta.

Key words – Egypt – Fusarium – *Linum usitatissimum* – soil-borne fungi

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Introduction

Flax (*Linum usitatissimum* L.) is the most important bast fiber crop in Egypt, it ranks second after cotton (seedy fiber) in terms of economic importance and production. There has been a steady increase in flax production owing to the growing trend back to natural fibers for textiles. Thus, the flax producing areas in Egypt were approximately 9505, 21267, and 40789 acres in 2000, 2002 and 2006, respectively (El-Hawary 2008). Flax production is currently confined to the Nile Delta governorates. Seedling blight and root rot are common in flax fields throughout the Nile

Delta. However, if affected seedlings are killed early in the season, they may become wind-blown or rained out and their loss is hardly noticed (AA Aly, personal observation). Flax seed is delicate and the outer coat is easily damaged during threshing. Small cracks, which may not be obvious unless the seed is inspected under a magnifying glass, allow easy penetration of microorganisms unless the seed is protected with a fungicide. Untreated cracked seeds may rot quickly without germinating, or they may germinate, producing weak seedlings that succumb quickly to attack by the microorganisms that cause blight. Plants affected by seedlings blight may occur singly or in patches. The patches may consist of only a few plants in a row, or they may contain many plants and cover a large area. Affected seedlings turn yellow, wilt, and die. The roots of recently attacked plants show red to brown lesions, but within a few days they shrivel and turn dark (Martens et al. 1984).

Root rot symptoms usually appear on older plants after the flowering stage. Plants turn brown prematurely and usually set few or no seeds. The underground portion of the stem and the roots are discolored and the root system may be stunted (Martens et al. 1984).

Fusarium species are economically important plant pathogens. Many species are also endophytic or saprophytic colonizers. As pathogens, *Fusarium* species cause a wide range of diseases on field, horticultural, and forest crops (Burgess et al. 1994, Moore et al. 2001, Ploetz 2001, Summerell et al. 2003). More than 80 economically important plants are affected by at least one disease caused by *Fusarium* (Leslie & Summerell 2006). *Fusarium* species; however, are often present as endophytes in many crops in agricultural ecosystems (Burgess 1981, Leslie et al, 1990, Kuldau & Yates 2000). They can occupy the internal plant tissue without causing any symptoms, but may induce disease symptoms when the plants are subjected to drought or other stress factors (Burgess 1981).

Fusarium spp. occur frequently among the fungal microflora associated with diseased flax roots and are a major cause of seedling blight and root rot in some countries (Nyvall 1981, Martens et al. 1984, Ligocka et al. 2002, Anonymous 2006, Gruzdevienė et al. 2008). In Egypt, although *Fusarium* spp. are frequently and easily isolated from blighted flax seedlings and rotted roots of adult plants, little attention has been given to their taxonomy and their role in the etiology of seedling blight and root rot.

Invasion of flax roots by soil-borne fusaria diminishes the plant's capacity for efficient nutrient and water uptake. Damage caused by these pathogens is difficult to assess from year to year due to differences in location, crop management, and climatic factors. Recognition of the role of soil-borne fusaria as a limiting factor for flax production potential in the Nile Delta is problematic due to a focus on more visible foliar diseases. Identification and quantification of root-invading fusaria involve more laborious procedures than the simple visual observations needed to detect and quantify the presence of foliar pathogens (Tunali et al. 2008).

Therefore, the main objectives of this investtigation were to identify *Fusarium* spp. associated with seedling blight and root rot of flax and to evaluate their pathogenicity to flax seedlings under greenhouse conditions. We also characterize effects of location and previous crop on their isolation frequencies. For comparison, isolation frequencies and pathogenicity of other fungi were also evaluated. The results of the present study will be used to develop more effective control strategies.

Materials and Methods

Isolation, identification, and quantification of *Fusarium* spp. and other fungi from flax roots

Diseased flax plants at the seedling stage of growth through maturity were collected at random from 55 fields (one sample/field) in six flax-growing governorates in the Nile Delta (Table 1) during 2007/2008 growing season. Each sample included from 20 to 30 seedlings affected with a variety of damping-off symptoms or rotted roots of 10 to 15 adult plants. The flax cultivars sampled were not determined for all fields because it is known that the cultivars grown in Egypt are all susceptible to seedling blight and root rot (AA Aly, *personal observation*). The seedlings and roots collected at each field were stored at 4°C until fungal isolation was performed. Seedlings and

Table 1 Flax samples[a] used in isolation of *Fusarium* spp. and other fungi.

Distribution based on						
Location				Previous crop		
Governorate	**Region**	**No.**	**%**	**Crop**	**No.**	**%**
Sharqiya	East Delta	9	16.3	Rice (*Oryza sativa* L.)	23	41.8
Daqahliya	East Delta	10	18.1	Corn (*Zea mays* L.)	20	36.3
Damietta	East Delta	9	16.3	Cotton (*Gossypium barbadense* L.)	12	21.8
KafrEl-Sheikh	North Delta	10	18.1	Total	55	100.0
Gharbiya	Middle Delta	10	18.1			
Beheira	West Delta	7	12.7			
Total		55	100.0			

[a]Each sample included from 20 to 30 seedlings affected with a variety of damping-off symptoms or rotted roots of 10 to 15 adult plants. There was one sample/field.

roots of mature plants were washed thoroughly under running tap water for 24 hr to remove any adhering soil. Small pieces (approximately 0.5 cm long) of necrotic root tissues were surface sterilized with 10% Clorox solution for 2 minutes, and washed several times with sterilized water. The surface-sterilized pieces were then blotted dry between sterilized filter papers and plated (5 pieces/plate) onto potato-dextrose agar (PDA) medium amended with streptomycin sulfate or penicillin G. and rose bengal (100–200 mg/L each) as bactericides. The plates were incubated at 26±3°C for 3–7 days. The developing colonies were identified according to Gilman (1966) or Barnett & Hunter (1972). Colonies of each fungus were expressed as percentage of the total developing colonies. Pure isolates, selected at random for pathogenicity tests, were grown on PDA in petri dishes.

Pathogenicity tests of *Fusarium* spp. and other fungi

Substrate for growth of each selected isolate was prepared in 500 mL glass bottle, each bottle contained 50 g of sorghum grains and 40 mL of tap water. Contents of bottles were autoclaved for 30 minutes. Isolate inoculum, taken from one-week-old culture on PDA, was aseptically introduced into the bottle and allowed to colonized sorghum for 3 weeks. The present tests were carried out by using autoclaved clay loam soil. Batches of soil were infested separately with inoculum of each isolate at a rate of 50 g/kg of soil. Infested soil was dispensed in 10 cm diameter clay pots and these were planted with 20 seeds per pot (cultivar Giza 7). In the control treatments, sterilized sorghum grains were mixed thoroughly with soil at a rate of 50 g/kg of soil. Pots were randomly distributed on greenhouse benches. The greenhouse was equipped with a heating system assuring that the minimum temperature in the greenhouse was maintained at 28°C; however, due to the lack of a cooling system, the maximum temperature could not be controlled fluctuating from 30 to 35°C depending on the prevailing temperature during the day (the tests were conducted in January and February 2008). Dead seedlings (combined pre-emergence and post-emergence damping-off) were recorded 45 days after planting. All pathogenicity tests were repeated once.

Identification of a selected group of *Fusarium* isolates to species level and evaluation of their pathogenicity.

A random sample of 36 isolates of *Fusarium* spp., purified by the single spore technique, was identified to species level according to Booth (1971). These isolate were subculture for the pathogenicity test, which was carried out as previously described.

Statistical analysis of the data

The experimental design of all the pathogenicity tests was a randomized complete block with five replicates. Analysis of variance (ANOVA) of the data was performed with the MSTAT-C Statistical Package. Duncan's multiple range test and least significant difference (LSD) were used to compare between isolate means. Percentage data were transformed into arcsine angles before carrying out the ANOVA to normalize data and stabilize variances throughout the data range. Correlation, regression, and cluster analyses were performed with the software package (SPSS 6.0).

Results

A total of 55 samples of blighted flax seedlings or rotted roots of adult plants were randomly obtained from six flax producing governorates in the Nile Delta (Table 1). Samples of east Delta governorates (Sharqiya, Daqahliya, and Damietta) represented 50.9% of the total samples. The samples were obtained from flax preceeded by rice, corn, or cotton (Table 1). Samples preceeded by rice, the predominant summer field crop in the Nile Delta, made up the highest percentage of samples (41.8%). Isolation frequencies of *Fusarium* spp. did not significantly differ from one governorate to another (Table 2). Data shown in Table 2 indicated that the nature of previous crop had an important bearing on the activity of flax-root-colonizing *Fusarium* spp. Thus, the samples preceeded by rice showed the lowest isolation frequency (23.7%), while those preceeded by cotton showed the highest isolation frequency (42.8%).

In the present study, root colonization severity (RCS) of flax by fungi was estimated by a method involving objective judgment (Table 3); however, the method was tedious and time-consuming. Estimating RCS indirectly from root colonization incidence (RCI), which was more easily acquired and more precise (Table 3), may reduce some of these problems. Regression analysis (Fig. 1) revealed that RCI and RCS relationship of root-colonization fungi of flax confirmed to the linear model. According to the generated model, RCI accounted for 81.7% of the total variation in RCS. Thirteen different fungi were associated with the necrotic root tissues of affected seedlings or older plants, but *Fusarium* spp. were isolated most frequently comprising 30.7% of all fungi isolated, while frequencies of isolation of the other fungi ranged from 0.3 to 12.1% (Table 3). No attempt was made to separate fungi on the basis of their association with seedlings versus older plants; however, the isolation of fungi from both seedlings and older plants suggests that prolonged colonization of roots is perhaps the greatest threat posed by fungi, particular *Fusarium* spp., for flax health.

Associations among the pairs of fungi isolated from flax roots were identified and the relative strength of these associations were measured by calculating Pearson's correlation coefficient (r) for each pair of fungi. A total of 78 fungal pairings were analyzed (Table 4). Thirteen (16.6%) of the fungal pairs were significantly associated. Of the 13 pairs, 8 were negatively associated and 5 were positively associated. No significant associations were found in the remainding fungal pairs.

A total of 112 randomly selected isolates were tested for pathogenicity under greenhouse conditions. Of these, 64 were tested in the first pathogenicity test (Table 5), while 48 were tested in the second one (Table 6). *Fusarium* spp. represented 53.1 and 50.0% of the tested isolates in the first and second tests, respecttively.

Under these favourable conditions, isolates of *Fusarium* spp. varied in virulence from nonpathogenic to highly pathogenic. For example, isolates no. 32, 38, and 52 in the first test (Table 5) were nonpathogenic (0.00% damping-off), while isolate no. 8 in the second test was highly pathogenic (100% damping-off). The results of pathogenicity tests demonstrated convincingly that *Fusarium* spp. are the major causal agents of flax seedling blight and root rot in the Nile Delta as they accounted for 57.0% of the pathogenic isolated in the two tests (Table 7).

A total of 36 randomly selected isolates of *Fusarium* spp. were identified to species level and tested for pathogenicity on seedlings of flax cultivar Giza 7 under greenhouse conditions (Table 8). *F. oxysporum* Schltdl. (50.0%) and *F. solani* (Mart.) Sacc. (33.3%) were the most predominant species. Other species were *F. lateritium* Nees (5.5%), *F. semitectum* Berk and Ravenel (2.7%), and unidentified *Fusarium* spp. (8.3%). Most of the pathogenic isolates (Table 9) belonged to *F. oxysporum* (47.0%) and *F. solani* (35.2%). Isolates of *F. oxysporum* tended to be pathogenic in the post-emergence stage, while *F. solani* isolates tended to be pathogenic in the pre-emergence stage (Table 10). There were no significant differences among the tested fusaria regarding post-emergence damping-off and survival (Table 11).

Discussion

In general, *Fusarium* spp. are most active and survive best in dry soils. Accordingly, *Fusarium* diseases are commonly more

Table 2 Effect of location and previous crop on frequency of *Fusarium* spp. isolated from diseased flax roots.

Location				Previous crop		
Governorate	**Region**	**No. of samples**	**Isolation Frequency (%)[a]**	**Crop samples**	**No. of samples**	**Isolation Frequency (%)[a]**
Sharqiya	East Delta	9	40.7A	Rice	23	23.7 A
Daqahliya	East Delta	10	30.8A	Corn	20	27.6 AB
Damietta	East Delta	9	34.3A	Cotton	12	42.8 B
Kafr El-Sheikh	North Delta	10	25.8A			
Gharbiya	Middle Delta	10	25.6A			
Beheira	West Delta	7	23.0A			

[a]Percentage data were transformed into arcsine angles before carrying out the analysis of variance to normalize data and stabilize variances throughout the data range. Means followed by the same letter are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test.

Table 3 Samples from which fungi were isolated and their isolation frequencies from roots of flax plants infected with postemergence damping-off or root rot.

Fungus	**Samples from which fungus was isolated**		**Isolation frequency[b] %**
	No.	**% [a]**	
Alternaria spp.	22	40.0	8.2 C-E
Aspergillus spp.	32	58.1	10.7 B-D
Chaetomium spp.	33	60.0	12.1 B
Cladosporium spp.	11	20.0	2.5 F-H
Fusarium spp.	46	83.6	30.7[c] A
Helminthosporium spp.	4	7.27	1.3 G-H
Macrophomina phaseolina	2	3.64	0.3 H
Mucor sp.	2	3.64	2.0 H
Penicillium spp.	14	25.4	5.0 E-G
Pythium spp.	18	32.7	6.7 E-F
Rhizoctonia solani	20	36.3	7.5 D-E
Rhizopus spp.	5	9.09	0.9 G-H
Unidentified	34	61.8	10.7 B-C

[a]Root colonization incidence.
[b]Root colonization severity, which is the number of colonies of a fungus expressed as percentage of the total developing colonies from a sample.
[c]Each value is the mean of 55 samples. Means followed by the same letter(s) are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test. Percentage data were transformed into arcsine angles before carrying out the analysis of variance to normalize data and stabilize variance throughout the data range.

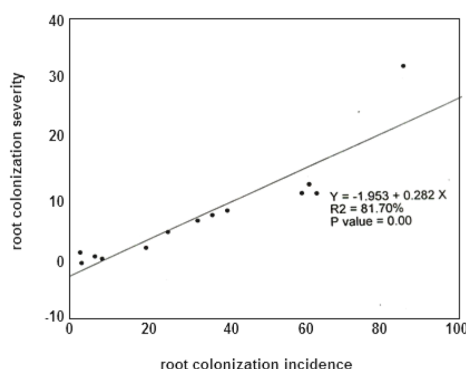

Fig. 1 – Relationship between root colonization incidence and severity.

Table 4 Correlation between frequencies of fungi isolated from flax roots.

Isolation frequency of	Isolation frequency of											
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
1-Fusarium spp.												
2-Aspergillus spp.	-0.271*											
3-Chaetomium spp.	-0.172	-0.061										
4-Penicillium spp.	-0.186	-0.067	-0.139									
5-Cladosporium spp.	-0.287*	0.189	-0.132	-0.181								
6-Alternaria spp.	-0.183	-0.256×	-0.194	0.100	-0.069							
7-Helminthosporium	-0.032	-0.021	-0.027	0.114	0.119	0.026						
8-Rhizoctonia solani	-0.187	-0.227×	-0.235×	-0.042	0.376**	-0.038	0.111					
9-Pythium spp.	0.040	-0.189	0.036	-0.118	-0.188	-0.280*	-0.112	-0.065				
10-Unidentified	-0.200	0.269*	-0.008	-0.234×	0.054	-0.216	-0.129	-0.225×	-0.082			
11-Rhizopus spp.	-0.149	-0.007	0.018	0.307*	-0.105	-0.040	0.356**	-0.076	-0.040	0.024		
12-Macrophomina phaseolina	-0.119	-0.057	0.086	-0.002	-0.064	-0.223×	-0.031	-0.066	-0.089	-0.043	-0.041	
13-Mucor sp.	-0.061	-0.103	-0.100	-0.086	-0.079	-0.009	-0.038	-0.43	-0.109	-0.165	-0.050	-0.030

[a]Pearson's correlation coefficient (r) is significant at $P \leq 0.10$ (x), $P \leq 0.05$ (*), or $P \leq 0.01$ (**)

Table 5 Pathogenicity of fungi isolated from flax roots on Giza 7 flax seedlings under greenhouse conditions (First group of isolates).

Fungus species	Isolate no.	Geographic origin		Dampping-off (%)[d]
		Governorate	Region	
Fusarium spp.	10	Daqahliya	East Delta	40 F-J*
	11	Kafr El-Sheikh	North Delta	10 M-Q
	14	Gharbiya	Middle Delta	28 H-M*
	15	Beheira	West Delta	25 H-N*
	16	Gharbiya	Middle Delta	7 L-Q
	17	Gharbiya	Middle Delta	3 N-Q
	18	Kafr El-Sheikh	North Delta	44 F-I*
	20	Beheira	West Delta	84 A-C*
	21	Daqahliya	East Delta	63 C-G*
	22	Beheira	West Delta	81 A-C*
	23	Sharqiya	East Delta	72 B-D*
	24	Kafr El-Sheikh	North Delta	92 A-B*
	25	Sharqiya	East Delta	63 C-G*
	26	Kafr El-Sheikh	North Delta	20 I-Q
	27	Sharqiya	East Delta	22 I-P*
	28	Kafr El-Sheikh	North Delta	40 G-K*
	29	Sharqiya	East Delta	52 D-H*
	30	Gharbiya	Middle Delta	5 N-Q
	31	Gharbiya	Middle Delta	13 J-Q
	32	Sharqiya	East Delta	0 Q
	33	Gharbiya	Middle Delta	3 N-Q
	36	Beheira	West Delta	19 H-P*
	38	Daqahliya	East Delta	0 Q
	46	Kafr El-Sheikh	North Delta	100 A*
	47	Sharqiya	East Delta	24 H-O*
	48	Gharbiya	Middle Delta	4 O-Q
	50	Kafr El-Sheikh	North Delta	8 N-Q
	51	Sharqiya	East Delta	21 I-P*
	52	Gharbiya	Middle Delta	0 Q
	54	Beheira	West Delta	18 I-Q
	56	Kafr El-Sheikh	North Delta	72 B-F*
	57	Sharqiya	East Delta	28 H-L*
	58	Daqahliya	East Delta	22 I-Q
	59	Daqahliya	East Delta	34 G-K*
Alternaria spp.	1	Beheira	West Delta	13 I-Q
	2	Gharbiya	Middle Delta	0 Q
	5	Gharbiya	Middle Delta	63 C-G*
	8	Sharqiya	East Delta	4 O-Q
	34	Daqahliya	East Delta	17 I-P*
	37	Beheira	West Delta	18 I-P*
	41	Sharqiya	East Delta	11 K-Q
Unidentified	3	Beheira	West Delta	6 I-Q
	6	Kafr El-Sheikh	North Delta	0 Q
	7	Sharqiya	East Delta	37 G-K*
	9	Kafr El-Sheikh	North Delta	25 I-P*
	12	Kafr El-Sheikh	North Delta	19 I-Q
	13	Gharbiya	Middle Delta	2 O-Q
	19	Kafr El-Sheikh	North Delta	74 B-E*
	35	Kafr El-Sheikh	North Delta	16 I-Q
	39	Sharqiya	East Delta	4 N-Q
	40	Beheira	West Delta	4 O-Q
	42	Beheira	West Delta	4 O-Q
	45	Kafr El-Sheikh	North Delta	0 Q
	49	Sharqiya	East Delta	35 G-K*
	53	Gharbiya	Middle Delta	5 L-Q
	55	Sharqiya	East Delta	1 P-Q

Table 5 (Continued) Pathogenicity of fungi isolated from flax roots on Giza 7 flax seedlings under greenhouse conditions (First group of isolates).

Fungus species	Isolate no.	Geographic origin		Dampping off (%)[d]
		Governorate	Region	
	60[a]	Giza	Middle Egypt	3 N-Q
	61[a]	Giza	Middle Egypt	91 A-B*
	62[a]	Giza	Middle Egypt	63 C-G*
Rhizoctonia solani	63	Gharbiya	Middle Delta	100 A*
	64	Sharqiya	East Delta	99 A*
Helminthosporium sp.	4	Kafr El-Sheikh	North Delta	41 F-J*
Stemphylium sp.	44[a]	Beheira	West Delta	3 N-Q
Macrophomina phaseolina	43	Sharqiya	East Delta	45 E-I*
Control[b]	65			

Table 6 Pathogenicity of fungi isolated from flax roots on Giza 7 flax seedlings under greenhouse conditions (Second group of isolates).

Fungal species	Isolate No.	Geographic origin		Damping off (%)
		Governorate	Region	
Fusarium spp.	1	Daqahliya	East Delta	75 A-E*
	2	Daqahliya	East Delta	79 A-D*
	3	Daqahliya	East Delta	84 A-C*
	4	Kafr El-Sheikh	North Delta	45 E-J*
	5	Beheira	West Delta	20 H-M
	6	Kafr El-Sheikh	North Delta	36 F-J*
	7	Kafr El-Sheikh	North Delta	32 G-K*
	8	Kafr El-Sheikh	North Delta	100 A*
	9	Sharqiya	East Delta	71 B-G*
	10	Beheira	West Delta	39 F-J*
	11	Gharbiya	Middle Delta	40 F-J*
	12	Gharbiya	Middle Delta	51 C-I*
	13	Beheira	West Delta	23 H-M
	14	Kafr El-Sheikh	North Delta	96 A-B*
	15	Gharbiya	Middle Delta	23 H-M
	16	Beheira	West Delta	43 C-I*
	17	Daqahliya	East Delta	63 C-I*
	18	Gharbiya	Middle Delta	76 A-E*
	19	Gharbiya	Middle Delta	71 A-E*
	20	Daqahliya	East Delta	32 H-M
	21	Daqahliya	East Delta	12 K-M
	22	Sharqiya	East Delta	12 F-J*
	23	Gharbiya	Middle Delta	32 J-M
	24	Kafr El-Sheikh	North Delta	11 H-M
Alternaria spp.	25	Gharbiya	Middle Delta	27 H-M
	26	Sharqiya	East Delta	33 E-J*
	27	Kafr El-Sheikh	North Delta	25 H-M
	30	Sharqiya	East Delta	11 H-M
	31	Beheira	West Delta	57 C-I*
	41	Daqahliya	East Delta	87 A-D*
Unidentified	32	Gharbiya	Middle Delta	31 H-M
	33	Kafr El-Sheikh	North Delta	11 I-M
	34	Daqahliya	East Delta	49 E-J*
	35	Daqahliya	East Delta	52 D-J*
	36	Kafr El-Sheikh	North Delta	40 C-I*
	37	Beheira	West Delta	60 C-H*
	39	Gharbiya	Middle Delta	40 F-J*
	40	Daqahliya	East Delta	58 B-F*
	42	Beheira	West Delta	75 B-F*
	43	Beheira	West Delta	12 J-M

Table 6 (Continued) Pathogenicity of fungi isolated from flax roots on Giza 7 flax seedlings under greenhouse conditions (Second group of isolates).

Fungal species	Isolate No.	Geographic origin		Damping off (%)
		Governorate	Region	
	44	Sharqiya	East Delta	31 H-M
	45	Beheira	West Delta	23 H-M
	46	Kafr El-Sheikh	North Delta	59 C-H*
	47	Beheira	West Delta	49 C-I*
Pythium sp.	28	Sharqiya	East Delta	14 J-M
Macrophomina phaseolina.	29	Beheira	West Delta	1 M
Penicillium sp.	38	Gharbiya	Middle Delta	73 B-F*
Rhizoctonia solani	48	Gharbiya	Middle Delta	100 A*
Control [a]	49	Gharbiya	Middle Delta	3 L-M

[a]Autoclaved soil was mixed with autoclaved sorghum.
[b]Combined pre-emergence and post-emergence. Percentage data were transformed into arcsine angles before carrying out the analysis of variance to normalize data and stabilize variances throughout the data range. Means followed by the same letter(s) are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test. An asterisk denotes a significant difference from the control.

Table 7 Distribution of pathogenic isolates from flax roots.

Fungal species	Total number of pathogenic isolates	Percentage of isolates within fungus	Percentage of total isolates [a]	Percentage of pathogenic isolates
Fusarium spp.	37	63.8	33.0	57.0
Alternaria spp.	6	46.1	5.3	9.3
Unidentified	15	46.9	13.3	23.4
Rhizoctonia solani	3	100.0	2.6	4.6
Helminthosporium sp.	1	100.0	0.8	1.5
Stemphylium sp.	0	0.0	0.0	0.0
Macrophomina phaseolina	1	50.0	0.8	1.5
Pythium sp.	0	0.0	0.0	0.0
Penicillium sp.	1	100.0	0.9	1.5
Total	64		57.1	100.0

[a]Total of 112 isolates were tested for pathogenicity on Giza 7 flax seedlings under greenhouse conditions in two tests, the first test included 64 isolates while the second one included 48 isolated. The details of the tests are shown in Tables 5 and 6, respectively.

important under dry rather than wet conditions (Cook 1981). As a result, wet soil, associated with rice irrigation systems, created an environment unfavorable to survival of *Fusarium* spp. and their subsequent coloni-zation of flax roots. This may indicate that *Fusarium* spp. are well adapted to colonize flax roots under a wide range of environmental conditions (edaphic factors, crop rotation, irrigation system, tempe-rature and so on). *Fusarium* spp. are the most frequently isolated fungi from roots of Egyptian cottons and roots are more subjected to colonization by *Fusarium* spp. as the plant matures (Aly et al., 1996). Therefore, it seems reasonable to assume that survival of *Fusarium* spp. in cotton root debris and their subsequent increase in inoculum density may contribute to the observed increase in frequency of *Fusarium* spp. isolated from flax roots when flax was preceeded by cotton.

Basic knowledge of the relationship between RCI and RCS must be obtained before RCI can be efficiently used as a measure of RCS (Rouse et al. 1981). Several models have been used to describe the relationship between disease incidence and disease severity in plant diseases (Seem 1984); however, there have been no reports of this type of study on root-colonization fungi of flax. The occurrence and associations of pathogen species are of a central importance in the ecology of host-pathogen interactions in complex pathosystems, i.e. those with multiple pathogens on a single or multiple hosts. Within such pathosystems, biotic and abiotic factors influence the distribution and abundance of pathogen species. Subsequently,

Table 8 Pathogenicity of *Fusarium* species isolated from flax roots on Giza 7 flax seedlings under greenhouse conditions.

Fusarium species	Isolate No.	Pre-emergence damping-off (%)	Post-emergence damping-off (%)	Survival (%)
F. oxysporum	3	80[b] A-E*	14 D-H	6 I-L*
	5	78 A-E*	15 C-H	7 J-L*
	7	94 A-C*	6 F-H	0 L*
	9	6 K-N	21 B-H	73 A-C
	10	4 K-N	50 A-C*	46 C-I*
	11	6 J-N	72 A*	22 G-L*
	12	8 I-N	34 A-G*	58 B-F*
	13	9 J-N	30 B-H	61 B-E*
	15	0 N	32 A-G*	68 B-D*
	16	54 D-I*	36 A-G*	10 G-L*
	17	9 J-N	54 A-C*	37 C-J*
	21	85 A-D*	11 E-H	4 J-L*
	26	36 E-M	46 A-E*	18 F-L*
	29	0 N	24 C-H	76 A-B
	31	42 E-M	46 A-E*	12 G-L*
	33	4 M-N	52 A-D*	44 C-H*
	34	100 A*	0 H	0 L*
	36	13 I-N	40 A-G*	47 B-G*
F. solani	1	67 B-H*	23 B-H	10 G-L*
	14	5 L-N	36 A-G*	59 B-F*
	18	16 I-N	62 A-B*	22 E-L*
	19	28 G-N	53 A-D*	19 F-L*
	20	37 F-N	32 B-H	31 D-K*
	22	49 D-J*	24 B-H	27 D-K*
	23	45 D-K*	27 B-H	28 C-J*
	25	67 A-F*	24 C-H	9 J-L*
	28	70 A-H*	15 C-H	15 G-L*
	30	79 A-E*	10 D-H	11 G-L*
	32	71 A-G*	19 C-H	10 H-L*
	35	33 H-N	30 B-H	37 C-J*
F. lateratium	2	83 A-E*	4 G-H	13 G-L*
	8	64 B-H*	26 B-H	10 G-L*
F. semitectum	24	47 E-L*	36 A-G*	17 G-L*
Unidentified	4	60 B-H*	27 B-H	13 G-L*
	6	98 A-B*	1 H	1 K-L*
	27	61 C-H*	20 C-H	19 G-L*
Control[c]		3 M-N	0 H	79 A

[a]Percentage data were transformed into arc sine angles before carrying out the analysis of variance to normalize data and stabilize variances throughout the data range.
[b]Values in a column followed by the same letter(s) are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test. An asterisk denotes a significant difference from the control.
[c]Autoclaved soil was mixed with autoclaved sorghum.

Table 9 Distribution of pathogenic isolates of *Fusarium* species from flax roots.

Fusarium species	Total number of Pathogenic isolates	Percentage of isolates within fungus	Percentage of total isolates [a]	Percentage of pathogenic isolates
F. oxysporum	16	88.8	44.4	47.0
F. solani	12	100.0	33.3	35.3
F. lateritium	2	100.0	5.5	5.9
F. semitectum	1	100.0	2.8	2.94
Unidentified	3	100.0	8.3	8.9
Total	34	–	94.4	100.0

[a]A total of 36 isolates were tested for pathogenicity on Giza 7 flax seedlings under greenhouse conditions.

Table 10 Distribution of *Fusarium* species based on their pathological effects on seedlings of flax cultivar Giza 7 under greenhouse condtions.

***Fusarium* species**	**Total number of tested isolstes**	**Percentage of isolates, which significantly affected [a]**		
		Pre-emergence damping-off (%)	**Post-emergence damping-off (%)**	**Survival (%)**
F. oxysporum	18	33.33	50.00	88.89
F. solani	12	58.33	33.33	100.00
F. lateritium	2	100.00	0.00	100.00
F. semitectum	1	100.00	100.00	100.00
Unidentified	3	66.67	0.00	100.00

[a]The tested isolates significantly increased pre- and post-emergence damping-off while they significantly decreased survival.

Table 11 The relative pathogenicity of *Fusarium* species on flax seedlings (Giza 7) under greenhouse conditions.

***Fusarium* species**	**Number of tested isolates**	**Pre-emergence damping-off (%)**	**Post-emergence damping-off (%)**	**Survival (%)**
F. oxysporum	18	34.89[c] A	32.29 A	32.72 A
F. oxysporum	12	47.25 AB	29.58 A	23.17 A
Other *Fusarium spp.*	6	68.83 B	19.00A	12.17 A

[a]Other *Fusarium* spp. included *F. lateritium*, *F. semitectum* and unidentified fusaria.
[b]Percentage data were transformed into arc sine angles before carrying out the analysis of variance to normalize data and stabilize variances throughout the data range.
[c]Values in a column followed by the same letter are not significantly different ($P \leq 0.05$) according to LSD test.

patterns of association result from interrelationships among organisms and from environmental factors. These patterns depend on whether or not organisms select or avoid the same habitat, have same mutual interaction or repulsion, or have no interaction (Nelson & Campbell 1992).

Organisms that have similar patterns of resource usage have a high degree of “niche overlap” (Ludwig & Reynolds 1988). Thus, pathogen species (e.g., root-colonizing fungi) in competition for a single resource (e.g., infection site of a root system) tend to occupy the same niche. Such a niche overlap generates affinity (or lack of affinity) for coexistence among species, known as interspecific association. Interspecific associations are of epidemiological interest, because they reflect spatial and temporal attributes of species diversity (Savary et al. 1988).

Patterns of association of pathogens involved in some complex pathosystems have been evaluated. These pathosystems included maize kernel-infecting fungi (Wicklow 1988), leaf spot on white clover (Nelson & Campbell 1992), and foliar pathogens of cucumber (Peterson & Campbell 2002). To the best of our knowledge, no attempts have been made to study the associations among fungi isolated from flax roots.

However, one should keep in mind that the significant r values should be interpreted with caution (Gomez & Gomez 1984) because the existence of a process may not be proved by the existence of a pattern (Nelson & Campbell 1992), i.e., the significant r value does not necessarily prove that one fungus is beneficial or detrimental to another. Thus, the primary utility of the correlation technique is to identify the potentially interactive fungi. However, the interpretation of the nature of such an interaction requires information on the ecological requirements and biological attributes of each member of the interacting pair (Wicklow 1988). In spite of these limitations, certain general conclusions could be drawn. A negative association between two fungi may have resulted because each fungus had distinct environmental and resource requirements or, perhaps displayed competitive exclusion or antagonism. Fungi that share specialized niche requirements often occur together and would

primarily exhibit a positive association (Peterson & Campbell 2002).

Our personal experience with seedling blight and root rot of flax indicated that the use of standard isolation techniques often results in the frequent recovery of fungi normally considered saprophytic. This was especially true of samples that included severely affected seedlings or older plants. Therefore, the pathogenicity tests employed in this study were not intended to simulate natural conditions. Quite the contrary, they were deliberately designed to provide for maximum expression of pathogenicity. The soil was autoclaved, the temperature was optimum most of the time, and the isolates inoculum was relatively high. Thus, those isolates incapable of producing statistically significant levels of damping-off, under these very favourable environmental conditions, were considered to be nonpathogenic. This seemed a reasonable approach to ensure inclusion of any potential incitants of seedling blight or root rot, which are widely considered to be complexes of several fungi including *Fusarium* spp. (Nyvall 1981, Martens et al. 1984, Ligocka et al. 2002, Anonymous 2006, Gruzdevienė et al. 2008). The nonspecialized *Fusarium* spp. involved in flax seedling blight have a wide host range, therefore, rotation of flax with other crops is a questionable practice for the disease control (Nyvall 1981). Control of flax seedling blight by selection of blight resistant cultivars has not been emphasized in the development of commercial flax cultivars because of the lack of sources of such a resistance (Anonymous 1972). Consequently, seedling blight of flax is controlled largely by using seed-dressing fungicides (Martens et al. 1984, Youssef et al. 1984, Amr et al. 1987, Khalil et al. 1992). Effective seed treatment relies on up-to-date information on the major causal agents of diseases. Results of this study indicate that the selection of seed-dressing fungicides to control flax seedling blight should include compounds targeted mainly at *Fusarium* spp.

Results of the damping-off pathogenicity tests (Tables 5 and 6) implicated several fungi not previously reported as root pathogens of flax. *Macrophomina phaseolina* was recorded on flax for the first time anywhere, and *Alternaria* spp., *Penicillium* sp., and *Helminthosporium* sp. for the first time in Egypt. It is worth noting that although *Penicillium* spp., *Helminthosporium* spp., and *M. phaseolina* were found in low frequencies (Table 3), they included pathogenic isolates capable of killing flax seedlings in the pathogenicity tests. *R. solani* has been described as non-specialized and highly virulent (Ogoshi 1987). In our pathogenicity tests (Tables 5 and 6), all *R. solani* isolates were highly pathogenic (99.00 to 100% damping-off).

Most of the unidentified isolates were found in their non-spore-producing forms. Therefore, we were unable to identify them based on these vegetative (mycelial) phases. Further research is needed to identify these isolates especially if one takes into account that they represented 23.44% of the total pathogenic isolates in the two tests (Table 7).

The results of pathogenicity tests also revealed that 36.2% of *Fusarium* spp. isolates were nonpathogenic (Table 7). This lack of pathogenicity does not necessarily mean that these isolates are unimportant in the agricultural ecosystem. In fact, they are either endophytic or saprophytic colonizers of flax root. Nonpathogenic isolates of *Fusarium* spp. have been reported to control *Fusarium* wilt on various crops (Alabouvette et al. 1998). Endophytic isolates can occupy the internal plant tissue without causing any symptoms, but may induce disease symptoms when the plants are subjected to drought or other stress factors (Burgess 1981).

The predominance of *F. oxysporum* in our random sample agrees with other reports, which indicate that this species makes up a major portion of the fungal flora. For example, Gordon (1956) found that *F. oxysporum* was by far the most prevalent species of *Fusarium* as it represented approximately 67% of *Fusarium* spp. in Canadian soil. Meyer (1967) showed that the relative abundance of *F. oxysporum* may be as high as 8–10% of the soil total fungal population. In the rhizosphere, the relative abundance of *F. oxysporum* may reach 43% of the total microfungal population while on the root surface or in its superficial layers, *F. oxysporum* is even more abundant, and its frequency among isolates may reach 97% (Meyer 1967).

The high frequency of *F. oxysporum* and *F. solani* and their ability to cause considerable

losses during seedling stage strongly suggest that they are the most important pathogenic fusaria involved in the etiology of seedling blight and root rot of flax in the Nile Delta.

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