IDENTIFICATION AND PATHOGENICITY OF *FUSARIUM* SPP. ISOLATED FROM FLAX ROOTS

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Abstract

Seven different fungi were isolated from the necrotic root tissues of infected Flax seedlings or older plants. Samples used in isolation were randomly collected from the experimental flax plots at Giza Agricultural Research Station. *Fusarium* spp. were isolated most frequently comprising 41.09%, while frequencies of isolation of the other fungi ranged from 1.00 to 23.97%. Regression analysis revealed that root colonization incidence (RCI) and root colonization severity (RCS) and relationship of root-colonizating fungi of flax conformed to the linear model. According to the generated model, RCI accounted for 89.8% of the total variation in RCS. A total of 52 randomly selected isolates from infected roots were tested for pathogencity on flax cultivar Sakha 1 under greenhouse conditions. The results of pathogenicity test demonstrated that *Fusarium* spp. are the major causal agents of flax seedling blight as they accounted for 54% of the pathogenic isolates in the test. A total of 103 monosporic *Fusarium* isolates were randomly colleced from eight governorates and identified to species level. *F.oxysporum* (63.60%) and *F.solani* (27.35%) were the most predominant species. Other species were *F.moniliforme* (3.88%), *F.lateritium*(1.04%), *F.semitectum* (0.66%) and unidentified *Fusarium* spp. (3.49%). RCI and RCS relationship of root-colonizing fusaria of flax conformed to the linear model. According to the generated model, RCI accounted for 75.3% of the total variation in RCS. Of the 103 isolates, a random sample of 32 isolates were tested for pthogenicity on seedlings of flax cultivar Giza 10 under greenhouse conditions. The results of the pathogenicity test showed that 66.67% of pathogenic isolates belonged to *F.oxysporum*, while 33.33% belonged to *F.solani*. The high frequencies *F.oxysporum* and *F.solani* and their ability to cause considerable losses during seedling stage, strongly suggest that they are the most important fusaria involved in the etiology of seedling blight and root rot of flax in Egypt. Grouping the isolates of *F.oxysporum, F.solani*, and *F.lateritium* by cluster analysis, based on their virulence patterns was neither related to their geographic origins nor to species.

Key words: *Linum usitatimum*, *Fusarium* spp., pathogenicity geographic distribuation, and root colonization.

INTRODUCTION

Flax (*Linum usitatissimum* L.) is the most important bast fiber crop in Egypt, ranking 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.(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 (A.A. 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 germination, 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 roots of recently attacked plants show red to brown lesions, but within a few days they shrivel and turn dark. 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 (Marten *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 (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 (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 (Gruzdeviene *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 disease. Identification and quantification of root-invading fusaria involve more laborious procedures than the simple visual observation needed to detect and quantify the presence of foliar pathogens (Tunali *et al*., 2008).

The main objectives of this investigation were to identify *Fusariam* spp. associated with seedling blight and root rot of flax and to evaluate their pathogenicity to flax seedlings under greenhouse conditions. For comparison, isolation frequencies and pathogenicity of other fungi from flax roots were also evaluated 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 seedling stage through maturity were collected at random from 20 experimental sites (one sample/site) at Giza Agricultural Research station during 2012. 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 genotypes sampled were not determined for all sites because it is known that the genotypes grown in Egypt are all susceptible to seedling blight and root rot (A.A.Aly, *personal observation*). The seedlings and roots collected at each site were stored at 4°C until fungal isolation was performed. Seedling and 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 (killebrew *et al*., 1993) or penicillin G. and rose bengal (100-200 mg/L each) as bacterial inhibitors. The plates were incubated at 26±3°C for 3-7 days. The developing colonies were identified according to Gilman (1966). Colonies of each fungus were expressed as percentage of the total developing

colonies. Pure isolates, selected at random for pathogenicity test, were grown on PDA in petri dishes.

Pathogenicity of *Fusarium* spp. and other fungi isolated from experimental plots.

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-weak-old culture on PDA, was aseptically introduced into the bottle and allowed to colonize sorghum for 3 weeks. The test was 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 Sakha 1). In the control treatments, sterilized sorghum grains were mixed throughtly 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 ambient temperature during the day (the tests were conducted in January and February 2013). Dead seedlings (combined pre-emergence and post-emergence damping-off) were recorded 45 days after planting. Pathogenicity test was repeated once.

Survey of *Fusarium* spp. isolated from commercial flax fields in eight governorates

A random sample of 103 *Fusarium* isolates were isolated from commercial flax fields in eight governorates . Isolation, Purification, and quantification of the isolates were carried out as previously mentioned. The isolates were identified to species level according to Booth (1971).

Pathogenicity of a selected group of *Fusarium* isolates from commercial flax fields

Of the 103 isolates, a random sample of 32 isolates were subcultured on PDA medium for 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 analysis were performed with the software package (SPSS 6.0).

RESULTS

Seven different fungi were isolated from the necrotic root tissues of affected seedlings or older plants, but *Fusarium* spp. were isolated most frequently comprising 41.09 % of all fungi isolated, while frequencies of isolation of the other fungi ranged from 1.00 to 23.97% (Table 1). 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 suggested that prolonged colonization of roots is perhaps the greatest threat posed by fungi, particularly *Fusarium* spp.,for flax health.

In the present study , root colonization severity (RCS) of flax by fungi was estimated by a method involving objective judgment (Table 2); 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 1), may reduce some of these problems. Regression analysis (Table 2 and Fig. 1) revealed that RCI and RCS relationship of root- colonization fungi of flax conformed to the linear model. According to the generated model, RCI accounted for 89.8% of the total variation in RCS.

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 21 fungal pairings were analysed (Table 3). Three (14.3%) of the fungal pairs were significantly associated. Of the three pairs, two were negatively associated and one was positively associated. No significant associations were found in the remaining fungal pairs.

A total of 52 randomly selected isolates were tested for pathogenicity under greenhouse condition (Table 4). *Fusarium* isolates represented 51.92% of the tested

isolates (Table 5). The prevailing environmental conditions during the test were favourable for infection. Thus, all the tested isolates, with isolates 28 and 35 of *Alternaria*, being less pathogenic (Table 4). The results of pathogenicity test demonstrated convincingly that *Fusarium* spp. are the major causal agents of flax seedling blight and root rot in the experimental sites as they accounted for 54% of the pathogenic isolates in the test.

A total of 103 *Fusarium* isolates were randomly collected from eight governorates and identified to the species level (Table 6). *F.oxysporum* Schlecht. (63.60 %) and *F.solani* (Mart.) Sacc. (27.35 %) were the most predominant species. Other species were *F.moniliforme* Sheld. (3.88 %), *F.lateritium* Nees (1.04 %), *F.semitectum* Berk and Ravenel (0.66 %), and unidentified *Fusarium* spp. (3.49 %)

RCI and RCS (Table 7) relationship of root-colonizing fusaria of flax conformed to the linear model. According to the generated model, RCI accounted for 75.3 % of the total variation in RCS (Table 8 and Fig. 2).

Isolation frequency of *F.oxysporum* was negatively correlated with each of isolation frequency of *F.solani* and *F.moniliforme*. The other correlation coefficients shown in (Table 9) were all nonsignificant.

A random sample of 32 monosporic isolates was tested for pathogenicity on seedlings of flax cultivar Giza 10 (Tables 10 and 11). Fourteen (43.75 %) of the tested isolates came from east Delta region.

Data in table (12) showed that the minority of *F.oxysporum* (33.33 %) and the majority of *F.solani* isolates (57.14 %) were pathogenic. On the other hand, the majority of the pathogenic isolates as a whole (66.67 %) belonged to *F.oxysporum*, while the minority (33.33 %) belonged to *F.solani*. Regarding any of the tested variables in table (13), the percentage of pathogenic isolates within *F.solani* was always greater than that in *F.oxysporum*. It is worth noting that some of the pathogenic isolates significantly reduced the percentage of surviving seedling. Therefore, less competition occurred among seedlings in the pots infested with these isolates leading to significant increases in dry weight. Data in table (14) are consistent with those of tables (12) and (13) in that *F.solani* appear to be the most pathogenic *Fusarium* to flax roots in Egypt. A highly significant negative correlation was always observed between survival and each of pre- and postemergence damping-off (Table 15). A significant negative correlation was observed between survival and dry weight only in the case of *F.solani* .

A phenogram based on dissimilarity distance (DD), generated from cluster analysis of virulence patterns of *Fuarium* isolates, is presented in Fig. (3). The smaller the DD, the more closely the isolates were related in their virulence patterns. Two groups of similar isolates were identified. The first group (DD=0.0) included 25 isolates while the second group (DD=11.0) included five isolates. The virulence patterns of isolates 10 and 14 were quite different from the others. Although the first group of isolates were identical in their virulence patterns, they belonged to *F.oxysporum, F.solani,* and *F.lateritium*. These isolates also came from different governorates. Thus, it seems reasonable to conclude that grouping the isolates by cluster analysis was neither related to their geographic origins nor species.

DISCUSSION

In the present study, *Fusarium* spp. were isolated from flax roots collected from widely separated governorates. This may indicate that *Fusarium* spp.are well adapted to colonize flax roots under a wide range of environmental conditions (edaphic factors, crop rotations, irrigation systems, temperature regimes, and so on).

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.

In the present study, regression analysis revealed that RCI and RCS relationship of root-colonization fungi of flax conformed to the linear model.

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 distribuation and abundance of pathogen species. Subsequently, patterns of association result from interrelationships among organisms and also 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).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 ,as we have demonstrated herein for some fungal pairs, shoud 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. 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.

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. On 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. (Gruzdeviene *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. Consequently, seedling blight of flax is controlled largely by using seed-dressing fungicides (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.

Survey of root-colonizing fungi of flax included several fungi not previously reported as root pathogens of flax. *Macrophomina phaseolina* was recorded on flax for the first time anywhere, and *Alternaria* spp., *Aspergillus* spp., and *Curvularia* spp. for the first time in Egypt. It is worth noting that although *Alternaria* spp. and *Aspergillus* spp. were found in low frequencies, they included pathogenic isolates capable of killing flax seedlings in the pathogenicity test (Table 4). *R. solani* has been described as non-specialized and highly virulent (Ogoshi, 1987). In our pathogenicity test (Table 4), all *R. solani* isolates were moderately pathogenic (37 to 55% damping off).

Most of unidentified isolates were found in their non-spore-producing forms. Therefore, we were unable to identify them based on these vegetative (mycelia) phases. Further research is needed to identify these isolates especially if one takes into account that they represented 16% of the total pathogenic isolates in the pathogenicity test (Table 4).

The results of pathogenicity test also revealed that 62.5% of *Fusarium* spp. isolates were nonpathogenic (Table 11). 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 flax in Egypt.

Table 1. Frequency of *Fusarium* spp. compared with frequencies of other fungi isolated from roots of flax plants infected with postemergence damping – off or root rot.

Fungus	Samples[a] from which fungus was isolated		Isolation frequency	
	NO.	%[b]	(%)[c]	transformed[d]
Fusarium spp.	18	90	41.09	38.13
Rhizoctonia solani	10	50	10.19	13.38
Alternaria spp.	6	30	6.22	8.02
Macrophomina phaseolina	9	45	14.55	15.38
Aspergillus spp.	1	5	1.00	1.33
Curvularia spp.	1	5	2.00	1.96
Unidentified	13	65	23.97	24.10
LSD ($p \leq 0.05$)				9.87

[a] Twenty samples of flax roots were randomly collected in 2012 from experimental flax plots at Giza Agricultural Research Station.
[b] Root colonization incidence
[c] Root colonization severity ,which indicates the number of colonies of a fungus expressed as the percentage of the total developing colonies from a sample, and each value is the mean of twenty samples.
[d] percentage data were transformed into arcsine angles before carrying out the analysis of variance to normalize data and stabilize variances throughout the data range.

Table 2. Regression equation[a] that describes the relationship between root Colonization incidence (X) and severity (Y) of flax plants infected with postemergence damping –off or root rot.

Regression equation	**F.value**	**P>F**	**R^2**	**r**
Y= - 3.8969 + 0.4355 X	44.216	0.001	0.898	0.948

[a] Regression equation was generated based on the number of samples from which *Fusarium* spp.and other fungi were isolated (Table 1).

Table 3. Correlation between frequencies of fungi isolated from roots of flax plants infected with postemergence damping – off or root rot.

		Isolation frequency of				
Isolation frequency of	1	2	3	4	5	6
1.*Fusarium* spp.						
2.*Rhizoctonia solani*	-0.084[a](0.726)[b]					
3.*Alternaria* spp.	-0.209(0.375)	-0.442(0.051)				
4.*Macrophomina phaseolina*	-0.123(0.606)	-0.021(0.931)	-0.345(0.137)			
5.*Aspergillus* spp.	-0.395(0.085)	-0.223(0.346)	0.728(0.000)	-0.185(0.436)		
6.*Curvularia* spp.	-0.395(0.085)	0.214(0.364)	-0.134(0.573)	-0.185(0.436)	-0.053(0.826)	
7.Unidentified	-0.558(0.010)	-0.160(0.500)	0.002(0.994)	-0.387(0.092)	-0.044(0.854)	0.177(0.456)

[a] Pearson's correlation coefficient.
[b] Probability level and n=20.

Table 4. Pathogenicity aof Fusarium spp. Compared with pathogenicity of other fungi isolated from roots of flax plants infected with postemergence damping-off or root rot.

Fungus	**Isolate no.**	**Damping-off(%)[c]**
***Fusarium* spp**.	2	34 A-F
	4	42 B-F
	5	54 A-E
	6	55 A-E
	7	32 D-F
	8	60 A-D
	9	34 C-F
	12	43 B-F
	13	60 AB
	14	56 A-D
	15	46 A-F
	17	51 A-F
	19	51 A-F
	20	62 A-C
	21	69 A
	23	57 A-D
	25	39 B-F
	26	52 A-F
	27	48 A-E
	31	32 D-F
	39	40 B-F
	40	40 B-F
	41	35 C-F
	42	42 B-F
	46	45 B-F
	49	40 B-F
	52	50 A-F

(Continued on next page)

Table 4. (Continued from preceding page)

Fungus	Isolate no.	Damping – off (%)
Alternaria spp.	3	41 B –F
	22	56 A-D
	24	46 A-F
	28	27 E-G
	34	39 B-F
	35	25 FG
	37	36 C-F
	50	51 A-F
	51	59 A-D
Rhizoctonia solani	29	47 A-F
	33	37 B-F
	38	40 B-F
	43	55 A-E
	47	45 B-F
Aspergillus spp.	16	56 A-D
	44	51 A-F
Stemphylium sp.	10	45 A-F
Unidentified	1	54 A-E
	11	51 A-F
	18	32 D-F
	30	49 A-F
	32	47 A-F
	36	47 A-F
	45	51 A-F
	48	47 A-F
Control[d]	…	10 G

[a] Pathogenicity of the tested fungi was evaluated on seedling of flax cultivar Sakha1 under greenhouse condition in 2013.

[b] samples of flax roots used in isolation were randomly collected in 2013 from experimental flax plots at Giza Agricultural Research Station.

[c] combined preemergence and postemergence.Percentage data were transformed into arcsine angles before carrying out the analysis of variance to normalize data and stabilize variances throughout the data range.Each value is the mean of five replicates(pots).Means followed by the same letter(s)are not significantly different ($P \leq 0.05$)according to Duncan's multiple range test.

[d] Autoclaved soil was mixed with autoclaved sorghum.

Table 5. Distribution of pathogenic isolates of *Fusarium* spp.and other fungi isolated from flax roots.

Fungus	Total number of Pathogenic isolates	Percentage of isolates Within fungus	Percentage of Total isolates[a]	Percentage of Pathogenic isolates
Fusarium spp.	27	100.00	51.92	54
Unidentified	8	100.00	15.38	16
Alternaria spp.	7	77.78	13.46	14
Rhizoctonia solani	5	100.00	9.62	10
Aspengillus spp.	2	100.00	3.85	4
Stemphylium sp.	1	100.00	1.92	2
Total	50		96.15	100

[a]A total of 52 isolates were tested for pathogenicity on sakha 1 flax seedlings under greenhouse conditions in 2013.The details of pathogenicity test are shown in table (4).

Table 6. Survey of *Fusarium* spp. isolated from flax roots in 2013.

Governorate	*Fusarium*						
	F. oxysporum	***F. solani***	***F. moniliforme***	***F. lateritium***	***F. semitectum***	***F. unidentified***	**Total**
Beheira	12[a]	3	1	0	0	0	16
(West Delta Region)	75.0%[b]	18.8%	6.3%	0.0%	0.0%	0.0%	
Damietta	4	3	0	0	0	0	7
(East Delta Region)	57.1%	42.9%	0.0%	0.0%	0.0%	0.0%	
Daqahliya	12	5	0	0	1	1	19
(East Delta Region)	63.2%	26.3%	0.0%	0.0%	5.3%	5.3%	
Gharbiya	6	8	3	0	0	0	17
(Middle Delta Region)	35.3%	47.1%	17.6%	0.0%	0.0%	0.0%	
Giza	9	1	0	1	0	1	12
(Middle Egypt Region)	75.0%	8.3%	0.0%	8.3%	0.0%	8.3%	
Kafr El sheikh	7	6	1	0	0	0	14
(North Delta Region)	50.0%	42.9%	7.1%	0.0%	0.0%	0.0%	
Sharqiya	9	2	0	0	0	0	11
(East Delta Region)	81.8%	18.2%	0.0%	0.0%	0.0%	0.0%	
Unknouwn	5	1	0	0	0	1	7
	71.5	14.3%	0.0%	0.0%	0.0%	14.3%	
Total	64	29	5	1	1	3	103

[a]Number of isolates of *Fusarium* sp. from a governorate.

[b] Number of isolates of *Fusarium* sp. expressed as the percentage of the total number of isolates of *Fusarium* spp. from a governorate

Table 7. Frequency of *Fusarium* spp isolated from roots of flax plants infected with postemergance damping-off or root rot in 2013.

Fusarium	Governorates[a] from which *Fusarium* was isolated		Isolation frequency	
	Number	%[b]	%[c]	Transformed[d]
F.oxysporum	8	100	63.60	52.21
F.solani	8	100	27.35	30.74
F.moniliforme	3	37.5	3.88	6.85
F.lateritium	1	12.5	1.04	2.09
F.Semitectum	1	12.5	0.66	1.66
Unidentified	3	37.5	3.49	6.53
LS D ($p \leq 0.05$)				10.05

[a] Isolates of *Fasarium* spp. were randomly isolated from commercial flax fields in eight governorates.
[b] Root colonization incidence.
[c] Root colonization serverity, which indicates the number of *Fusarium* sp. isolates recovered from a governorate expressed as the percentage of the total number of isolates of *Fusarium* spp. recovered from the governorate, and each value is the mean of eight replicates (governorates).
[d] Percentage data were transformed into arcsine angles before carrying out the analysis of variance to normalize data and stabilize variances throughout the data range.

Table 8. Regression equation[a] that describes the relationship between root colonization incidence(X) and severity (Y) of flax plants infected with postemergence damping-off or root rot.

Regression equation	F.value	P>F	R^2	r
Y=-10.355 + 0.5405 X	12.162	0.025	0.753	0.867

[a] Regression equation was generated based on the number of governorates from which *Fusarium* spp. were isolated (Table 7).

Table 9. Correlation between frequencies of *Fusarium* spp. Isolated from roots of flax plants infected with postemergence dmping – off or root rot.

Isolation frequency of					
Isolation frequency of	1	2	3	4	5
1. F.oxysporum					
2.F.solani	-0.897[a](0.003)[b]				
3.F.moniliforme	-0.763(0.028)	0.611(0.108)			
4.F.lateritium	0.297(0.475)	-0.515(0.192)	-0.248(0.554)		
5.F.semitectum	-0.010(0.980)	-0.028(0.947)	-0.248(0.554)	-0.143(0.736)	
*6.*Unidentified	0.348(0.398)	-0.620(0.101)	-0.452(0.260)	0.360(0.381)	0.136(0.749)

[a] Pearson's correlation coefficient.

[b] Probability level and n=8.

Table 10. *Fusarium* isolates used in pathogenicity test.

Fusarium	Isolate serial No.	*Fusarium* Collection no.	Geographic origin	
			Governorate	Region
F.oxysporum	1	5/1	Daqahliya	East Delta
	2	5/2	Daqahliya	East Delta
	3	5/3	Daqahliya	East Delta
	4	10	Giza	Middle Egypt
	5	17	Giza	Middle Egypt
	6	21	Unknown	Unknown
	7	34	Giza	Middle Egypt
	8	36	Unknown	Unknown
	9	37	Daqahliya	East Delta
	10	44	Beheira	West Delta
	11	47	Sharqiya	East Delta
	12	49	Sharqiya	East Delta
	13	53	Sharqiya	East Delta
	14	56	Daqahliya	East Delta
	15	57	Gharbiya	Middle Delta
	16	64	Daqahliya	East Delta
	17	66	Beheira	West Delta
	18	76	Beheira	West Delta
	19	84	Beheira	West Delta
	20	85	Kafr El-Sheikh	North Delta
	21	88	Daqahliya	East Delta
	22	104	Sharqiya	East Delta
	23	141	Daqahliya	East Delta
	24	154	Kafr El-Sheikh	North Delta
F.solani	25	30	Unknown	Unknown
	26	35	Giza	Middle Egypt
	27	50	Kafr El-Sheikh	North Delta
	28	90	Gharbiya	Middle Delta
	29	90/1	Gharbiya	Middle Delta
	30	91	Daqahliya	East Delta
	31	140	Daqahliya	East Delta
F.lateritium	32	2	Giza	Middle Egypt

Table 11. Pathogenicity of *Fusarium* spp. Isolated from flax roots on flax seedlings (cultivar Giza 10) under greenhouse conditions in 2014.

Fusarium	Isolate No.	Preemergence damping-off		postemergence damping-off		survival		Dry weight (mg/plant)
		%	Transformed[b]	%	Transformed	%	Transformed	
F.oxysporum	5/1	7[a]	13.65*	2	5.17	91	73.04	44.80
	5/2	10	16.49*	1	2.58	89	70.92*	41.80
	5/3	2	5.17	7	13.65	91	72.90	53.0٠
	10	5	9.96	2	5.17	93	76.59	48.80
	17	1	2.58	17	19.60*	82	69.29*	48.80
	21	3	7.75	13	14.74	84	72.47	48.80
	34	2	5.17	5	9.73	93	76.94	46.80
	36	5	9.96	3	7.75	92	75.48	44.0٠
	37	2	3.69	3	6.27	95	82.10	50.60
	44	5	7.90	88	72.23*	7	9.87*	22.0٠
	47	9	14.86*	38	36.84*	53	47.92*	73.60*
	49	30	30.96*	55	47.90*	15	20.2٠*	65.20*
	53	3	6.27	11	14.12	86	71.32	49.0٠
	56	58	50.02*	21	27.14*	21	18.59*	21.60
	57	22	26.05*	45	41.42*	33	34.53*	68.60*
	64	4	8.86	5	9.73	91	73.13	59.20*
	66	3	4.56	3	4.56	94	80.88	51.80
	76	4	8.86	3	7.75	93	76.59	44.40
	84	4	7.14	4	10.34	92	75.83	46.40
	85	7	13.65*	1	2.58	92	75.48	46.20
	88	8	11.81	10	13.01	82	69.77*	55.20*
	104	6	10.48	1	2.58	93	76.94	47.60
	141	1	2.58	5	8.25	94	80.88	57.0٠*
	154	9	13.38*	1	2.58	90	74.04	44.80

(continued to next page)

Table 11. (continued from preceding page).

Fusarium	**Isolate**	**Preemergence damping-off**		**postemergence damping-off**		**survival**		**Dry weight**
	No.	**%**	**Transformed**	**%**	**Transformed**	**%**	**Transformed**	**(mg/plant)**
F.solani	30	5	9.96	2	5.17	93	78.30	49.0٭
	35	5	12.92	5	9.96	90	72.03	42.60
	50	46	42.29*	21	24.07*	33	31.70*	57.60*
	90	6	10.86	16	18.45*	78	66.01*	41.80
	90/1	4	8.86	7	11.17	89	73.89	47.20
	91	52	46.15*	40	39.18*	8	16.0٭*	76.40*
	140	16	15.64*	6	10.83	78	68.84*	67.0٭*
F.lateritium	2	5	11.44	1	2.58	94	75.98	42.20
Control[c]	…	0	0.00	1	2.58	99	87.42	29.60
LSD (P ≤ 0.05)		13.29		14.00		16.51	23.46	
LSD (P ≤ 0.01)		17.56		18.50		21.82	31.01	

a Mean of five replicates (pots).

b percentage data were transformed into arcsine angles before carrying out the analysis of variance to normalize data and stabilize variances throughout the data range.

* Significant difference from the control.

c Autoclaved soil was mixed with autoclaved sorghum.

Table 12 . Distribution of pathogenic isolates of Fusarium spp. from flax roots.

Fusarium sp.	**Total number of pathogenic isolates**	**Percentage of isolates within fungus**	**Percentage of total isolates [a]**	**Percentage of pathogenic isolates**
F.oxysporum	8	33.33	25	66.67
F.solani	4	57.14	12.5	33.33
F.lateritium	0	0.00	0.00	0.00
Total	12	0.00	37.5	100.0

[a]A total of 32 isolates were tested for pathogenicity on flax seedings (cultivar Giza 10) under greenhouse conditions.14

Table 13 . Distribution of Fusarium spp. based on their pathological effects on seedlings of flax cultivar Giza 10 under greenhouse conditions.

Fusarium	Total number of tested isolate	Percentage of isolate ,which significantly affected[a]			
		Pre emergence damping –off(%)	post emergence damping –off(%)	Survival (%)	Dry weight
F.oxysporum	24	33.33	25.0	33.33	25.0
F.solani	7	42.86	42.86	57.14	42.86
F.lateritium	1	0.00	0.00	0.00	0.00

[a] The tested isolates significantly increased pre- and postemergence damping-off and Dry weight, subsequently significantly decreased survival.

Table 14. The relative pathogenicity of Fusarium oxysporum and F. solani on flax seedlings (cultivar Giza 10) under greenhouse conditions.

Fusarium	number of tested isolates	Damping-off (%)[a]
F. oxysprum	24	25.88
F.solani	7	33.94
LSD (p≤0.05)		N.S.

[a] Combined pre- and postemergence damping-off, and each value is the mean of the tested isolates.

Table 15. Correlation among variables used for evaluating pathogenicity of Fusarium spp. Isolates on flax seedlings (cultivar Giza 10) under greenhouse conditions.

Fusarium	**Variable**		**Variable**	
		1	**2**	**3**
F.oxysporum	1.preemergence damping –off (%)			
(n=24)	2.postemergence damping –off (%)	0.320[a] (0.127)[b]		
	3.Survival (%)	-0.688 (0.000)	-0.908 (0.000)	
	4.Dry weight (mg/plant)	-0.212 (0.320)	-0.091 (0.672)	0.164 (0.445)
F.solani	1.preemergence damping –off (%)			
(n=7)	2.postemergence damping –off (%)	0.865 (0.012)		
	3.Survival (%)	-0.979 (0.000)	-0.949 (0.001)	
	4.Dry weight (mg/plant)	0.784 (0.037)	0.658 (0.108)	-0.759 (0.048)
All fusaria[c]	1.preemergence damping –off (%)			
(n=32)	2.postemergence damping –off (%)	0.386 (0.029)		
	3.Survival (%)	-0.778 (0.000)	-0.880 (0.000)	
	4.Dry weight (mg/plant)	0.100 (0.587)	0.016 (0.933)	-0.062 (0.736)

[a] Pearson's correlation coefficient.

[b] Probability level.

[c] All fusaria included *F. oxysporum* (24 isolates) , *F.solani* (seven isolates) , and *F.lateritium* (one isolate)

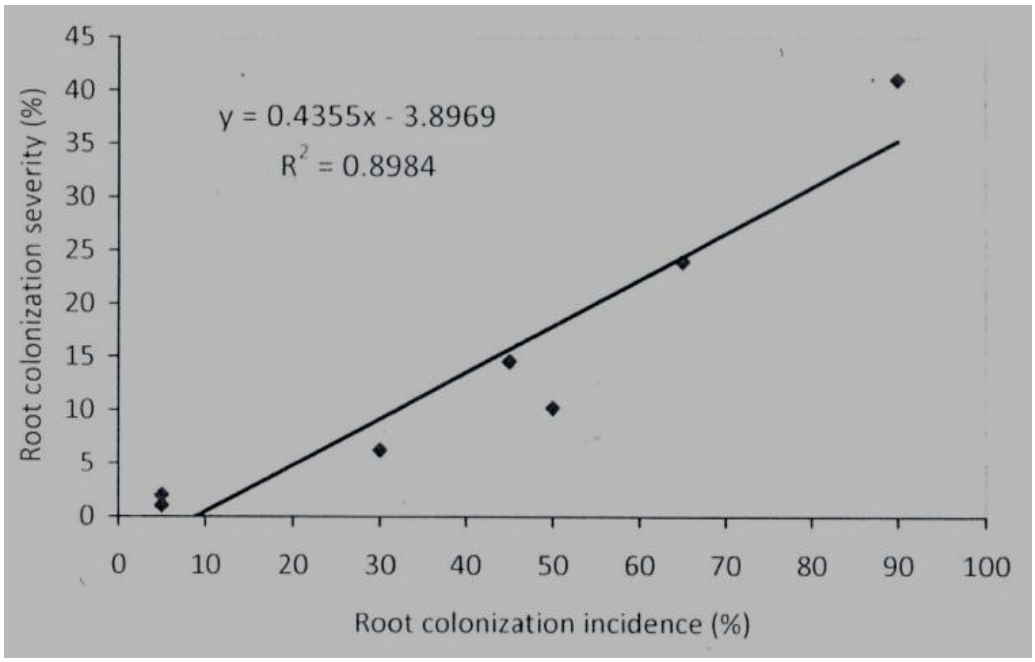

Fig. 1. Relationship between root colonization incidence and severity of flax plants infected with *Fusarium* spp. and other fungi involved in postemergence damping-off and root rot.

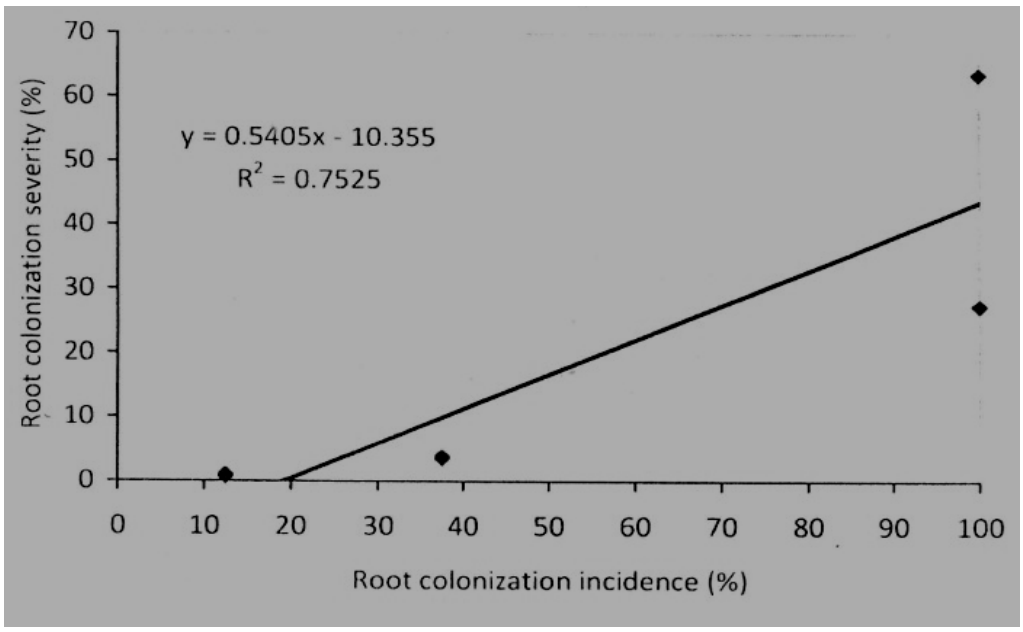

Fig. 2. Relationship between root colonization incidence and severity of flax plants infected with Fusarium spp. involved in postemergenc damping-off and root rot.

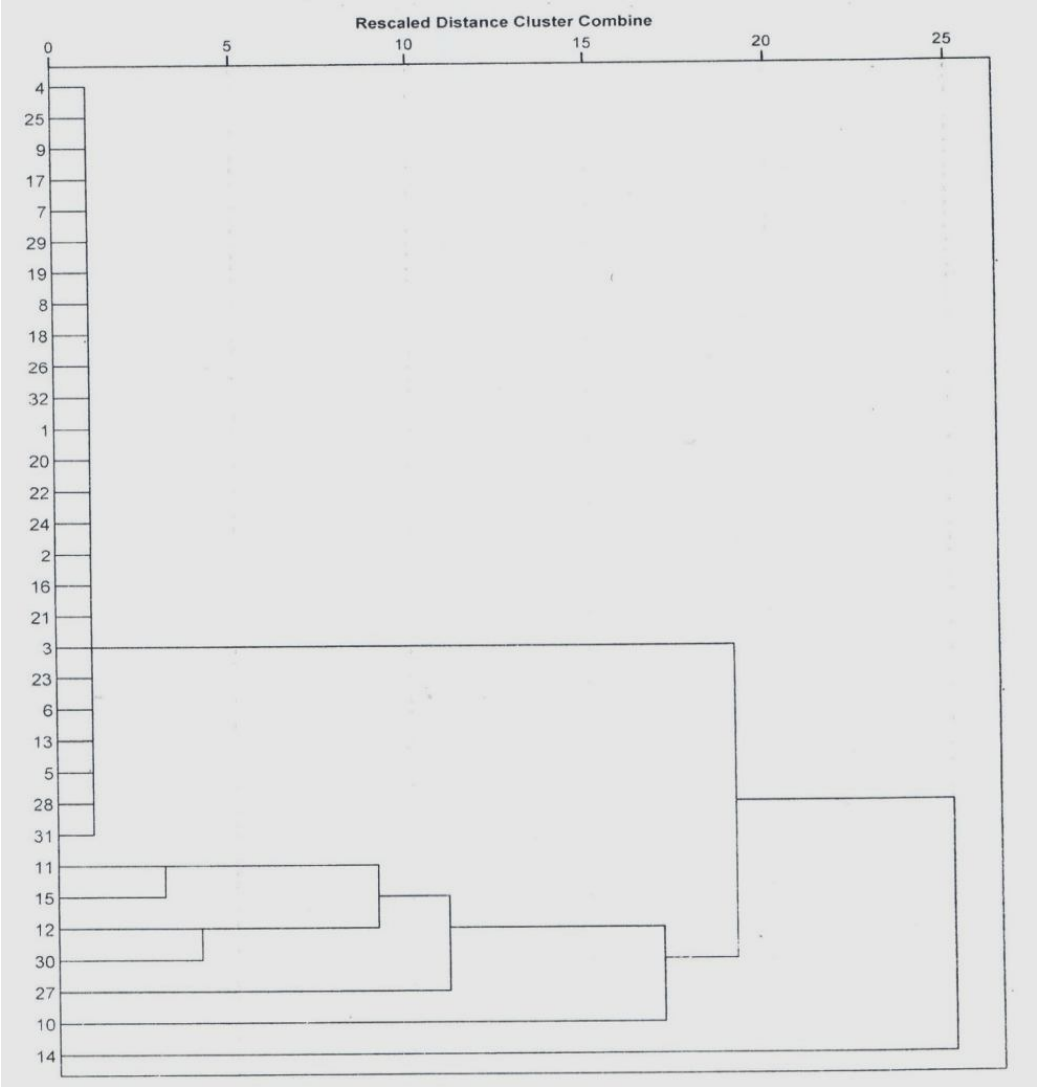

Fig. 3. Phenogram based on average linkage cluster analysis of virulence of 32 isolates of Fusarium spp. on flax seedlings (cultivar Giza 10) under greenhouse conditions. Virulence of the isolates was evaluated based on pre- and postemergence damping-off, survival, and dry weight. Identification of the tested Fusarium spp isolates and their geographic origins are shown in Table 10.

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تصنيف وتقييم القدرة المرضية لأنواع الفيوزاريوم المعزولة من جذور الكتان

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عزلت سبعة فطريات من الأنسجة الميته لجذور بادرات الكتان أو النباتات البالغة. العينات المستعملة فى العزل جمعت بطريقة عشوائية من القطع التجريبية المزروعة بالكتان فى محطة البحوث الزراعية بالجيزة.كانت الأنواع التابعه لجنس الفيوزاريوم هى الأعلى تكراراً فى العزل بنسبه وصلت الى٤١,٠٩% من إجمالى الفطريات المعزولة، فى حين تراوح تكرار عزل الفطريات الأخرى من ١,٠٠ إلى ٢٣,٩٧ %.أظهر تحليل الإنحدار أن العلاقة بين حدوث إستعمار للجذر و شدة إستعمار الجذر بواسطة الفطريات المعزولة هى علاقة خطية . أظهر نموذج الإنحدار المتحصل عليه أن حدوث إستعمار للجذر كان مسئولا عن ٨٩,٨٠%. من التباين الكلى فى شدة إستعمار الجذر. أجرى إختبار القدرة المرضية، تحت ظروف الصوبة، على صنف الكتان سخا ١ ، لعدد ٥٢ عزلة أختيرت بطريقة عشوائية من إجمالى العزلات التى أمكن الحصول عليها من جذور الكتان المصابة . أظهر إختبار القدرة المرضية أن عزلات جنس الفيوزاريوم هى المسبب الرئيسى لمرض لفحة بادرات الكتان إذ أنها مثلت ٥٤% من إجمالى العزلات الممرضة فى هذا الإختبار. جمعت ١٠٣ عزلة فيوزاريوم بطريقة عشوائية من ثمانى محافظات ، و عرفت إلى مستوى النوع بعد تنقيتها بطريقة الجرثومة الفردية . أظهرت النتائج المتحصل عليها أن فيوزاريوم أوكسيسبورم و فيوزاريوم سولانى كانا أكثر الأنواع تكراراً فى العزل حيث وصل تكرار عزلهما إلى ٦٣,٦٠ و ٢٧,٣٥%. على الترتيب ، أما الأنواع الأخرى و هى فيوزاريوم مونيليفورمى و فيوزاريوم لاتيريتيم و فيوزاريوم سيميتيكتم وأنواع لم يمكن تعريفها فقد كانت تكرارت عزلها هى ٣,٨٨ و ١,٠٤ و ٠,٦٦ و ٣,٤٩%. على الترتيب. أظهر تحليل الإنحدار أن العلاقة بين حدوث إستعمار للجذر و شدة إستعمار الجذر بواسطة أنواع الفيوزاريوم هى علاقة خطية. أظهر نموذج الإنحدار المتحصل عليه أن حدوث إستعمار للجذر كان مسئولاً عن ٧٥,٣٠% من التباين الكلى فى شدة إستعمار الجذر. أختبرت عينة عشوائية، تتكون من ٣٢ عزلة من ال ١٠٣ عزلة سالفة الذكر، من حيث القدرة المرضية على صنف جيزة ١٠، تحت ظروف الصوبة. أظهر إختبار القدرة المرضية أن ٦٦,٦٧ و ٣٣,٣٣ % من العزل االممرضة كانت تتبع نوعى فيوزاريوم أوكسيسبورم و فيوزاريوم سولانى، على الترتيب. إن التكرار المرتفع لعزلات هذين النوعين، بالاضافة إلى قدرتهما. على إحداث مستوى مرتفع من الإصابة للبادرات فى إختبار القدرة المرضية ، يدل على أن هذه العزلات هى المسبب الأساسى لمرضى لفحة بادرات الكتان و عفن جذور النباتات البالغة . أمكن – باستخدام التحليل العنقودى – تقسيم ال٣٢ عزلة إلى مجموعات ، بناءً على ما بينها من تباين فى أنماط القدرة المرضية ، إلا أن هذه المجموعات لم ترتبط بالأصل الجغرافى للعزلات أو نوعها.