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COTTON SEEDBORNE FUNGI AND THEIR EFFECT ON INCIDENCE OF COTTON SEEDLING DISEASE

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ABSTRACT

Surface-sterilized and nonsterilized seeds from eight commercial cultivars of cotton (*Gossypium barbadense* L.) were examined for qualitative and quantitative estimates of seedborne fungi. The observed fungi were *Alternaria alternata*, *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus* spp., *Cephalosporium* sp., *Cladosporium* sp., *Drechslera* spp., *Fusarium moniliforme*, *Fusarium oxysporum*, *Fusarium semitectum*, *Fusarium solani*, *Fusarium* sp., *Nigrospora oryzae*, *Penicillium* spp., *Rhizoctonia solani*, *Rhizopus stolonifer*, *Trichoderma* spp. and *Trichothecium roseum*. The quantitative estimates of the fungi showed that *A. niger* (71%), *Penicillium* sp. (34%) and *Cladosporium* sp. (25.63%) were the most dominant fungi isolated from the nonsterilized seeds. Other fungi occurred at frequencies ranged from 0.13 to 22.50%. The isolation frequencies of *A. niger*, *Cephalosporium* sp., *Cladosporium* sp., and *T. roseum* were significantly decreased by surface sterilization, while the isolation frequencies of the other fungi were not affected. These results may suggest that *A. niger*, *Cephalosporium* sp., *Cladosporium* sp., and *T. roseum* tended to colonize the outer seed coat more than embryos, while the other fungi tended to colonize the internal parts of the seeds. Cultivar and cultivar x treatment interactions were all very highly significant or significant sources of variation in frequencies of the isolated fungi except *F. oxysporum*. Cultivar was the first in importance as a source of variation in frequencies of 6 (40%) of the isolated fungi, while cultivar x treatment interaction was the first in importance as a source of variation in frequencies of 5(33.33%) of the isolated fungi. No single cultivar yielded all the 18 fungi. Giza 70 yielded the highest number of fungi (14 fungi), while Giza 85 yielded the lowest number (9 fungi). The other cultivars yielded a number of fungi ranged from 10 to 13. *A. alternata*, *A. niger*, *F. moniliforme* and *Penicillium* sp. were the only fungi, which were isolated from all the tested cultivars. The present study showed that the role of seedborne fungi of cotton, as seedling disease incitants, was more evident in the preemergence stage compared with the postemergence stage. Pearson correlation coefficient was calculated to evaluate the degree of association among 153 pairs of the isolated fungi. Eleven (7.19%) of the fungal pairs were significantly associated. Of the 11 pairs, 9 were positively associated, and 2 were negatively associated. No significant associations were found in the remainder fungal pairs. Cluster analysis divided the isolated fungi into distinct groups. One group consisting of *Drechslera* spp., *F. solani*, *Cephalosporium* sp., *F. semitectum*, *A. alternata*, *F. moniliforme*, *A. niger*, *Cladosporium* sp., *A. flavus*, *Fusarium* sp., *Penicillium* spp. and *Trichoderma* spp., and a second group consisting of *R. solani*, *T. roseum*, *Aspergillus* spp., *N. oryzae*, *R. stolonifer,* and *F. oxysporum*. Within each group, fungi were associated strongly and positively, whereas between groups, fungi were associated weakly or negatively. This result implies the potential existence of cultivar related groups of fungi. Four regression models, derived from stepwise multiple regression analysis, were constructed to describe the effect of the isolated fungi (independent variables) on seedling disease variables (dependent variables). These models showed that differences in seedling disease variables were due largely to the effects of *N. oryzae*, *F. semitectum*, *R. stolonifer*, *R. solani,* and *Trichoderma* spp. It is worth noting that no regression model was constructed to predict postemergence damping-off, which reconfirms that soilborne fungi of cotton are more important, as seedling disease incitants, in the preemergence stage compared with the postemergence stage.

INTRODUCTION

When a cotton boll opens, the seeds within it are in the best condition they will ever be. Most are well supplied with nutrients; a few less so. However, without special precautions, cottonseed begins at once to deteriorate; I.e., decline in quality and quantity of stored nutrients and consequently in potential for vigorous germination (Halloin and Bourland, 1981). The most serious economic losses from seed deterioration occur when the quality of planting seed is reduced. Deteriorated planting seed may produce sparse stands, necessitating expensive replanting. Young seedlings grown from deteriorated seeds have necrotic cotyledons, abnormal roots and increased susceptibility to seedling disease organisms. Plants that survive the seedling stage have decreased vigor and delayed maturation (Halloin and Bourland, 1981).

Deterioration also lowers substantially the value of seeds for processing. Oil from deteriorated seeds is high in free fatty acids and is discolored. Discolored oil is commercially undesirable and may require additional processing. Deteriorated seeds may contain mycotoxins that render them unsuitable for consumption (Halloin and Bourland, 1981; Amer, 1986 and Cotty, 2001).

Cottonseed deterioration requires high seed moisture and conditions that favor the growth of microorganisms, particularly fungi. Under Egyptian conditions, these fungi include *Alternaria* spp., *Aspergillus* spp., *Cephalosporium* spp., *Cladosporium* spp., *Curvularia* spp., *Fusarium moniliforme*, *F. oxysporum*, *F. semitectum*, *F. solani*, *Helmenthosporium* spp., *Nigrospora* spp., *Pythium* spp., *Rhizoctonia solani*, *Trichothecium* spp., *Epicoccim* spp., *Penicillium* spp., *Chaetomium* sp., *Diplodia gossypii*, *Rhizopus* sp. and others (Mostafa, 1959; El-Helaly *et al.*, 1966; Bakry and Rizk, 1967; Abd El-Aziz and Morsey, 1969; Abd El-Aleem, 1979; Waked *et al.*, 1981; Amer, 1986 and Mohamed, 1999).

Fungal microflora of cottonseed are classified into two groups; field and storage fungi. Field fungi usually invade the maturing cottonseeds on the developing plants in the field before harvest of bolls. These fungi require a moisture content in equilibrium with a relative humidity of more than 90% to grow. The storage fungi are those that grow on stored seeds. Most of them are able to grow without free water, and on media with high osmotic pressure (Amer. 1986).

The main objectives of this investigation were to identify fungi associated with seeds of some Egyptian cotton cultivars, and to evaluate their relationship to incidence of cotton seedling disease under greenhouse conditions. Patterns of association of the isolated fungi were also examined.

MATERIALS AND METHODS

Isolation of seedborne fungi:

Random seed samples for cotton (*Gossypium barbadense* L.) cultivars were obtained from Cotton Research Institute, Agric. Res. Center, Giza, Egypt. A random subsample of 100 seeds for each cultivar was surface sterilized in 10% Clorox solution for 2 minutes, and washed several times in sterilized water. The surface sterilized seeds were then blotted dry between sterilized filter papers.

Occurrence of seedborne fungi was determined by the standard blotter method (ISTA, 1993). Ten nonsterilized or surface-sterilized seeds for each cultivar selected at random were placed on three layers of damp 9-cm Whatman No. 1 filter paper in Petri dishes and each was replicated ten times. The plates were incubated in 12-hr light and 12-hr darkness at 20±2°C for 7 days. After incubation, each colony was examined macroscopically or microscopically for identification to genus or species level according to Gilman (1966), Booth (1971) or Barnet and Hunter (1979). Isolation frequency of each fungus was expressed as the percentage of seeds from which the fungus grew. If more than one fungus grew from the same seed, each was counted.

Assessment of cotton seedling disease variables:

Autoclaved soil was dispensed in 10-cm-diameter clay pots and these were planted with 10 nonsterilized seeds per pot for each cultivar. Pots were distributed on a greenhouse bench under temperature regime ranged from 23±5°C to 37±6°C. Percentage of preemergence damping off was recorded 15 days after planting. Postemergence damping-off, infection, plant height and dry weight were recorded 40 days after planting.

Statistical analysis of the data:

Percentage data of isolation frequencies were transformed into $\sqrt{x}$, $\sqrt{0.5+x}$, or arc sine angles before carrying out analysis of variance (ANOVA) to normalize and stabilize variance. The least significant difference (LSD) was used to identify differences in frequencies among fungi. ANOVA of the data was performed with MSTAT-C statistical package (A Microcomputer Program for The Design, Management and Analysis of Agronomic Research Experiments, Michigan State Univ., USA). Pots were distributed on a greenhouse bench in a completely randomized block design of ten replications. Linear correlation coefficients were calculated to evaluate the degree of association among fungi, among seedling disease variables and between fungi and seedling disease variables. Stepwise regression technique with greatest increase in R^2 as the decision criterion was used to describe the effect of seedborne fungi on seedling disease variables. Correlation and regression analyses were performed with a computerized program.

RESULTS AND DISCUSSION

The mean percentage of fungal recovery from cottonseeds (Table 1) showed that *Aspergillus niger* (71%), *Penicillium* sp. (34%) and *Cladosporium* sp. (25.63%) were the most dominant fungi isolated from the nonsterilized cottonseeds. Other fungi occurred at frequencies ranged from 0.13 to 22.5%. The dominance of *A. niger* relative to the other fungi isolated from cottonseeds is consistent with the findings of Simpson *et al.* (1973) who found that *A. niger* was a dominant fungus at several locations in their study, infecting up to 23% of the seeds. *Cladosporium* sp. and *Penicillium* sp. are among the fungi involved in cotton boll rot and may cause deterioration in fiber quality under favourable environmental conditions (Abd El-Rehim *et al.*, 1993). *Cladosporium* sp. is also involved in sooty mold of cotton (Zayed, 1997). *Alternaria* has been reported as a dominant member of the mycoflora of cottonseed by Davis (1977). However, *Alternaria* was listed as an infrequent fungus by Roncadori *et al.* (1971), and was present in more than 10% of the seeds from only one location in the study by Simpson *et al.* (1973). Klich (1986) found *A. alternata* in more than 10% of the seed.

Table 1. Frequencies of fungi isolated from nonsterilized and sterilized cottonseeds.

Fungus	Isolation frequency			
	%		Transformed[a]	
	T1[b]	T2	T1	T2
Alternaria alternata	13.88	8.63	20.24	15.22
Aspergillus flavus	17.13	9.75	21.60	17.60
Aspergillus niger	71.00	42.50	58.55	40.64
***Aspergillus* spp.**	9.25	13.25	13.89	17.14
***Cephalosporium* sp.**	22.5	1.25	24.92	4.78
***Cladosporium* sp.**	25.63	1.63	28.26	5.96
***Drechslera* spp.**	0.50	0.00	1.44	0.00
Fusarium moniliforme	9.50	3.25	17.16	7.62
Fusarium oxysporum	0.75	0.50	2.88	1.96
Fusarium semitectum	1.75	2.00	4.16	5.46
Fusarium solani	1.75	0.63	4.21	2.26
***Fusarium* sp.**	5.75	1.88	10.54	5.91
Nigrospora oryzae	0.13	0.25	0.72	3.21
***Penicillium* spp.**	34.00	25.63	34.99	28.83
Rhizoctonia solani	0.38	1.50	1.25	2.53
Rhizopus stolonifer	13.00	14.25	16.74	19.56
***Trichoderma* spp.**	10.75	2.13	12.58	3.99
Trichothecium roseum	11.13	0.50	12.63	1.96
L.S.D. (P= 0.05)			10.04	
L.S.D. (P= 0.01)			13.27	

[a] Percentage data were transformed into arc sine angles before carrying out the analysis of variance to produce approximately constant variance.
[b] T1= Nonsterilized seeds and T2= Sterilized seeds.

In the present study, *A. alternata* was found in 13.88% of the seed. Generally, fusaria were major components of the fungal flora in earlier studies (Simpson *et al.*, 1973 and Roncadori *et al.*, 1971). In the present study, *Fusarium* spp. collectively was found in 19.5% of the seed. However, one should keep in mind that taxonomic changes in the genus *Fusarium* makes comparisons to earlier studies difficult. The isolation frequencies of *A. niger*, *Cephalosporium* sp., *Cladosporium* sp. and *Trichothecium roseum* were significantly decreased by surface sterilization. However, isolation frequencies of the other fungi were not significantly affected by surface sterilization.

These results may indicate that *A. niger*, *Cephalosporium* sp., *Cladosporium* sp., and *T. roseum* tended to colonize the outer seed coat more than embryos, while the other fungi tended to colonize the internal parts of the seed.

Cultivar and cultivar x treatment interactions were all very highly significant or significant sources of variation in frequencies of fungi isolated from seeds except *F. oxysporum* (Table 2). Cultivar was the first in importance as a source of variation in frequencies of 6(40%) of the isolated fungi, while cultivar x treatment interaction was the first in importance as a source of variation in frequencies of 5(33.33%) of the isolated fungi (Table 3). Due to the significance of this interaction, an interaction LSD was used to compare between means of nonsterilized and sterilized seeds within each cultivar for each of the tested fungi (Table 4). These comparisons showed that the effect of surface sterilization on frequencies of fungi isolated from seeds varied depending upon the cultivar used in isolation. For example, sterilization significantly reduced the frequency of *Penicillium* isolated from Giza 70, while it did not affect the frequency when *Penicillium* was isolated from Giza 83. Frequencies of *A. niger* isolated from Giza 70 and Giza 86 were significantly reduced by sterilization, while the isolation frequencies were not affected by sterilization in the case of Giza 89 and Giza 90. The significant role of cotton cultivar in determining the frequencies of fungi isolated from cottonseed, as we have demonstrated herein, could by attributed to the heritable anatomical characteristics of the seed, which may vary from one cultivar to another, however, our results are in sharp contrast with the findings of Davis (1982) and Klich (1986) who reported that fungal infection of cottonseed was apparently not substantially influenced by cultivar.

A total of 18 fungi were identified among the 8 cultivars that were tested (Table 5). No single cultivar yielded all the 18 fungi. Giza 70 yielded the highest number of fungi (14 fungi), while Giza 85 yielded the lowest number (9 fungi). The other cultivars yielded a number of fungi ranged from 10 to 13. *A. alternata*, *A. niger*, *F. moniliforme* and *Penicillium* sp. were the only fungi, which were isolated from all the tested cultivars.

In the present study, nonsterile seeds were planted in autoclaved soil, therefore, it seems reasonable to conclude that the seedborne fungi of cotton were the only sources of seedling infection. Disease pressure during pre-emergence stage was higher than that during postemergence stage for all the tested cultivars in particular Giza 70 and Giza 80. In addition, preemergence damping-off was positively correlated with infection (Table 6). Taken together, these results imply that the role of seedborne fungi of cotton, as seedling disease incitants, was more evident in the preemergence stage compared with the postemergence stage.

Table 2. Analysis of variance of effects of sterilization, cultivar and their interaction on frequencies of fungi isolated from cottonseeds.

Fungus	Source of variation	d.f.	Mean square	F. value	P > F
Rhizopus stolonifer	Treatment (T)	1	0.374	0.0764	
	Cultivar (C)	7	81.577	16.6515	0.0000
	T X C	7	18.428	3.7616	0.0009
	Error	144	4.899		
Penicillium spp.	Treatment (T)	1	1038.921	3.6603	0.0577
	Cultivar (C)	7	3355.837	11.8232	0.0000
	T X C	7	586.001	2.0646	0.0510
	Error	144	283.833		
Alternaria alternata	Treatment (T)	1	24.258	5.9016	0.0164
	Cultivar (C)	7	44.989	10.9452	0.0000
	T X C	7	20.364	4.9543	0.0000
	Error	144	4.110		
Aspergillus flavus	Treatment (T)	1	5.347	0.9943	
	Cultivar (C)	7	25.094	4.6660	0.0001
	T X C	7	34.859	6.4817	0.0000
	Error	144	5.378		
Aspergillus niger	Treatment (T)	1	8163.448	24.07	0.0000
	Cultivar (C)	7	2147.142	6.331	0.0000
	T X C	7	1122.688	3.3114	0.0027
	Error	144	339.037		
Fusarium sp.	Treatment (T)	1	69.116	24.2112	0.0000
	Cultivar (C)	7	20.273	7.1015	0.0000
	T X C	7	20.960	7.3423	0.0000
	Error	144	2.855		
Trichothecium roseum	Treatment (T)	1	161.805	139.8560	0.0000
	Cultivar (C)	7	29.028	25.0903	0.0000
	T X C	7	28.930	25.0053	0.0000
	Error	144	1.157		
Aspergillus spp.	Treatment (T)	1	3.136	0.9530	
	Cultivar (C)	7	69.268	21.0509	0.0000
	T X C	7	27.480	8.3513	0.0000
	Error	144	3.290		
Cladosporium sp.	Treatment (T)	1	468.883	69.8247	0.0000
	Cultivar (C)	7	30.164	4.4920	0.0001
	T X C	7	28.636	4.2643	0.0003
	Error	144	6.715		
Cephalosporium sp.	Treatment (T)	1	503.117	135.7123	0.0000
	Cultivar (C)	7	24.275	6.5473	0.0000
	T X C	7	19.806	5.3420	0.0000
	Error	144	3.708		

Table 2. Cont.

Fungi	Source of variation	d.f.	Mean square	F. value	P > F
Fusarium oxysporum	Treatment (T)	1	0.021	0.0479	
	Cultivar (C)	7	0.402	0.9192	
	T X C	7	0.559	1.2777	0.2654
	Error	144	0.437		
Fusarium semitectum	Treatment (T)	1	0.010	0.0163	
	Cultivar (C)	7	3.860	6.2546	0.0000
	T X C	7	5.808	9.4109	0.0000
	Error	144	0.617		
Fusarium solani	Treatment (T)	1	1.016	2.2507	0.1357
	Cultivar (C)	7	2.348	5.2019	0.0000
	T X C	7	3.496	7.7439	0.0000
	Error	144	0.451		
Trichoderma spp.	Treatment (T)	1	70.610	64.8116	0.0000
	Cultivar (C)	7	20.538	18.8512	0.0000
	T X C	7	20.906	19.1895	0.0000
	Error	144	1.089		
Fusarium moniliforme	Treatment (T)	1	109.809	30.1332	0.0000
	Cultivar (C)	7	23.269	6.3853	0.0000
	T X C	7	8.164	2.2402	0.0342
	Error	144	3.644		

Table 3. Relative contribution of treatment, cultivar and their interaction to variation in frequency of fungi isolated from cottonseeds.

Fungus	Relative contribution to variation in isolation frequency[a]		
	Treatment (T)	Cultivar (C)	T x C
Rhizopus stolonifer	0.05	81.53	18.42
Penicillium spp.	3.63	82.04	14.33
Alternaria alternata	5.04	65.37	29.59
Aspergillus flavus	1.26	41.33	57.68
Aspergillus niger	26.29	48.40	25.31
Fusarium sp.	19.32	39.67	41.01
Trichothecium roseum	28.51	35.80	35.68
Aspergillus spp.	0.46	71.27	28.27
Cephalosporium sp.	53.25	23.98	22.77
Cladosporium sp.	61.99	20.93	17.08
Fusarium oxysporum	0.31	41.72	57.98
Fusarium semitectum	0.01	39.92	60.07
Fusarium solani	2.42	39.21	58.37
Trichoderma spp.	19.57	39.85	40.57
Fusarium moniliforme	33.29	49.38	17.33

[a] Calculated as percentage of sum of squares of the explained (model) variation.

Table 4. Frequencies of fungi isolated from nonsterilized and sterilized cottonseeds of eight cultivars.

Cultivar	Isolate frequency of																			
	Rhizopus stolonifer				*Penicillium spp.*				*Alternaria alternata*				*Aspergillus flavus*				*Aspergillus niger*			
	T1 [a]		T2		T1		T2		T1		T2		T1		T2		T1		T2	
	%	t	%	t	%	t	%	t	%	t	%	t	%	t	%	t	%	t	%	t
Giza 70	10	(2.42)	1	(0.32)	35	(34.45)	15	(18.75)	13	(2.84)	4	(0.89)	20	(3.15)	3	(0.95)	79	(66.56)	39	(36.90)
Giza 80	38	(6.38)	39	(5.16)	53	(41.68)	27	(29.05)	3	(0.63)	6	(1.71)	2	(0.63)	8	(1.94)	18	(20.95)	36	(34.93)
Giza 83	42	(5.18)	27	(4.21)	33	(30.47)	30	(32.49)	29	(4.45)	1	(0.32)	9	(1.24)	17	(3.83)	96	(53.73)	24	(28.65)
Giza 85	0.0	(0.00)	11	(2.26)	66	(51.48)	39	(38.35)	1	(0.32)	4	(0.89)	54	(6.23)	18	(3.42)	85	(46.16)	38	(37.78)
Giza 86	0.0	(0.00)	0.0	(0.00)	17	(18.90)	25	(23.53)	20	(3.93)	5	(1.18)	21	(3.47)	7	(2.03)	86	(54.73)	41	(33.54)
Giza 88	6	(1.53)	0.0	(0.00)	37	(36.73)	60	(51.28)	23	(4.70)	42	(6.34)	12	(2.58)	0.0	(0.00)	61	(51.90)	28	(22.30)
Giza 89	3	(0.76)	17	(3.97)	21	(22.13)	7	(10.84)	17	(2.55)	6	(1.71)	19	(3.14)	4	(1.26)	68	(60.40)	62	(52.20)
Giza 90	5	(0.71)	7	(1.84)	10	(12.92)	2	(3.69)	5	(1.21)	6	(1.34)	0.0	(0.00)	21	(4.07)	75	(62.25)	78	(56.09)
L.S.D.(transformed data) at P=0.05	1.96				14.89				1.79				2.05				16.28			
L.S.D.(transformed data) at P=0.01	2.58				19.67				2.37				2.71				21.50			

[a] T1 and T2 were nonsterilized and sterilized seeds, respectively.
[b] Percentage data were transformed into $\sqrt{X}$ for *Rhizopus* spp., *Alternaria alternata*, *Aspergillus flavus*, *Fusarium* spp., *Aspergillus* spp., *Cephalosporium* sp., *Cladosporium* sp., and *Fusarium moniliforme*, $\sqrt{X+0.5}$ for *Trichothecium roseum*, *Fusarium oxysporum*, *Fusarium semitectum*, *Fusarium solani*, and *Trichoderma* spp., and arc sine angles for *Penicillium* sp. and *Aspergillus niger* before carrying out analysis of variance to produce approximately constant variance. Transformed means are shown in parentheses.
[c] Transformed data

Table 4. Cont.

Cultivar	Isolate frequency of																			
	Fusarium sp.				*Trichothecium roseum*				*Aspergillus* spp.				*Cephalosporium* sp.				*Cladosporium* sp.			
	T1		T2		T1		T2		T1		T2		T1		T2		T1		T2	
	%	t	%	t	%	t	%	t	%	t	%	t	%	t	%	t	%	t	%	t
Giza 70	11	(2.68)	2	(0.63)	1	(0.96)	0.0	(0.71)	17	(3.30)	21	(2.47)	2	(2.65)	1	(0.32)	33	(5.27)	2	(0.63)
Giza 80	2	(0.63)	1	(0.32)	26	(4.52)	0.0	(0.71)	17	(3.10)	13	(3.15)	9	(2.26)	5	(1.40)	0.0	(0.00)	1	(0.32)
Giza 83	5	(5.46)	0.0	(0.00)	0.0	(0.71)	3	(0.96)	0.0	(0.00)	1	(0.32)	21	(4.62)	0.0	(0.00)	21	(3.39)	1	(0.32)
Giza 85	15	(3.20)	3	(0.95)	0.0	(0.71)	0.0	(0.71)	0.0	(0.00)	5	(1.21)	24	(4.30)	0.0	(0.00)	42	(6.03)	6	(1.31)
Giza 86	0.0	(0.00)	0.0	(0.00)	11	(2.84)	0.0	(0.71)	0.0	(0.00)	3	(0.32)	12	(2.81)	1	(0.32)	14	(2.81)	1	(0.32)
Giza 88	0.0	(0.00)	7	(1.18)	0.0	(0.71)	0.0	(0.71)	12	(2.65)	66	(7.96)	54	(7.27)	2	(0.63)	21	(4.11)	0.0	(0.00)
Giza 89	13	(2.26)	2	(0.63)	0.0	(0.71)	1	(0.96)	6	(1.94)	0.0	(0.00)	58	(7.46)	1	(0.32)	22	(3.83)	2	(0.63)
Giza 90	0.0	(0.00)	0.0	(0.00)	51	(6.83)	0.0	(0.71)	22	(4.32)	10	(2.13)	0.0	(0.00)	0.0	(0.00)	52	(6.46)	0.0	(0.00)
L.S.D.(transformed data) at P=0.05	1.49				0.95				1.60				2.29				1.70			
L.S.D.(transformed data) at P=0.01	1.97				1.26				2.12				3.03				2.25			

[a] T1 and T2 were nonsterilized and sterilized seeds, respectively.
[b] Percentage data were transformed into $\sqrt{X}$ for *Rhizopus* spp., *Alternaria alternata*, *Aspergillus flavus*, *Fusarium* spp., *Aspergillus* spp., *Cephalosporium* sp., *Cladosporium* sp., and *Fusarium moniliforme*, $\sqrt{X + 0.5}$ for *Trichothecium roseum*, *Fusarium oxysporum*, *Fusarium semitectum*, *Fusarium solani*, and *Trichoderma* spp., and arc sine angles for *Penicillium* sp. and *Aspergillus niger* before carrying out analysis of variance to produce approximately constant variance. Transformed means are shown in parentheses.
[c] Transformed data

Table 4. Cont.

Cultivar	Isolate frequency of																			
	Fusarium oxysporum				*Fusarium semitectum*				*Fusarium solani*				*Trichoderma spp.*				*Fusarium moniliforme*			
	T1		T2		T1		T2		T1		T2		T1		T2		T1		T2	
	%	t	%	t	%	t	%	t	%	t	%	t	%	t	%	t	%	t	%	t
Giza 70	4	(1.28)	0.0	(0.71)	0.0	(0.71)	0.0	(0.71)	1	(0.96)	0.0	(0.71)	1	(1.02)	3	(1.35)	11	(2.29)	0.0	(0.00)
Giza 80	1	(0.96)	1	(0.96)	0.0	(0.71)	0.0	(0.71)	0.0	(0.71)	2	(1.22)	0.0	(0.71)	0.0	(0.71)	6	(1.10)	1	(0.32)
Giza 83	0.0	(0.71)	0.0	(0.71)	1	(0.96)	3	(1.45)	0.0	(0.71)	0.0	(0.71)	14	(3.83)	0.0	(0.71)	18	(3.15)	0.0	(0.00)
Giza 85	0.0	(0.71)	0.0	(0.71)	0.0	(0.71)	0.0	(0.71)	0.0	(0.71)	0.0	(0.71)	43	(4.56)	0.0	(0.71)	4	(0.86)	3	(0.76)
Giza 86	0.0	(0.71)	0.0	(0.71)	0.0	(0.71)	0.0	(0.71)	4	(1.12)	0.0	(0.71)	0.0	(0.71)	0.0	(0.71)	14	(3.25)	13	(2.49)
Giza 88	0.0	(0.71)	3	(1.35)	2	(1.09)	4	(1.20)	8	(2.74)	0.0	(0.71)	28	(5.25)	1	(0.96)	4	(1.18)	7	(1.58)
Giza 89	1	(0.96)	0.0	(0.71)	11	(2.92)	0.0	(0.71)	0.0	(0.71)	3	(1.47)	0.0	(0.71)	0.0	(0.71)	16	(3.09)	3	(0.95)
Giza 90	0.0	(0.71)	0.0	(0.71)	0.0	(0.71)	8	(2.44)	1	(0.96)	0.0	(0.71)	0.0	(0.71)	0.0	(0.71)	3	(0.55)	0.0	(0.00)
L.S.D. (transformed data) at P=0.05	N.S.				0.69				0.59				0.92				1.69			
L.S.D. (transformed data) at P=0.01	N.S.				0.92				0.78				1.22				N.S.			

[a] T1 and T2 were nonsterilized and sterilized seeds, respectively.
[b] Percentage data were transformed into √X for *Rhizopus* spp., *Alternaria alternata*, *Aspergillus flavus*, *Fusarium* spp., *Aspergillus* spp., *Cephalosporium* sp., *Cladosporium* sp., and *Fusarium moniliforme*, √X + 0.5 for *Trichothecium roseum*, *Fusarium oxysporum*, *Fusarium semitectum*, *Fusarium solani*, and *Trichoderma* spp., and arc sine angles for *Penicillium* sp. and *Aspergillus niger* before carrying out analysis of variance to produce approximately constant variance. Transformed means are shown in parentheses.
[c] Transformed data

Table 5. Frequencies of fungi isolated from nonsterilized seeds of eight cotton cultivars and their effects on cotton seedling disease variables when the seeds were grown inautoclaved soil.

Cultivars	Isolation frequency of[a]																		Seedling disease variables				
	Alternaria alternata	*Aspergillus flavus*	*Aspergillus niger*	*Aspergillus* spp.	*Cephalosporium* sp.	*Cladosporium* sp.	*Drechslera* spp.	*Fusarium moniliforme*	*Fusarium oxysporum*	*Fusarium semitectum*	*Fusarium solani*	*Fusarium* sp.	*Nigrospora oryzea*	*Penicillium* spp.	*Rhizoctonia solani*	*Rhizopus stolonifer*	*Trichoderma* spp.	*Trichithecium roseum*	Preemergence damping-off %	Postemergence damping-off %	Infection[b] %	Plant height (cm)	Plant weight (g/plant)
Giza 83	29	9	96	0	21	21	0	18	0	1	0	5	0	33	0	42	14	0	26	17	43	17.51	0.27
Giza 88	23	12	61	12	54	21	4	4	0	2	8	0	0	37	0	6	28	0	20	9	29	18.59	0.30
Giza 85	1	54	85	0	24	42	0	4	0	0	0	15	0	66	0	0	43	0	29	10	39	18.82	0.34
Giza 90	5	0	75	22	0	52	0	3	0	0	1	0	0	10	3	5	0	51	21	14	35	13.27	0.29
Giza 89	17	19	68	6	58	22	0	16	1	11	0	13	0	21	0	3	0	0	13	8	21	17.03	0.30
Giza 80	3	2	18	17	9	0	0	6	1	0	0	2	1	53	0	38	0	26	40	7	47	18.96	0.36
Giza 86	20	21	86	0	12	14	0	14	0	0	4	0	0	17	0	0	0	11	23	9	32	20.05	0.28
Giza 70	13	20	79	17	2	33	0	11	4	0	1	11	0	35	0	10	1	1	31	6	37	17.98	0.25

[a] Frequency (%) of fungi isolated from 100 nonsterilized seeds of each cultivar by the standard blotter method and examined 7 days from incubation at 20°C and alternative cycle of cool white light/darkness.
[b] Infection by pre- and postemergence damping-off (cotton seedling damping-off).

The occurrence and associations of pathogen species are of central importance in the ecology of host-pathogen interactions in complex pathodydtems, 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, 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 attraction or repulsion, or have no interaction (Nelson and Campbell, 1992).

Organisms that have similar patterns of resource usage have a high degree of "niche overlap" (Ludwig and Reynolds, 1988). Thus, pathogen species (e.g., seedborne pathogens) in competition for a single resource (e.g., a seed) tend to occupy the same niche. Such niche overlap generates affinity (or lake 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 were evaluated. These pathosystems are maize kernel-infecting fungi (Wicklow, 1988), leaf spot on white clover (Nelson and Campbell, 1992) and foliar pathogens of cucumber (Peterson and Campbell, 2002). To the best of our knowledge, no attempts have been made to study the associations among fungi isolated from cottonseeds in Egypt.

Table 6. Correlation among variable used for evaluating effects of seedborne fungi on incidence of cotton seedling disease on eight cotton cultivars under greenhouse conditions.

Variable	Variable			
	2	3	4	5
1. Preemergence damping-off (%)	-0.222[a]	0.897**	0.353	0.396
2. Postemergence damping-off (%)		0.231	-0.487	-0.225
3. Infection (%)			0.132	0.293
4. Plant height (cm)				0.214
5. Dry weight (g/plant)				

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

In the present study, associations among the pairs of fungi isolated from cottonseeds were identified and the relative strength of these associations were measured by calculating Pearson correlation coefficient (r) for each pair of the fungi. A total of 153 fungal pairings were analyzed (Table 7). Eleven (7.19%) of the fungal pairs were significantly associated. Of the 11 pairs, 9 were positively associated and 2 were negatively associated. No significant associations were found in the remainder fungal pairs. However, one should keep in mind that significant r values should be interpreted with caution (Gomez and Gomez, 1984). The existence of a process may not be proved by the existence of a pattern (Nelson and Campbell, 1992) -that is, 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.

Table 7. Correlation among frequencies of fungi isolated from nonsterilized seeds of eight cotton cultivars.

Isolation frequency (%) of	Isolation frequency of																
	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1. *Alternaria alternata*	-0.250[a]	0.423	-0.420	0.414	-0.316	0.363	0.683x	-0.114	0.250	0.430	-0.241	-0.432	-0.407	-0.353	0.193	-0.074	-0.507
2. *Aspergillus flavus*		0.412	-0.602	0.175	0.295	-0.123	-0.064	-0.011	0.005	-0.117	0.720*	-0.362	0.548	-0.409	-0.496	0.682x	-0.556
3. *Aspergillus niger*			-0.543	-0.036	0.553	-0.168	0.406	-0.103	-0.044	-0.017	0.262	-0.891**	-0.293	0.067	-0.326	0.245	-0.321
4. *Aspergillus* spp.				-0.346	0.189	0.125	-0.544	0.409	-0.167	0.031	-0.321	0.351	-0.198	0.577	0.051	-0.429	0.654x
5. *Cephalosporium* sp.					-0.204	0.571	0.162	-0.277	0.761*	0.374	0.227	-0.245	0.043	-0.408	-0.225	0.352	-0.565
6. *Cladosporium* sp.						-0.115	-0.365	0.002	-0.124	-0.152	0.284	-0.634x	-0.166	0.653x	-0.515	0.279	0.263
7. *Drechslera* spp.							-0.370	-0.218	0.027	0.881**	-0.368	-0.143	0.065	-0.143	-0.166	0.423	-0.242
8. *Fusarium moniliforme*								0.154	0.431	-0.258	0.204	-0.236	-0.346	-0.438	0.270	-0.390	-0.477
9. *F. oxysporum*									0.014	-0.233	0.399	0.073	0.056	-0.218	0.018	-0.377	-0.204
10. *F. semitectum*										-0.111	0.401	-0.186	-0.278	-0.186	-0.210	-0.183	-0.315
11. *F. solani*											-0.548	-0.247	-0.179	-0.106	-0.349	0.225	-0.161
12. *Fusarium* sp.												-0.240	0.431	-0.368	-0.217	0.317	-0.547
13. *Nigrospora oryzea*													0.412	-0.143	0.594	-0.363	0.324
14. *Penicillium* spp.														-0.521	0.247	0.673x	-0.379
15. *Rhizoctonia solani*															-0.190	-0.263	0.869**
16. *Rhizopus stolonifer*																	0.044
17. *Trichoderma* spp.																	-0.446
18. *Trichothecium roseum*																	

[a] Linear correlation coefficient (r) is significant at $P < 0.10$ (x), $P < 0.05$ (*), or $P < 0.01$ (**).

However, 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, display competitive exclusion or antagonism. Fungi that share specialized niche requirements often occur together and would primarily exhibit a positive association (Peterson and Campbell, 2002).

The phenogram shown in Fig. 1 indicates that fungi isolated from cottonseeds appear to form two distinct groups. One group consisting of 12 fungi, and a second group consisting of 6 fungi. Within each group, fungi were associated strongly and positively, whereas between groups, fungi were associated weakly or negatively. This phenogram implies the potential existence of cultivar related groups of fungi. These results are in agreement with those of ANOVA, which also indicate that cotton cultivar plays a significant role in determining frequencies of fungi isolated from seeds.

Isolation frequencies of some fungi were significantly correlated with seedling disease variables (Table 8). The significance of some r values may indicate the presence of causal relationship between seedborne fungi and the incidence of cotton seedling disease because such a relationship is consistent with biological expectations. For example, the significant positive correlations between each of *N. oryzae* and *Penicillium* spp. and preemergence damping-off, the significant positive correlation between *R. stolonifer* and infection, and the highly significant negative correlation between *R. solani* and plant height are in agreement with the previous reports (Davis *et al.,* 1981 and Waked *et al.,* 1981), which indicated that these fungi are involved in cotton seedling disease. On the other hand, no immediate biological explanation is available for some of the significant r values between frequencies of seed borne fungi and seedling disease variables. For example, it was surprising to find a negative significant correlations between frequency of *F. semitectum* and each of preemergence damping-off and infection because this fungus is pathogenic on cotton seedlings (El-Samawaty, 1999).

Data for seedling disease variables and frequencies of the fungi isolated from nonsterilized seeds were entered into a computerized stepwise multiple regression analysis. The analysis constructed a predictive model by adding predictors, in this case, frequencies of the isolated fungi, to the model in order of their contribution to R^2. The analysis was effective in eliminating those variables with little or no predictive value by incorporating into the model only those variables that made a satisfactory significant contribution to the R^2 value of the model (Podleckis *et al.,* 1989). Using the predictors supplied by stepwise regression, four models were constructed to predict seedling disease variables (Table 9). These models showed that differences in seedling disease variables were due largely to the effects of *N. oryzae*, *F. semitectum*, *R. stolonifer*, *R. solani*, and *Trichoderma* spp. (Table 10). It is worth nothing that no regression model was constructed to predict postemergence damping-off, which reconfirms that soilborne fungi of cotton are more important, as seedling disease incitants, in the preemergence stage compared with the postemergence stage.

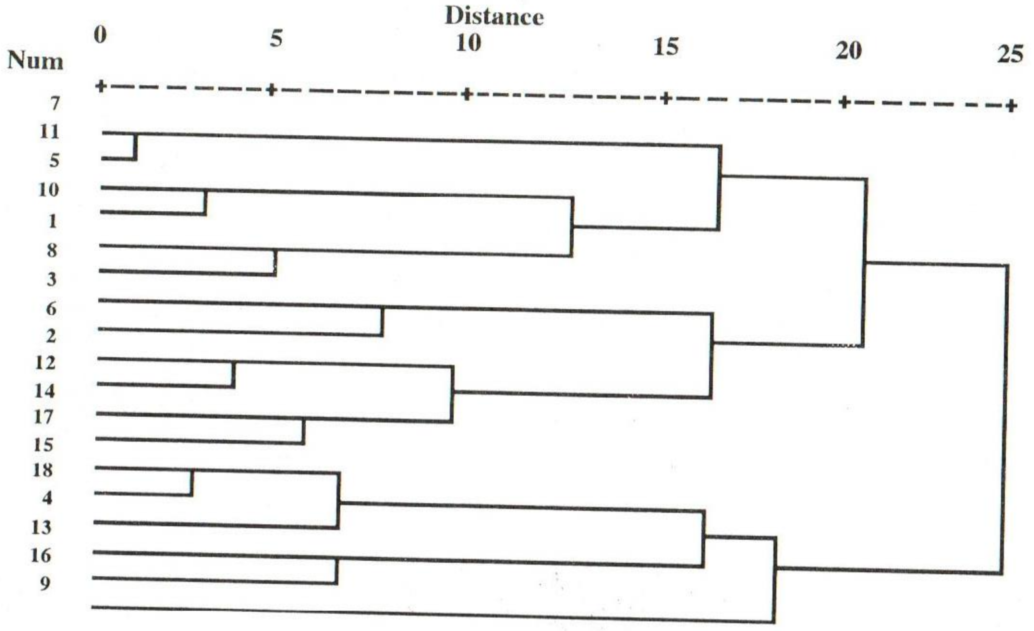

Fig. 1: Phenogram based on average linkage cluster analysis of isolation frequencies (%) of 18 fungi isolated from nonsterilized seeds of 8 cotton cultivars by the standard blotter method. The isolated fungi were *Allernaria alternata* (1), *Aspergillus flavus* (2), *A. niger* (3), *Aspergillus* spp. (4), *Cephalosporium* sp. (5), *Cladosporium* sp. (6), *Drechslera* spp. (7), *Fusarium moniliforme* (8), *F. oxysporum* (9), *F. semitectum* (10), *F. solani* (11), *Fusarium* sp. (12), *Nigrospora oryzae* (13), *Penicillium* spp. (14), *Rhizoctonia solani* (15), *Rhizopus stolonifer* (16), *Trichoderma* spp. (17), and *Trichothecium roseum* (18).

Table 8. Correlation among cotton seedling disease variables and frequencies of fungi isolated from nonsterilized seeds of eight cotton cultivars.

Isolation frequency (%) of	Preemergence damping-off (%)	Postemergence damping-off (%)	Infection[a] (%)	Plant height (cm)	Dry weight (g/plant)
Alternaria alternata	-0.456[b]	0.323	-0.309	0.196	-0.629x
Aspergillus flavus	-0.007	-0.215	-0.104	0.429	0.179
Aspergillus niger	-0.456	0.512	-0.223	-0.125	-0.678x
Aspergillus spp.	0.214	-0.238	0.106	-0.550	0.019
Cephalosporium sp.	-0.624	-0.122	-0.677x	0.183	0.134
Cladosporium sp.	-0.316	0.340	-0.162	-0.671x	-0.278
Dershslera spp.	-0.266	-0.109	-0.315	0.0161	0.014
Fusarium moniliforme	-0.2641	0.167	-0.188	0.214	-0.519
Fusarium oxysporum	0.312	-0.583	0.047	0.063	-0.378
Fusarium semitectum	-0.673x	-0.172	-0.749*	-0.126	-0.013
Fusarium solani	-0.313	-0.162	-0.386	0.275	-0.170
Fusarium sp.	-0.034	-0.250	-0.147	0.102	0.049
Nigrospora oryzea	0.724*	-0.327	0.575	0.234	0.687x
Penicillium spp.	0.648x	-0.257	0.530	0.517	0.634x
Rhizoctonia solani	-0.217	0.436	-0.019	-0.890**	-0.098
Rhizopus stolonifer	0.563	0.336	0.714*	0.066	0.140
Trichpderma spp.	0.021	0.154	0.091	0.272	0.326
Trichothecium roseum	0.129	0.231	0.233	-0.654	0.191

[b] Infection by pre and postemergence damping-off (cotton seedling damping-off).
[a] Linear correlation coefficient (r) is significant at $P < 0.10$(x), $P < 0.05$ (*), or $P < 0.01$ (**).

Table 9. Stepwise regression models that describe the relationship between cotton seedling disease variables and frequencies of fungi isolated from nonsterilized seeds of eight cotton cultivars.

Dependent variable (Y)	Stepwise linear regression model[a]	Coefficient of determination (R^2)	F value [b]
Preemergence damping-off (%)	Y= 19.11 + 17.96x**13** – 1.66x**10** + 0.65x**12** + 0.18x**1** + 0.59x**9** + 0.019x**18**	100.00	213903.28***
Postemergence damping-off (%)	[c]	...	...
Infection (%)[d]	Y= 34.33 - 1.17x**10** + 0.34x**16** – 0.19x**1** + 0.09x**2** - 0.21x**7** – 0.12x**11**	100.00	176568.98***
Plant height (cm)	Y= 18.74 - 1.82x**15** - 0.16x**10**	87.97	18.28**
Dry weight (g/plant)	Y= 0.28 + .08x**13** - 0.001x**17**	74.81	7.42*

[a] Identification of the predictors and their relative contributions to R^2 are shown in Table 10.
[b] F. value is significant at $P < 0.05$ (*), $P < 0.01$ (**), or $P < 0.005$ (***).
[c] No regression model could be constructed.
[d] Infection by pre and postemergence damping-off (cotton seedling damping-off).

Table (10): Identification of the predictors included in stepwise regression models shown in Table 9 and their relative contributions to R^2.

Predictor	Variable and number	Relative contribution to R^2 (%)
Isolation frequency (%) of	Preemergence damping-off	
Nigrospora oryzae	X 13	52.47
Fusarium semitectum	X 10	29.98
Fusarium sp.	X 12	14.11
Alternaria alternata	X 1	2.56
Fusarium oxysporum	X 9	0.84
Trichothecium roseum	X 18	0.04
	Infection[a] (%)	
Fusarium semitectum	X 10	56.12
Rhizopus stolonifer	X 16	32.44
Alternaria alternata	X 1	7.98
Aspergillus flavus	X 2	3.12
Derchslera spp.	X 7	0.33
Fusarium solani	X 11	0.01
	Plant height (cm)	
Rhizoctonia solani	X 15	79.21
Fusarium semitectum	X 10	8.76
	Dry weight (g/plant)	
Nigrospora oryzae	X 13	47.18
Trichoderma spp.	X 17	27.63

[a] Infection by pre- and postemergence damping-off (cotton seedling damping-off).

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فطريات بذرة القطن وتأثيرها على حدوث مرض موت البادرات

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أظهر التقدير النوعى للفطريات المعزولة من بذور ثمانية من أصناف القطن التجارية – وذلك بعد تعقيم البذرة سطحيا أو بدون تعقيم سطحى – وجود الفطريات التالية: ألترناريا ألترناتا وأسبرجلس فلافس وأسبرجلس نيجر وأسبرجلس وسيفالوسبوريم وكلادوسبوريم ودريشليرا وفيوزاريوم مونيليفورمى وفيوزاريوم أوكسيسبورم وفيوزاريوم سيميتكتم وفيوزاريوم سولانى وفيوزاريوم ونجروسبورا أوريزا وبنسليوم وريزوكتونيا سولانى وريزوبس ستولونيفر وترايكودرما وترايكوثيسيم روزيم. أظهر التقدير الكمى أن فطريات أسبرجلس نيجر (٧١ %) وبنسليوم (٣٤%) وكلادوسبوريم (٢٥,٦٣%) هى الأكثر شيوعا فى العزل من البذرة غير المعقمة، أما الفطريات الأخرى فقد تراوح تكرار عزلها من ٠,١٣ إلى ٢٢,٥%. أدى التعقيم السطحى للبذرة إلى حدوث إنخفاض معنوى فى تكرارات عزل فطريات أسبرجلس نيجر وسيفالوسبوريم وكلادوسبوريم وترايكوثيسيم رزويم، فى حين لم يتأثر تكرار عزل باقى الفطريات، من الممكن تفسير هذه النتيجة على أساس أن مجموعة الفطريات الحساسة للتعقيم السطحى تميل إلى استعمار الأغلفة الخارجية للبذرة أكثر من ميلها لاستعمار أنسجة الجنين، أما المجموعة الغير حساسة للتعقيم السطحى فتميل إلى إستعمار الأنسجة الداخلية للبذرة. أظهر تحليل التباين أن الصنف وتفاعل الصنف × المعاملة هى مصادر معنوية أو عالية المعنوية للتباين فى تكرارات الفطريات المعزولة باستثناء فطر فيوزاريوم أوكسيسبورم. احتل الصنف المرتبة الأولى فى الأهمية كمصدر للتباين فى تكرار عزل ستة (٤٠%) من الفطريات، فى حين احتل تفاعل الصنف × المعاملة المرتبة الأولى فى الأهمية كمصدر للتباين فى تكرار عزل خمسة (٣٣,٣٣%) من الفطريات. لايوجد صنف قطن أعطى جميع الفطريات (١٨ فطر) عند العزل منه. أكبر عـــدد من الفطريـــات (١٤ فطر) أمكن عزله من صنف جيزة ٧٠، أما أقل عدد من الفطريـــات (٩ فطريات) فقد أمكن عزله من جيزة ٨٥، أما باقى الأصناف فقد أعطت عند العزل عدداً من الفطريات تراوح من ١٠ إلى ١٣. فطريات ألترناريا ألترناتا وأسبرجلس نيجر وفيوزاريوم مونيليفورمى وبنسيليم هى الوحيدة التى أمكن عزلها من جميع الأصناف المختبرة. أظهرت الدراسة الحالية أن الدور الذى تلعبه فطريات البذرة – كمسببات لمرض موت البادرات– أكثر وضوحاً فى مرحلة ما قبل ظهور البادرات فوق سطح التربة مقارنة بمرحلة ما بعد ظهور البادرات فوق سطح التربة. عند استعمال معامل إرتباط بيرسون لتقييم درجة الإرتباط بين ١٥٣ زوج من الفطريات المعزولة ، ظهر أن هناك إرتباط معنوى بين ١١ (٧,١٩%) زوج من الفطريات ، كان الإرتباط المعنوى موجبا بين ٩ أزواج وسالبا بين زوجين من الفطريات ، أما باقى الأزواج فقد أظهرت إرتباطا غير معنويا . أمكن – باستعمال التحليل العنقودى – تقسيم الفطريات المعزولة إلى مجموعتين محددتين، إشتملت المجموعة الأولى على فطريات دريشسليرا وفيوزاريوم سولانى وسيفالوسبوريم وفيوزاريم سيميتكتم والترناتا وفيوزاريوم مونيليفورمى وأسبرجلس نيجر وكلادوسبوريم وأسبرجلس فليفس وفيوزاريوم وبنيسليم وتريكوديرما، فى حين إشتملت المجموعه الثانية على فطريات ريزوكتونيا سولانى وترايكوتيسيم روزيم وأسبرجلس ونيجروسبورا أوريزا وريزوبس ستولونيفر وفيوزاريوم أوكسيسبورم. كان الارتباط قويا وموجبا بين الفطريات داخل كل مجموعة، فى حين كان ضعيفا أو سالبا بين فطريات المجموعتين. تدل هذه النتيجة على إحتمال وجود فطريات تتخصص فى إصابة بذور أصناف بعينها. إستعمل الإنحدار المتعدد المرحلى لوصف العلاقة بين فطريات البذرة (متغيرات مستقلة) والمتغيرات المستعملة لوصف المرض (متغيرات تابعة)، أظهرت نماذج الإنحدار الأربعة التى أمكن التوصل إليها أن الجانب الأكبر من التباين فى المتغيرات المستعملة لوصف المرض من الممكن أن يعزى إلى تأثير فطريات نيجوسبورا أوريزا وفيوزاريوم سيميتكتم وريزوبس ستولونيفر وريزوكتونيا سولانى وتريكوديرما. الجدير بالذكر أنه لم يمكن التوصل إلى أى نموذج إنحدار لوصف العلاقة بين فطريات البذور وحدوث موت البادرات بعد ظهورها فوق سطح التربة، مما يؤكد ما سبق التوصل اليه من أن الدور الذى تلعبه فطريات البذرة – كمسببات لمرض موت البادرات – أكثر وضوحاً فى مرحلة ماقبل ظهور البادرات فوق سطح التربة مقارنة بمرحلة ما بعد ظهور البادرات فوق سطح التربة.