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CHARACTERIZATION OF *Fusarium moniliforme* INVOLVED IN COTTON SEEDLING DAMPING-OFF BY PATHOGENICITY AND ELECTROPHORETIC PROTEIN PROFILES

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ABSTRACT

Pathogenicity of fourteen isolates of *Fusarium moniliforme*, from different cotton-growing areas of Egypt was evaluated on cotton cultivar Giza 86 under greenhouse conditions. All the tested isolates were pathogenic. They affected all disease variables. Isolates no$_s$. 3, 13, 1, and 10 were the most pathogenic isolates in pre-emergence stage, while isolates no$_s$. 2, 10, and 3 were the most pathogenic isolates in post-emergence stage. All isolates were more pathogenic in pre-emergence stage than post-emergence stage except isolate no. 2, which was more pathogenic in post-emergence stage. All tested isolates significantly reduced both of plant height and dry weight of seedlings. There were no relationship between pathogenicity of the isolates and their geographic origins. There was a negative significant correlation between pre-emergence damping-off and the dry weight of seedlings. Also, there was a negative significant correlation between post-emergence damping-off and survival. Cluster analysis of isolates based on pathogenicity patternes showed that there was no relationship between virulence patternes of isolates and their geographic origin. Electrophoretic protein profiles of isolates showed that the population of Upper Egypt was genetically differed from that of Nile Delta. There was a correlation between protein profiles and geographic origin of isolates, while there was no correlation between protein profiles and pathogenicity of isolates.

INTRODUCTION

Seedling disease is a major problem in cotton production. *Fusarium moniliforme* is often associated with diseased cotton plants, but its pathogenicity is controversial. Several researchers have reported only mild virulence and consider the fungus a secondary invader (Fulton and Bollenbacher, 1959; Johnson *et al.*, 1978; and Johnson and Doyle, 1986), while others have reported a high degree of virulence (Woodroof, 1927; Roy and Bourland, 1982; and Batson and Borazjani, 1984). Nevertheless, researchers agree on the frequent association of *F. moniliforme* with diseased seedlings (Johnson *et al.*, 1978; Roy and Bourland, 1982; and Johnson and Doyle, 1986). *F. moniliforme* and *F. solani* were isolated from diseased seedlings in Oklahoma at frequencies of 61% and 19% respectively (Ray and Mclaughlin, 1942). Jakob (1969) isolated 97 isolates of *Fusarium* spp. from seedling of Egyptian cottons infected with damping-off. Isolates of *F. moniliforme* represented 23.7% of these isolates. *F. oxysporum* and *F. moniliforme* were the only species capable of infecting and killing cotton seedling, while other species were categorized as wound parasites or saprophytes. Colyer (1988) reported that *Fusarium* spp. frequently isolated

from 42% of all fungi isolated from cotton diseased seedlings. He demonistrated that the high level of virulence and the frequency of isolates indicated that *Fusarium* spp. are important in the etiology of cotton seedling diseases in Louisiana. Aly *et al.* (1996) demonestrated that *F. moniliforme* appear to be the most pathogenic species to cotton seedlings in Egypt. Armanious (2000) found that *F. moniliforme, F. oxysporum, F. semitectum* and *F. solani* were associated with damping-off, root rot, and wilt diseases influcing cotton plants in Minia governorate. Abd-Elsalam *et al.* (2006) reported that pathogenic *Fusarium* spp. associated with cotton damping-off in Egypt are similar to those in other cotton-producing countries of the world, except for the absence of *F. equiseti* (Chimbekujwo, 2000). Abd-Elsalam *et al.* (2006) isolated 46 isolates of *Fusarium* spp. from seven different cotton growing-arease in Egypt. 19.6% of those isolates identified as *F. moniliforme*.

Proteins electrophoresis has been widely used as a biochemical tool in fungal taxonomy. This is because amino acids sequences of polypeptides (components of proteins) are depended on nucleotide sequences of their coding genes; therefore, an analysis of protein variation among fungal isolates by electrophoresis approximates an analysis of their genetic variation (Markert and Faulhaber, 1965). However, a problem with proteins as markers is the vast number, which can be generated from an organism. Faced with so much data, only sophistecated analysis can help to draw meaningful conclusion. The ready availability of computers has made numerical taxonomy more accessible and same letter stduies of fungi have proven useful (Manicom *et al.*, 1990). Lo and Sun (1986) found that protein patterns in vertical slab electrophoresis of *F. oxysporum* from radish were different from that of *F. oxysporum* from mustard and *F. oxysporum* from kale. Scala *et al.* (1989) distinguished pathotype 2 of *F. oxysporum* f.sp. *dianthi* by electrophoresis of native protein due to a unique band with MM 30 kDa. Aly *et al.* (1997) used polyacrylamide gel electrophoresis (PAGE) and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) to characterize *F. oxysporum, F. moniliforme,* and *F. solani* isolated from cotton seedlings infected with damping-off. On the basis of electrophoretic similarities among protein banding patterns, species were grouped by cluster analysis and the results were expressed as phenograms. Delineation of isolates of each species was not possible on the basis of electrophoretic banding patterns of native proteins. On the contrary, banding patterns of SDS-dissociated proteins proved a reliable method for grouping isolates for each species. Moreover, phylogenetic relationships between the resulting clusters matched that based on morphological taxonomy. Aly *et al.* (2000) reported that electrophoretic banding patterns of dissociated proteins provided a reliable method for grouping the four Egyptian races of *F. oxysporum* f.sp. *ciceris*. Aly *et al.* (2001) compared protein patterns of five isolates of *F. oxysporum* f.sp. *vasinfectum* (FOV) and a nonpathogenic isolate of *F. oxysporum* by PAGE and SDS-PAGE. On the basis of electrophoretic dissimilarities among protein patterns, isolates were grouped by cluster analysis and the results were expressed as phenograms. Both PAGE and SDS-PAGE could not be used to distinguish the highly pathogenic isolates of FOV.

The objective of the present study was to characterize isolates of *F. moniliforme* involved in cotton seedling damping-off by pathogenicity and electrophoretic protein profiles.

MATERIALS AND METHODS

Fungal Isolates

Isolates of *F. moniliforme* (Sheldom), used in this study, were recovered from roots of cotton seedling infected with damping-off disease (Table 1). Isolation, purification and identification of these isolates were carried out at Cotton Pathology Lab., Plant Path. Res. Inst., Agri. Res. Center, Giza.

Table 1: Geographical origins of *Fusarium moniliforme* isolates used in this study

Isolate No.	Governorate	Region
1	Dumitta	East Delta
2	Daqahliya	East Delta
3	Minofiya	Mid-Delta
4	Sharqiya	East Delta
5	Daqahliya	East Delta
6	Beheira	West Delta
7	Daqahliya	East Delta
8	Qaleobiya	South Delta
9	Sharqiya	East Delta
10	Kafr-Elshiekh	North Delta
11	Assiut	Upper Egypt
12	Beheira	West Delta
13	Sohag	Upper Egypt
14	Gharbiya	Mid-Delta

Pathogenicity test of *F. moniliforme* isolates on cotton cultivar Giza 86 under greenhouse conditions

Substrate for growth of each isolate was prepared in 500-ml glass bottles, each bottle contained 50g of sorghum grains and 40 ml of tap water. The bottles were autoclaved for 30min. Isolate inoculum, taken from one-week-old culture on PDA, was aseptically introduced into the bottle and allowed to colonize sorghum for 3weeks. Batches of autoclaved clay loam soil were inoculated separately with inoculum of each isolate at the rate of 50 g/kg of soil. Infested soil was dispended in 15-cm-diameter clay pots and these were planted with 10 seeds per pot (cultivar Giza 86). In the control treatment, sterilized sorghum grains were mixed thoroughly with soil at a rate of 50g/kg of soil. Pots were randomly distributed on a greenhouse bench under a temperature regime ranged from 20±2C° to 24±2.5C°. Pre-emergence damping-off was recorded 15 days after planting. Post-emergence damping-off, survival, plant height (cm), and dry weight (mg/plant) were recorded 45 days after planting.

Statistical analysis of greenhouse study

The experimental design of greenhouse study was a randomized complete block design with five replicates. Analysis of variance (ANOVA) of

the data was performed with the MSTAT-C statistical package. Least significant difference (LSD) was used to compare treatment means. Percentage data were transformed into arc sine angles before carrying out ANOVA to produce approximately constant variance. Cluster analysis of pathogenicity test was performed with the software package SPSS 6.0.

Extraction of proteins from *F. moniliforme* isolates

Proteins were prepared according to the methods described by Guseva and Gromova (1982). The grown mycelium - for 12 days at 20-30C° on liquid potato dextrose medium - was harvested by filtration through cheesecloth, washed with distilled water several times, and freeze-dried. This frozen mycelium was suspended in phosphate buffer pH 8.3 (1-3 ml/g mycelium), mixed thoroughly with glass beads and ground in liquid nitrogen to a fine powder. The ground mycelium was centrifuged at 19,000 rpm for 30 min. at 0C°. The protein content in supernatant was estimated according to Bradford (1976) by using bovine serum albumin as a standard protein.

Electrophoresis of dissociated protein by SDS-PAGE

Each supernatant was mixed with an equal volume of a solution consisting of (by volume) 64% buffer (0.15 M Tris-HCl, pH6.8), 20% glycerol; 6% SDS; 10% 2-mercaptoethanol and 0.1% bromophenol blue, before boiling in a water bath for 3 minutes. Twenty-microliter samples (40 µg of protein) were subjected to electrophoresis in a 7.5% polyacrylamide gel prepared in 0.1% SDS with a 3.5% stacking gel (Laemmli, 1970). Electrophoresis was conducted at 10°C for 4hrs. in a 7.5% polyacrylamide gel with a 3.5% stacking gel, at 15 and 30mA, respectively, until the dye band reached the bottom of the separating gel (Laemmli, 1970). Electrophoresis was performed in a vertical slab mold (16x18x0.15cm). Gels were stained with silver nitrate for the detection of protein bands (Sammons *et al.*, 1981).

Gel analysis

Protein patterns obtained by SDS-PAGE were clustered by gel documentation system (Uvitec, Cambridge, UK) by the unweighted pair group method of arithmetic means (UPGMA) according to Sneath and Sokal (1973). Similarity coefficient matrix among protein banding patterns was calculated based on the number of shared bands (Nei and Li 1979).

RESULTS AND DISCUSSION

Pathogenicity test of *F. moniliforme* isolates on cotton cultivar Giza 86

Pathogenicity test of *F. moniliforme* isolates on cotton cultivar Giza 86 were shown in Table 2. All *F. moniliforme* isolates were pathogenic and affected all seedling growth variables. Isolates no$_s$. 3, 13, 1 and 10 were the most pathogenic isolates at pre-emergence stage. They caused moe than 50% pre-emergence damping-off. At post-emergence stage, isolates no$_s$. 2, 10 and 3 were the most pathogenic isolates, while isolates no$_s$. 14, 11 and 13 were the least pathogenic isolates at this stage. It is noteworthy that all isolates were more pathogenic in pre-emergence stage except isolate no. 2, which was more pathogenic in post-emergence stage. Isolate no. 5 showed the same level of pathogenicity (28%) at both pre- and Post-emergence stages. On the contrary, Aly *et al.* (1996) tested pathogenicity of some

Fusarium spp. included 8 isolates of *F. moniliforme* , 7 of the 8 isolates were pathogenic to cotton seedlings and 5 of the 7 isolates caused Post-emergence damping-off, they concluded that *F. moniliforme* was the most pathogenic species in terms of mortality during Post-emergence stage. Regarding survival, isolates nos. 10, 2 and 3 were the most pathogenic isolates as they caused 96, 94 and 94% mortality, respectively, while isolate no. 11 was the least pathogenic isolate (40% infection). It is noticable that the pathogenicity of the isolates was not related to their geographic origin, for instant, isolate no. 2 from Daqahliya was highly pathogenic (94% infection), while isolates nos. 5 and 7 also from Daqahliya were moderatly pathogenic (56% infection). Similary, the two isolates of Upper Egypt (isolates nos. 11 and 13) differed in their pathogenicity level. Thus, isolate no. 11 from Assiute caused 40% infection, while isolate no. 13 from Sohag caused 68% infection. The most pathogenic isolates nos. 10, 2, and 3 were from North Delta, East Delta, and Mid-Delta. All isolates of *F. moniliforme* significantly reduced plant height of seedlings. Isolates nos. 3, 10, and 2 were the most effective isolates in reducing plant height. Woodroof (1927) reported that *F. moniliforme* caused dwarfing of cotton plants. Dry weight of seedling significantly decreased as a result of infection by all the tested isolates. The most effective isolates on dry weight were isolates nos. 10 and 3. Abd-Elsalam *et al.* (2006) mintioned that *F. moniliforme* caused decrease in dry weight. It is noteworthy that isolates nos. 3 and 10 were the most pathogenic isolates as they influnced all seedling growth variables.

Table 2. Pathogenicity of *Fusarium moniliforme* isolates on cotton cultivar Giza 86 under greenhouse conditions

Isolate No.	Seedling growth variable							
	Pre-emergence damping-off		Post-emergence damping-off		Survival		Plant height	Dry weight
	%	T[a]	%	T[a]	%	T[a]	(cm)	(mg/plant)
1	52	(46.15)	20	(26.27)	28	(31.88)	14.38	116.80
2	40	(39.18)	54	(45.05)	6	(14.31)	7.30	103.60
3	56	(48.69)	38	(37.85)	6	(14.31)	5.12	73.60
4	42	(40.38)	32	(34.43)	26	(30.55)	13.28	128.00
5	28	(31.75)	28	(31.88)	44	(41.68)	11.24	129.40
6	46	(42.69)	28	(31.75)	26	(30.55)	13.70	121.20
7	26	(30.44)	20	(26.27)	44	(45.00)	13.78	154.40
8	30	(33.21)	28	(31.33)	42	(40.28)	11.26	141.80
9	48	(43.84)	34	(35.62)	18	(24.94)	12.78	142.80
10	52	(46.15)	44	(41.49)	4	(07.38)	6.30	58.40
11	28	(31.88)	12	(18.00)	60	(50.82)	14.26	141.00
12	38	(38.03)	18	(24.94)	44	(41.54)	13.04	154.80
13	56	(48.46)	12	(20.06)	32	(34.29)	12.96	123.20
14	44	(41.54)	10	(16.38)	46	(42.69)	11.28	130.60
Control	6	(11.06)	00	(00.00)	94	(78.94)	23.30	266.40
LSD(P<0.05)	6.14		7.70		8.91		4.48	56.24
(P<0.01)	8.17		10.24		11.84		5.96	74.8

[a] **Percentage data were transformed into arc sine angles before carrying out the analysis of variance to produce approximately constant variance.**

Aly *et al.*, (1996) stated that *F. oxysporum* and *F. moniliforme* were important pathogens in the etiology of cotton damping-off in Egypt. The importance of *F. oxysporum* is due to its high frequency of isolation, while the importance of *F. moniliforme* is due to its high virulence.

The correlation between variables used to evalute pathogenicity of *F. moniliforme* isolates were shown in Table 3. A significant negative correlation (r= -0.615, P<0.05) was observed between Pre-emergence damping-off and dry weight. This correlation implies that higher disease pressure during the Pre-emergence stage, the less the surviving seedlings would be in vigorous. A highly significant negative correlation (r= -0.770, P<0.01) was found between Post-emergence damping-off and survival. Survival was significantly correlated (r= 0.536, P<0.05) with plant height and highly correlation (r= 0.719, P<0.01) with dry weight. A highly significant correlation (r= 0.814, P<0.01) was observed between plant height and dry weight.

Table 3: Correlation [a] between variables used to evaluate pathogenicity of *Fusarium moniliforme* isolates under greenhouse conditions

Variable	Variable			
	2	3	4	5
1. Pre-emergence damping-off %	-0.165	-0.502	-0.295	-0.615*
2. Post-emergence damping-off %		-0.770**	-0.393	-0.366
3. Survival %			0.536*	0.719**
4. Plant height (cm)				0.814**
5. Dry weight (mg/plant)				

[a] Linear correlation coefficient (r) is significant at P< 0.05 (*) or P< 0.01 (**)

Cluster analysis of *F. moniliforme* isolates based on their pathogenicity patterne (Table 4 and Fig.1) showed that the isolates classified into two main groups at 52% similarity level. The first group included isolates no_s. 3 and 10 from Minofiya and Kafr-Elshiekh respectively. The second group included the raimender of the isolates. it is clear that grouping the isolates based on the pathogenicity patterns was not related to their geographic origins.

Table 4. Similarity of *Fusarium moniliforme* isolates involved in cotton seedling damping-off based on their virulence patterns

Isolate	Isolate No.												
No.	1	2	3	4	5	6	7	8	9	10	11	12	13
1	1.000												
2	0.744												
3	0.701	0.848											
4	0.967	0.852	0.687										
5	0.892	0.689	0.424	0.945									
6	0.986	0.848	0.786	0.981	0.870								
7	0.871	0.585	0.311	0.908	0.992	0.828							
8	0.911	0.718	0.462	0.962	1.000	0.859	0.988						
9	0.963	0.884	0.746	0.996	0.902	0.991	0.858	0.925					
10	0.435	0.771	0.952	0.452	0.143	0.560	0.000	0.185	0.528				
11	0.766	0.947	0.646	0.905	0.825	0.849	0.754	0.846	0.913	0.508			
12	0.946	0.664	0.465	0.958	0.985	0.913	0.985	0.990	0.925	0.164	0.785		
13	0.994	0.652	0.640	0.932	0.873	0.957	0.866	0.890	0.922	0.350	0.681	0.940	
14	0.951	0.565	0.45	0.915	0.946	0.891	0.956	0.949	0.877	0.128	0.666	0.983	0.966

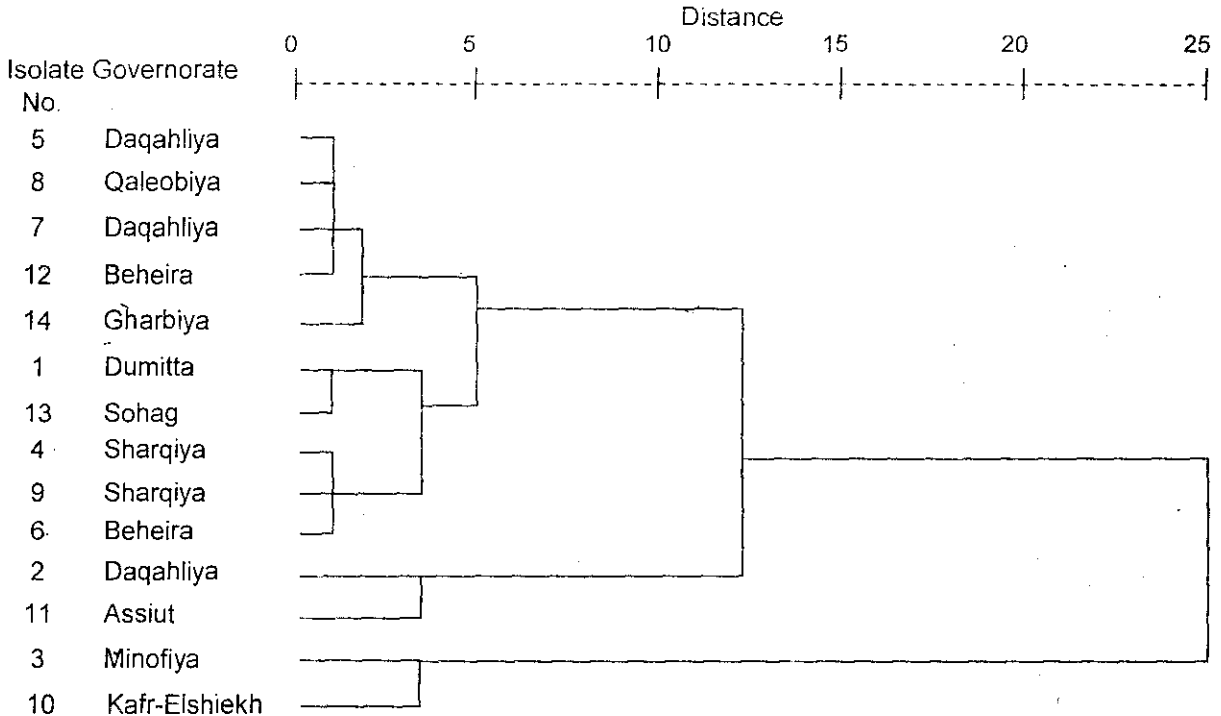

Figure 1. Phenogram of *Fusarium moniliforme* isolates involved in cotton seedling damping-off based on their virulence patterns.

For example, isolates nos. 1 and 13 showed the same pathogenicity patterns although isolate no.1 was obtained from Dumitta, while isolate no. 13 was obtained from Sohag. Similarity between isolate no. 11 from Upper Egypt (Assuite) and isolate no. 2 from East Delta (Daqahliya) was higher than the similarity between the two isolates of Upper Egypt (isolates nos. 11 and 13). These results suggest that populations of *F. moniliforme* are not geographically isolated, because the fungus is not pathologically specialized and it is easily spread by seeds, soil, or air.

Correlation among *F. moniliforme* isolates based on their protein profiles were shown in Table 5 and Figs. 2 and 3. The isolates divided into two main groups at 10% similarity level.

Table 5. Similarity of *Fusarium moniliforme* isolates involved in cotton seedling damping-off based on their electrophoretic protein profiles

Isolate No.	Isolate No.													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	1.00													
2	0.30	1.00												
3	0.10	0.00	1.00											
4	0.27	0.42	0.00	1.00										
5	0.53	0.17	0.13	0.54	1.00									
6	0.47	0.30	0.10	0.40	0.47	1.00								
7	0.28	0.22	0.18	0.48	0.40	0.34	1.00							
8	0.17	0.00	0.33	0.13	0.40	0.25	0.27	1.00						
9	0.44	0.32	0.11	0.36	0.56	0.56	0.37	0.27	1.00					
10	0.43	0.27	0.00	0.32	0.41	0.55	0.17	0.21	0.45	1.00				
11	0.10	0.22	0.00	0.17	0.13	0.10	0.18	0.00	0.11	0.13	1.00			
12	0.39	0.33	0.00	0.36	0.34	0.49	0.13	0.15	0.46	0.56	0.09	1.00		
13	0.10	0.22	0.00	0.17	0.13	0.10	0.18	0.00	0.11	0.13	1.00	0.09	1.00	
14	0.47	0.32	0.17	0.27	0.38	0.40	0.48	0.13	0.29	0.24	0.17	0.30	0.17	1.00

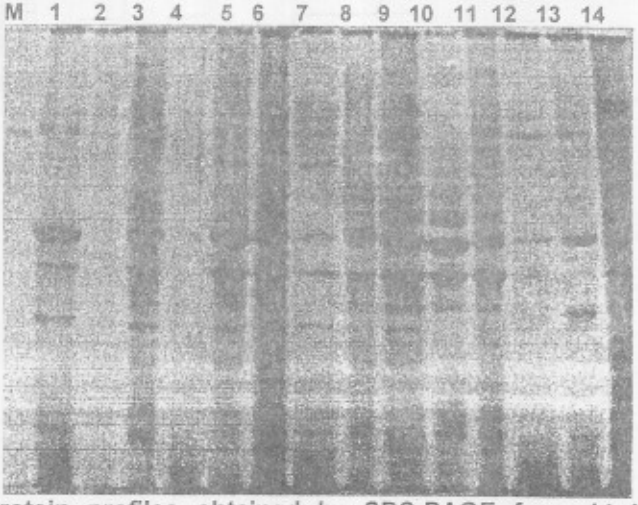

Fig. 2. Protein profiles obtained by SDS-PAGE from 14 isolate of *Fusarium moniliforme*

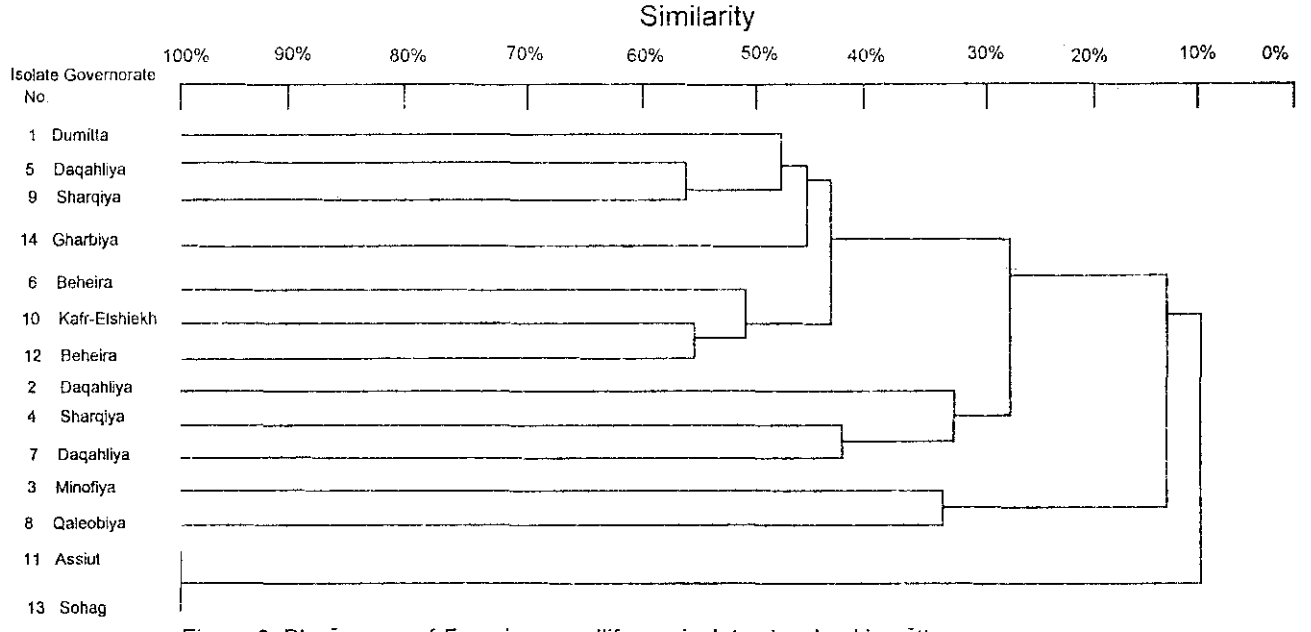

Figure 3. Phenogram of *Fusarium moniliforme* isolates involved in cotton seedling damping-off based on their electrophoretic protein profile.

The first group included isolates of Upper Egypt (11 and 13). The second group included isolates of Lower Egypt (Nile Delta). The second group divided into three sub-groups. The first sub-group included isolates no. 3 from Minofiya and isolate no. 8 from Qaleobiya. The second sub-group included three isolates from East Delta (no$_s$. 2 and 7 from Daqahliya and isolate no. 4 from Sharqiya). The third sub-group included the raimender isolates which divided into three sub-groups. The first sub-group included isolates no$_s$. 6 and 12 from Beheira (West Delta) and isolate no. 10 from Kafr-Elshekh (North Delta). The second sub-group included isolates of East Delta (isolates no$_s$. 1, 5 and 9). The third sub-group included only one isolate (no. 14) from Gharbiya (Mid-Delta). It is noteworthy that isolates from East Delta divided into 2 sub-groups, that is, they are not homogenous. However, the number of Mid-Delta and West Delta isolates was too limited to draw conclusive results concerning the homogeneity of their populations. It is clear that protein profiles were somewhat related to the geographic origins of the isolates. Thus, fungal population in Upper Egypt was genetically different from that in Nile Delta. Aly *et al.*(2003) dimonestrated that there were low correlation between clustering in the protein dendogram and geographic origin. The cluster of isolates based on protein profiles was not related to their pathogenicity. The highly pathogenic isolate no. 10 (caused 96% infection) was placed in the same group with the mild isolate no. 12 (56% infection). Isolates no$_s$. 11 and 13 showed 100% similar in protein profiles, but they were different in their pathogenic potential (40% and 68% infection, respectively).

Our study indicates that *F. moniliforme* is important pathogen in the etiology of cotton damping-off in Egypt due to its high virulence. Future control strategies for cotton damping-off should include control of *F. moniliforme*.

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توصيف عزلات فطر فيوزاريوم مونيليفورمي المسبب لمرض موت بادرات القطن باستعمال القدرة المرضية وأنماط البروتين الناتجة من التفريد الكهربي

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تم تقييم القدرة المرضية لأربع عشرة عزلة من فطر فيوزاريوم مونيليفورمي عزلت من مناطق زراعة القطن المختلفة في مصر على الصنف جيزة ٨٦ تحت ظروف الصوبة. جميع العزلات كانت ممرضة حيث أثرت على جميع المتغيرات المستخدمة في تقييم القدرة المرضية لهذه العزلات. كانت العزلات ٣، ١٣، ١، ١٠ أقوى العزلات من حيث النسبة المئوية للبادرات الميتة قبل ظهورها فوق سطح التربة. في حين كانت العزلات ٢، ١٠، ٣ أقوى العزلات من حيث النسبة المئوية للبادرات الميتة بعد ظهورها فوق سطح التربة. جميع العزلات كانت أكثر مرضية في مرحلة ما قبل ظهور البادرات فوق سطح التربة ما عدا العزلة ٢ والتي كانت أكثر مرضية في مرحلة ما بعد ظهور البادرات فوق سطح التربة. سببت جميع العزلات نقص معنوي في طول البادرات وكذلك في الوزن الجاف للبادرات. لم يكن هناك ارتباط بين القدرة المرضية للعزلات والموقع الجغرافي لهذه العزلات. وجد ارتباط معنوي سالب بين موت البادرات قبل ظهورها فوق سطح التربة وبين الوزن الجاف للبادرات. كما وجد ارتباط سالب عالي المعنوية بين موت البادرات بعد ظهورها فوق سطح التربة وبين البادرات السليمة الباقية على قيد الحياة. أظهر التحليل العنقودي لعزلات الفطر– بناء على نمط القدرة المرضية لها – عدم وجود علاقة بين نمط القدرة المرضية للعزلات وأصلها الجغرافي. عند تقسيم العزلات على أساس أنماط البروتين الناتجة من التفريد الكهربي اتضح أن عشيرة الفطر في مصر العليا مختلفة وراثياً عن عشيرة الفطر في دلتا النيل.