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QUALITATIVE AND QUANTITATIVE ESTIMATION OF SEEDS MYCOFLORA AND THEIR INFLUENCE ON COTTON SEEDLING DAMPING –OFF DISEASE.

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ABSTRACT

Surface and non-surface sterilized seeds of commercial cotton cultivars were examined for qualitative and quantitative estimates of seed-borne fungi. *Aspergilus flavus, A.niger* and *Alternaria alternata* were the most dominant species isolated. Cultivar and cultivar treatment interactions were very highly significant source of variation in frequencies of fungi. Both cultivar and cultivar x treatment interactions contributed most to the variation in frequencies of the isolated fungi. The effect of surface sterilization on frequencies of fungi isolated from seeds varied depending on the cultivar used. Giza 85 cultivar yielded the lowest number of fungi (5 fungi). Other cultivars yielded a number of fungi ranging from 7 to 13. The role of seed borne fungi in cotton seedling disease incidence was more evident in the post-emergence stage compared with the pre-emergence stage. Also, Cluster analysis divided the cotton cultivars into two distinct groups, one group consisting of 6 cultivars (Giza 45,Giza 70,Giza 85,Giza88,Giza89 and Giza90) and a second group consisting only one cultivar Giza90.Constructed regression models showed that difference in seedling disease variables were due largely to the effects of *A. alternata, Cladosporium* spp. and *Fusarium semitectum* .
Keywords: Cotton seeds, seed-borne fungi, surface sterilization.

INTRODUCTION

Fungi associated externally or internally with cotton seeds are classified into field and storage fungi. Field fungi usually invade the maturing cotton seeds on the developing plants in the field before harvest. These fungi required moisture content in equilibrium with relative humidity 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 and Aly *et al.*, 2004). Under Egyptian conditions, fungi associated with seeds of cotton cultivars included species of *Alternaria*, species of *Aspergillus*, *Apiocrea chrysospermum*, *Cephalosporium* spp., Cladosporium spp., *Curvularia* spp., *Chaetomium* sp., *Derchslera* spp., *Diplodia gossypii,* species of *Fusarium spp, Helminthosporium* spp., *Microascus longistris, Nigrospora oryzae,* species of *Penicillium, Paecilomyces variotii. Pythium* spp., *Rhizoctonia solani, Rhizopus stolonifer, Trichothecium roseum, Trichoderma* spp. *and others* (Bakry and Rizk, 1967; Abd El-Aleem,1979; Amer,1986; El-Naghy *et al.*, 1991 and Aly, 2004). The economic value of cotton seed is greatly influenced by the presence of fungi carried on the seed. Fungi or associated metabolites may reduce the vigor of planting seeds (Davis, 1982). The increasing in amount of free fatty acid in the seed thereby reducing the quality of the extracted oil (Roncadori *et al.*,

1971), or even the presence of mycotoxins that render the seed unsuitable for consumption (Diener *et al.*,1976).

The main objective of this investigation was to identify the fungi associated with seeds of some Egyptian cotton cultivars and to evaluate their effects on incidence cotton seedling damping –off disease.

MATERIALS AND METHODS

Seed borne fungi isolation:

Seeds of cotton cultivars Giza45, Giza70, Giza85, Giza86, Giza88, Giza 89, and Giza90 were obtained from Cotton Research Institute, Agri. Rec. Center, Giza Egypt. Random subsamples of 100 cotton seeds for each cultivar were surface sterilized in 5.2% hypochlorite sodium solution for 3 minutes and washed in sterilized water. The surface sterilized seeds were blotted between filter paper. Seed-borne fungi of cotton seeds were counted according to the standard blotter method . Ten seeds of surface sterilized or non-sterilized for each treatment and cultivars were blotted on five layers of filter paper in Petri dishes and each one was replicated 10 times for each cultivar. The incubation at 20±2°C for7 days. Examination for each colony was done using stereo-binocular microscope or light microscope.

Pre-treated cotton seeds were also grown on potato dextrose agar (PDA). Each plate included ten seeds was incubated at 20±2°C for 4 days . Isolation and purification was made using hyphal tip and single spore techniques . The purified culture was kept at 5°C for the further studies. Identification to genus and/or species was done according to Booth (1971), Barent and Hunter,(1979). Isolation frequency for each fungus was recorded as the percentage of seeds from which fungi grew (if there was more than one fungus from one seed each of them was calculated individually).

One hundred cotton seeds of each tested cultivars were used to measure the germination %, Cotton seeds were sown between five layers of sterilized moisted paper towels and incubated at 22±2°C with 12h near ultraviolet light and followed by darkness for 12 h for ten days. Percent of germination was calculated for all the plates (when radical growth were very poor, there was no germination).

Assessment of cotton seedling disease variables:

Autoclaved soil was infested individually with each of the tested fungi, then dispensed in 10cm diameter clay pots. Soil infestation was made according to the method mentioned by Klich (1986) pots were planted each with 10 non sterilized seeds of each cultivar. Pots were distributed on greenhouse bench under temperature ranged from 22 to 38±5°C and after 15 days from planting percentage of pre-emergence damping off was calculated. Survival%, plant height and dry weight were recorded 40 days after planting.

Statistical analysis of the data:

Percentage data of isolation frequencies were transformed into 'arc sin angles' before carrying out analysis of variance (ANOVA) to normalize and stabilize variance. Duncan's multiple range test 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). The experimental design of greenhouse experiments was a randomized complete block with five replications. Stepwise regression technique with greatest increase in R^2 as the decision criterid was used to describe the effect of seed borne fungi on seedling disease. Cluster analysis was performed with the software package SPSS 6.0. Correlation and regression analysis were performed with a computerized program.

RESULTS AND DISCUSSION

Fungi isolated:

The mean percentage of fungi associated with cotton seeds (Table 1) showed that *Aspergillus flavus* (16.28%), *Alternaria alternata* (13.71%), *Aspergillus niger* (11.71%) and *Cladosporium* spp. (11.28%) were the most dominant fungi isolated from the non sterilized cotton seeds.

Other fungi occurred at frequencies ranging from 1.28 to 8.14%. Data of Table (1) showed that the frequency of fungi isolated by blotter method was higher than the PDA method except *A. flavus, Cladosporium* spp., *Trichoderma harzianum*, *Trichoderma* spp. and *Trichothecium* spp. Cotton seedlings abnormalities were shown in Fig. (1) which due to mixed infection of cotton seed mycoflora under greenhouse studies.

Table (1): Isolated from non-sterilized and sterilized cotton seeds of 7 cotton cultivars

Fungi	Frequencies (%) of isolated Fungi		
	T1[a]	T2[b]	T3[C]
Alternaria alternate	13.71	15.86	22.57
Aspergillus flavus	16.28	13.28	24.71
Aspergillus fumigatus	4.43	10.14	10.14
Aspergillus niger	11.71	15.28	19.57
Cladosporium spp.	11.28	10.14	13.57
Fusarium oxysporum	7.14	11.14	10.71
Fusarium moniliforme	3.14	6.28	4.86
Fusarium semitectum	2.00	6.00	0.14
Macrophomina phasolina	8.14	3.43	0.00
Penicillium spp.	7.43	7.86	13.71
Rhizoctonia solani	4.00	6.14	7.14
Trichoderma harzianum	1.28	1.14	1.57
Trichoderma spp.	5.00	3.14	6.71
Trichothecium spp.	6.28	3.00	8.71

[a] PDA Method(sterilized seeds) [b] Blotter method (sterilized seeds)
[c] Blotter method(non sterilized seeds)

Fig (1): A photograph showing different stages of cotton seedlings abnormalities obtained from greenhouse experiments.

The dominance of genus *Aspergillus* (*A. favus* and *A. niger*) relative to the other fungi isolated from cottonseeds is consistent with the findings of Simpson *et al.* (1973) and El-Naggar (2007). *Alternaria* has been reported as a dominant member of the mycoflora of cottonseeds 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 in the study of Klich (1986). *Cladosporium* spp. is also involved in sooty mold of cotton (Zayed, 1997) and is among the fungi involved in cotton boll rot and may cause deterioration in fiber quality under suitable environmental condition (Abd El-Rehim *et al.*, 1996).

In the three isolation methods , *Aspergillus flavus*, *A. niger* and *Alternaria alternata* were the most dominant fungi isolated from non-sterilized and sterilized cotton seeds. These results were in the same trend with Simpson *et al.*, (1973); Davis(1977) and Aly(2004). Other fungi were affected with isolation methods. Thus the frequency of *Penicillium* spp. was increased with sterilization, while the frequency of *Fusarium semitectum* was decreased with the same treatment. On the other hand, isolation protocol has no effect on isolation frequency of *Trichoderma harzianum*. These results may indicate that some fungi tended to colonize on outer coat of seed more than embryo , while the other fungi tended to establish in the internal parts of seeds.

Cultivar and cultivar x treatment sterilization interaction were all very highly significant sources of variation in frequencies of fungi isolated from seeds (Table2). Cultivar was the first in importance as a source of variation in isolation frequencies of *Penicillium* spp., *Trichoderma* spp. and *Rhizoctonia solani*, while cultivar x treatment interaction was the first in importance as a source of variation in isolation frequencies of *Fusarium moniliforme*, *Aspergillus niger*, *F. oxysporum* and *A. flavus*. (Table 3)

Table (2): Analysis of variance of effects of sterilization, cultivar and their interaction on frequencies of fungi isolated from cotton seeds.

Fungus	Source of variation	D.F	Mean square	F. value	P >F
Alternaria alternate	Treatment (T)	2	13.576	6.4944	0.0024
	Cultivar (C)	6	15.988	7.6486	0.0000
	T x C	12	4.979	2.3818	0.0111
	Error	80			
Aspergillus flavus	Treatment (T)	2	1107.062	14.4404	0.0000
	Cultivar (C)	6	264.962	3.4561	0.0044
	T x C	12	416.359	5.4310	0.0000
	Error	80			
Aspergillus fumigatus	Treatment(T)	2	11.501	10.5528	0.0001
	Cultivar (C)	6	21.883	20.0789	0.0000
	T x C	12	12.069	11.0743	0.000
	Error	80			
Aspergillus niger	Treatment (T)	2	8.378	4.1714	0.0189
	Cultivar (C)	6	17.959	8.9420	0.0000
	T x C	12	12.588	6.2678	0.0000
	Error	80			
Cladosproum spp.	Treatment (T)	2	2.329	1.0812	0.3441
	Cultivar (C)	6	26.540	12.3233	0.0000
	T x C	12	10.197	4.7348	0.0000
	Error	80			
Fusarium oxysporum	Treatment (T)	2	7.796	3.0262	0.0541
	Cultivar (C)	6	15.035	5.8361	0.0000
	T x C	12	11.267	4.3732	0.0000
	Error	80			
Fusarium moniliforme	Treatment (T)	2	3.778	2.7109	0.0726
	Cultivar (C)	6	8.528	6.1203	0.0000
	T x C	12	5.983	4.2934	0.0000
	Error	80			
Fusarium semitectum	Treatment (T)	2	9.397	26.5814	0.0000
	Cultivar (C)	6	16.554	46.8277	0.0000
	T x C	12	6.186	17.4982	0.0000
	Error	80			
Macrophomina phaseolina	Treatment (T)	2	29.467	73.9635	0.0000
	Cultivar (C)	6	6.949	17.4423	0.0000
	T x C	12	7.375	18.5107	0.0000
	Error	80			
Penicillum spp.	Treatment (T)	2	6.884	4.84410	0.0103
	Cultivar (C)	6	35.476	24.9620	0.0000
	T x C	12	2.854	2.0084	0.0339
	Error	80			
Rhizoctonia solani	Treatment (T)	2	1.982	1.8789	0.1594
	Cultivar (C)	6	22.139	20.9855	0.0000
	T x C	12	4.092	3.8786	0.0001
	Error	80			
Trichoderma harzianum	Treatment (T)	2	0.405	1.29850	0.2786
	Cultivar (C)	6	6.730	21.5596	0.0000
	T x C	12	4.231	13.5559	0.0000
	Error	80			
Trichoderma spp.	Treatment (T)	2	4.303	4.5961	0.0129
	Cultivar (C)	6	21.764	23.2463	0.0000
	T x C	12	3.281	3.5045	0.0003
	Error	80			
Trichothecium spp.	*Treatment (T)*	*2*	*7.973*	*6.6682*	*0.0021*
	Cultivar (C)	*6*	*26.280*	*21.9807*	*0.0000*
	T x C	*12*	*2.801*	*2.3428*	*0.0125*
	Error	*80*			

Table (3): Relative contribution of treatment, cultivar and their interaction to variation in frequency of fungi isolated from cotton seed

Fungus	Relative contribution to variation in isolation frequenc		
	Treatment (T)	Cultivar(C)	T x C
Alternaria alternate	12.15	42.92	26.73
Aspergillus flavus	22.90	16.45	51.68
Aspergillus fumigatus	7.51	42.88	47.30
Aspergillus niger	6.03	38.79	54.38
Cladosporium spp.	1.38	54.79	42.11
Fusarium oxysporum	6.06	35.04	52.52
Fusarium moniliforme	5.78	39.15	54.93
Fusarium semitectum	9.68	51.16	38.23
Macrophomina phasolina	30.93	21.88	46.45
Penicillium spp.	5.22	80.69	12.99
Rhizoctonia solani	2.05	68.71	25.40
Trichoderma harzianum	0.85	42.51	53.46
Trichoderma spp.	4.58	69.39	20.92
Trichothecium spp.	7.47	73.91	15.75

***calculated as percentage of sum of squares of the explained (model) variation.**

Due to significance of this interaction, Duncan's multiple range test was used to compare between means of non sterilized and sterilized seeds within each cultivar for each of the tested fungi (Table4). These comparison showed that, the effect of surface sterilization on frequencies of fungi isolated from seeds varied depending on the cultivar used in isolation. For example, sterilization significantly reduced the frequencies of *Alternaria alternata* isolated from Giza89, while it showed no effect on the frequency of the same fungus isolated from Giza45 and Giza70.

The frequency of *Penicillium* isolated from Giza89 was significantly reduced by sterilization, while the isolation frequencies were not affected by sterilization in case of Giza86. The significant role of cotton cultivar in determining the frequencies of fungi isolated from cotton seeds, as we have demonstrated herein, could by attributed to the heritable anatomical characteristic of the seeds, which may vary from one cultivar to another, however these results are in sharp contrast with the findings of Davis, (1982); Klich, (1986) and Aly *et al.*, (2004) who reported that, fungal infection of cotton seeds was apparently not substantially influenced by cultivar.

A total of 14 fungi were identified from the 7 cultivars that were tested (Table 5). Only Giza 85 cultivar yielded 14 fungi, while Giza 86 and Giza89 yielded the lowest number (5 fungi). The other cultivars yielded a number of fungi ranged from 7 to 13. *A. alternata* and *A. flavus* were the only fungi, which were isolated from all tested cultivars.

Table (4): Frequencies of Fungi isolated from non sterilized and sterilized cotton seeds of seven cultivars

Fungi		% Frequency						
		Giza 45	Giza 70	Giza 85	Giza 86	Giza 88	Giza 89	Giza 90
Alternaria alternate	T_1	12 cd[a]	10 d	17 bd	19 bd	0 e	22 bd	16 bd
	T_2	15 cd	16 bd	8 d	20 bd	I5 bd	27 be	10 d
	T_3	15 bd	16 bd	13 cd	30 ab	15 bd	51 a	18 bd
Aspergillus flavus	T_1	17 ad	13 cd	1 e	11 d	17 ad	36 ab	20 ad
	T_2	8 d	15 ad	23 ac	16 ad	0 e	I5 ad	16 ad
	T_3	25 ac	13 bd	30 ac	33 a	19 ad	31 ab	22 ac
Aspergillus fumigatus	T_1	0 e	0 c	21 ab	0 e	10 cd	0 e	0 e
	T_2	8 d	0 c	17 ad	0 e	20 ac	26 a	0 e
	T_3	0 e	20 ac	23 ac	15 ad	0 e	13 bd	0 c
Aspergillus niger	T_1	0 e	6 de	18 ac	13 bd	I5 ac	15 ad	15 ac
	T_2	14 ac	10 cd	0 e	26 ab	18 ac	14 ac	25 ac
	T_3	30 a	18 ac	0 e	24 ac	13 bd	22 ac	30 ab
Cladosporium spp.	T_1	13 ce	22 ac	0 f	IS be	0 f	11 ce	18 ae
	T_2	6 df	12 cf	0 f	10 cf	7 cf	36 a	0 f
	T_3	20 ad	0 f	4 ef	18 ad	0 f	34 ab	19 ad
Fusarium oxysporum	T_1	10 ad	0 d	0 d	10 ac	18 ab	12 ab	0 d
	T_2	7 bd	0 d	18 ab	13 ab	20 ab	11 ac	9 ac
	T_3	24 a	10 ac	21 ab	2 cd	10 ac	8 ac	0 d
Fusarium moniliforme	T_1	0 c	15 a	0 c	0 c	0 c	7 ab	0 c
	T_2	4 bc	0 c	9 ab	8 ab	16 a	0 c	7 ac
	T_3	7 ab	0 c	0 c	5 bc	18 a	4 bc	0 c
Fusarium semitectum	T_1	0 da	11 e	0 d	0 d	0 d	14 b	0 d
	T_2	0 d	0 d	0 d	11 e	0 d	31 a	0 d
	T_3	0 d	0 d	0 d	0 d	0 d	1 d	0 d
Macrophomina phaseolina	T1	0 c	20 a	0 e	6 b	9 b	6 b	16 a
	T_2	0 c	0 c	0 e	0 e	0 c	24 a	0 c
	T_3	0 c	0 c	0 e	0 e	0 c	0 e	0 c
Penicillium spp.	T_1	8 de	0 e	10 ed	13 bd	0 e	10 bd	1l bd
	T_2	7 de	0 e	0 e	12 bd	0 e	23 b	13 bd
	T_3	8 d	0 e	9 d	16 bd	0 e	43 a	20 bc
Rhizoctonia solani	T_1	0 c	0 c	0 e	8 ab	8 ab	12 ab	0 c
	T_2	0 c	6 bc	10 ab	10 ab	0 c	17 a	0 c
	T_3	0 c	0 c	16 ab	19 a	0 c	15 ab	0 c
Trichoderma harzianum	T_1	0 c	0 c	0 c	9 a	0 c	0 c	0 c
	T_2	0 c	0 c	8 b	0 e	0 c	0 c	0 c
	T_3	0 e	0 c	11 a	0 e	0 c	0 e	0 c
Trichoderma spp	T_1	0 e	0 e	15 ab	11 bd	0 e	6 ce	3 de
	T_2	0 e	0 e	0 e	12 ac	0 e	10 bd	0 e
	T_3	0 e	0 e	8 bd	19 a	0 e	20 a	10 ad
Trichothecium spp.	T_1	0 e	0 e	15 ac	11 cd	0 e	15 ac	0 e
	T_2	0 e	0 e	0 e	12 ac	0 e	9 ac	0 e
	T_3	0 e	0 e	8 bd	22 a	0 e	21 ab	0 e

[a] **Percentage data were transformed into arc sine angles before carrying out the analysis of variance to produce approximately constant variance. Values in a column followed by the same latter M not significantly different (P>0.05) according to Duncan's multiple range test However, the environmental conditions under which these cultivars were grown and subsequently the seeds produced could not be ignored as a contributing factor for these differences.**

Table (5): Frequency of fungi isolated from non-sterilized seeds of seven cotton cultivars and their effects on cotton seedling disease variables when the seeds were grown in autoclaved soil

	Fungi	Cultivars						
		Giza 45	Giza 70	Giza 85	Giza 86	Giza 88	Giza 89	Giza 90
Isolation frequency of Fungi [a]	*Alternaria alternate*	13.0	16.0	30.0	16.0	18.0	16.0	51.0
	Aspergillus flavus	30.0	25.0	33.0	13.0	22.0	19.0	31.0
	Aspergillus fumigatus	13.0	0.0	15.0	20.0	0.0	0.0	13.0
	Aspergillus niger	0.0	30.0	24.0	18.0	30.0	18.0	22.0
	Cladosporium spp.	4.0	20.0	18.0	0.0	19.0	0.0	34.0
	Fusarium oxysporum	20.0	15.0	2.0	10.0	0.0	10.0	8.0
	Fusarium moniliforme	0.0	7.0	5.0	0.0	0.0	18.0	4.0
	Fusarium semitectum	1.0	4.0	3.0	0.0	0.0	0.0	0.0
	Macrophomina phaseolina	0.0	0.0	13.0	0.0	0.0	0.0	1.0
	Penicillium spp.	9.0	8.0	16.0	0.0	13.0	0.0	13.0
	Rhizoctonia solani	16.0	0.0	14.0	0.0	0.0	0.0	15.0
	Trichoderma harzianum	11.0	0.0	19.0	0.0	0.0	0.0	21.0
	Trichoderma spp.	8.0	0.0	20.0	0.0	10.0	0.0	20.0
	Trichothecium spp.	4.0	20.0	18.0	0.0	19.0	0.0	34.0
Seedling disease variables								
	Seed germination (%)	98	94	85	95	97	95	75
	Pre-emergence damping off (%)	15.0	15.3	5.7	4.3	4.7	5.7	4.3
	Post-emergence damping off (%)	14.3	24.7	24.0	5.0	5.0	34.0	25.0
	Survival (%)	70.7	60.0	70.3	90.7	90.3	60.3	70.7
	Plant height (cm)	16.3	16.6	13.9	15.14	15.33	12.70	11.50
	Dry weight (g/plant)	233	240	165	187	187	150	142

[a] **Isolation frequency of fungi isolated from 100 non sterilized seeds of each cultivar by the blotter method and examined 7 days from incubation at 25°C and alternative cycle of cool white light /darkness**

Disease Incidence:

In this study, non-sterilized seeds were planted in autoclaved soil, therefore it seems reasonable to conclude that the cotton seed borne fungi were the only source of seedling infection. Disease pressure during post-emergence stage was higher than in pre-emergence stage for Giza70, Giza 85, Giza89 and Giza90. In addition, post-emergence damping-off showed highly significant positive correlation with survival and highly significant negative correlation with plant height (Table 6). These results imply that, the role of seed-borne fungi, in seedling disease incidence was more evident in the post-emergence stage compared with the pre-emergence stage.

The occurrence and associations of pathogen species are of central importance in the ecology of host-pathogen interactions in complex disease *i.e.*, multiple pathogen on one host. During this dynamic cycle biotic and abiotic factors play a role in pathogen distribution and concentration. Subsequently, patterns of association result from interrelationships among organisms and environmental factors (Nelson and Campbell, 1992).Patterns of pathogens association involved in some complex patho-systems were evaluated. These patho-systems are foliar pathogens of cucumber (Peterson and Campbell, 2002) and fungi associated with cotton seeds (Aly *et al.*, 2004).

Table (6): Correlation among variable used for evaluating seed borne fungi on incidence of cotton seedling disease on eight cotton cultivars under greenhouse conditions

	Parameters	Variable				
		2	3	4	5	6
1	Seed germination	-0.257	-0.598	0.558	-0.862*	0.792*
2	Pre-emergence damping-off (%)		0.562	-0.627	0.487	-0.241
3	Post-emergence damping-off (%)			-0.990**	0.877**	0.852
4	Survival				-0.892	-0.893
5	Plant height (cm)					-0.893
6	Dry weight (g/plant)					

[a] **liner correlation coefficient(r) is significant at P<0.5(*),or P<0.01(**)**

In the present study, the cluster analysis of 14 fungi isolated from non-sterilized seeds of 7 cotton cultivars based on isolation frequencies (Fig.2) showed that, two unrelated groups of fungi were identified. The first group includes fungi nos. 4, 11, 13, 9, 10, 1, 12, 7, 2, 5, 14 and 8. Within this group, fungi were classified to three subgroups. The fungi under every subgroup were associated positively, while the second group includes only fungi no.3 and 6. This phonogram implies the potential existence of cultivar related groups of fungi. These results are in harmony with those of the ANOVA which also indicated that cotton cultivar plays a significant role in determining fungi frequencies.

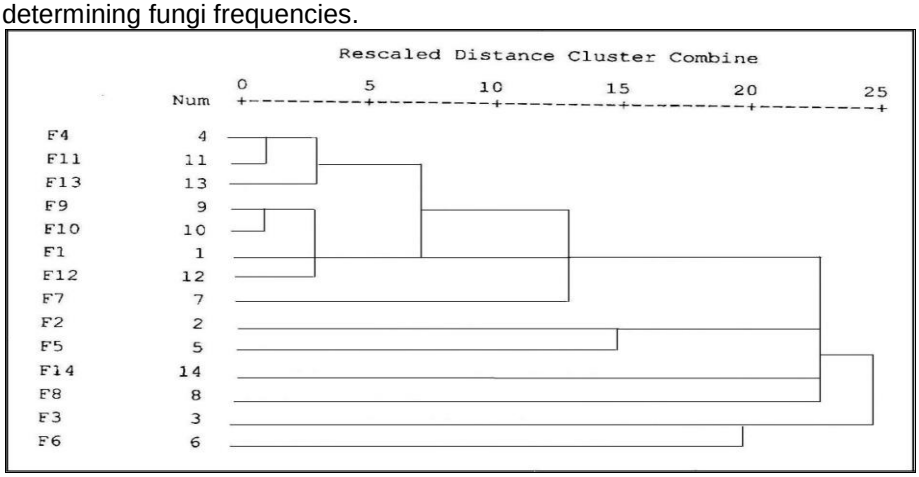

Fig (2): phonogram based on average linkage cluster analysis of isolation frequencies (%) of 14 fungi isolated from non-sterilized seeds of 7 cotton cultivars by the standard blotter method. The isolated fungi were *Alternaria alternate*(1), *Aspergillus flavus*(2), *Aspergillus fumigatus* (3), *Aspergillus nigar*(4), *Cladosporium* spp.(5), *Fusarium oxysporum* (6), *Fusarium moniliforme*(7), *Fusarium semitectum*(8), *Macrophomina phasolina*(9), *Penicillium* spp.(10), *Rhizoctonia solani*(11), *Trichoderma harzinum*(12), *Trichoderma* spp. (13) and *Trichothecium* spp.(14)

On the other hand, the cluster analysis of 7 cotton cultivars based on fungal profiles (Fig.3) indicate that cotton cultivar appear to form two distinct groups. One group consisting of 6 cultivars (Giza45, Giza 70, Giza85, Giza88, and Giza89) while the second group consisting of only one cultivar (Giza90).

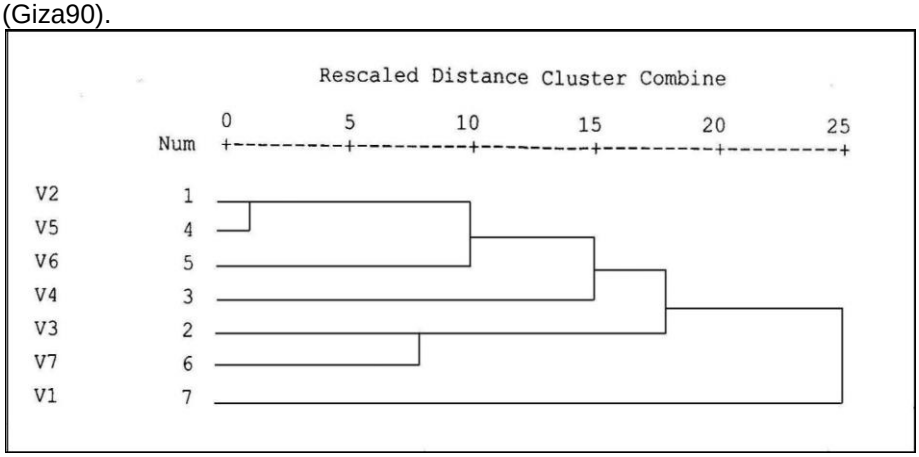

Fig (3): Phenogram based on average linkage cluster analysis of fungal profiles (%) from 7 cotton cultivars. The tested cultivars were (1) Giza45, (2) Giz70, (3) Giza85, (4) Giza86, (5) Giza88,(6) Giza89 and (7) Giza90.

Table (7): Stepwise regression models that describe the relationship between cotton seedling disease variables and frequencies of fungi isolated from non sterilized and seed of seven cultivars.

Dependent variable (Y)	Stepwise liner regression model [a]	Coefficient of determination (R^2)	F value [b]
Seed germination (%)	Y = 105.0786 - 0.634393 X1	96.38	133.16"''
Pre-emergence damping-off (%)	Y = 6.361745 + 0.7161738 X6	98.57	138.08"'
Post-emergence damping-off (%)	Y = 15.11667 + 55.58333 X8	77.76	17.48"
Survival (%)	Y = 73.71666 + 57.41667 X8	74.79	14.84"
Plant height (cm)	Y = 10.91615 + 216.1775 X8 + 0.1732622 X9 + $9.053945E^{-20}$ Xl4 + $7.194705\ E^{-20}$ XS	100	181067.88"'
Dry weight (g/plant)	Y = 150.6045 - 188.5674 X8 + 2.920225 *X2*	96.35	52.76"'

[a] **Identification of the predictors and their relative contributions to R^2 are shown in Table 8**
[b] **F. value is significant at P<(0.05(*),P<0.01(**)or P<0.005(***)**

These results agreed with the geographical distribution of the cultivar as the first group cultivars are cultivated in Delta- governates while cotton cultivar of second group (Giza90) is cultivated in Upper-Egypt governates. Data for seedling disease variables and frequencies of fungi isolated from non-sterilized seeds were processed by computerized stepwise multiple regression analysis which , 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 satisfactory contribution to the R^2 value of the model (Poddbckis *et al.,* 1989). Using the predictors supplied by stepwise regression, 6 models were constructed to predict seedling disease variables (Table 7). The models showed that differences in seedling disease variables were due largely to the effect of *A. alternata*, *Cladosporium* spp. and *F. semitectum* (Table 8). These results may suggest that these seed-borne fungi affect seedling disease variables.

Table (8): Identification of the predictors included in stepwise regression models shown in Table 8 and their relative contributions to R^a

Predictors	Variable and number	Relative contribution R^a %
Seed germination (%)		
Alternaria alternate	XI	96.38
Pre-emergence damping-off (%)		
Fusarium oxysporum	X6	44.84
Cladosporium spp.	X14	53.73
Post-emergence damping-off (%)		
Fusarium semitectum	X8	77.76
Survival (%)		
Fusarium semitectum	X8	74.79
Plant high (cm)		
Fusarium semitectum	X8	99.96
Aspergillus fumigatus	X3	3.49
Cladosporium spp.	X14	4.75
Fusarium oxysporum	X6	2.83
Dry weight (g/plant)		
Fusarium semitectum	X8	81.34
Aspergillus flavus	X2	15.01

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التقدير النوعي والكمي لفطريات بذره القطن وتأثيرها علي مرض موت البادرات.

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استعملت بذور معقمه سطحيا وبذور غير معقمه لبعض أصناف القطن التجاريه في التقدير النوعي والكمي لفطريات البذرة. أظهرت النتائج أن أكثر الفطريات تكرارا في العزل أسبرجلس فليفس و أسبرجلس نيجر والترناريا الترناتا . إن الصنف وتفاعل الصنف في المعامله كانا مصادر عاليه المعنويه للتباين في تكرار الفطريات المعزوله والمساهمان الاكثر اهميه في تكرار العزل. والملاحظ أن تأثير التعقيم السطحي علي تكرار الفطريات المعزوله اختلف بإختلاف الصنف ،فالصنف جيزة ٨٥ اعطي أقل عدد من الفطريات (٥ فطريات) أما الاصناف الأخري فقد أعطت عدد من الفطريات تتراوح (٧-١٣) . إن الدور الذي لعبته فطريات البذرة في حدوث مرض موت البادرات كان أكثر وضوحا في مرحله ما بعد ظهور البادرات فوق سطح التربه . أمكن بإستخدام التحليل العنقودي تقسيم أصناف القطن الي مجموعتين محددتين بناءا علي ما بينها من تباين في نسبه الفطريات المعزوله من كل صنف ،إشتملت المجموعه الاولي علي الاصناف جيزة ٤٥ ، جيزة ٧٠، جيزة ٨٥ ، جيزة ٨٦، جيزة ٨٨، جيزة ٨٩ ، أما المجموعه الثانيه اشتملت علي صنف واحد هو جيزة ٩٠ ، أظهرت نماذج الإنحدار التي أمكن الحصول عليها أن معظم الفروق في المتغيرات داله علي حدوث مرض موت البادرات تعزي في المقام الأول إلي تأثير فطريات الترناريا الترناتا وكلادوسبوريم وفيوزاريوم سيمتكتم.

قام بتحكيم البحث

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