J. Agric. Sci. Mansoura Univ., 29 (11): 6197 - 6211, 200

USE OF PROTEIN ELECTROPHORESIS TO QUANTIFY COTTON SEEDBORNE FUNGI

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ABSTRACT

A total of 14 seedborne fungi were identified among the cotton (*Gossypium barbadense* L.) cultivars Giza 80, Giza 83, Giza 85, Giza 86, Giza 88, and Giza 89. No single cultivar yielded all the 14 fungi. Giza 88 and Giza 89 yielded the highest number of fungi (12 fungi), while Giza 86 yielded the lowest number (8 fungi). The other cultivars yielded a number of fungi ranged from 9 to 11. *Penicillium* spp., *Alternaria alternata*, *Aspergillus flavus*, *Aspergillus niger*, *Cephalosporium* sp., and *Fusarium moniliforme* were isolated from all the tested cultivars. Cluster analysis of the cultivars, based on their fungal profiles, showed that Giza 80 had a unique fungal profile different from the profiles of the other cultivars. On the other hand, Giza 86 and Giza 89 showed a very high similarity level in their fungal profiles. Proteins of the cultivars were separated by SDS-PAGE, and the obtained banding patterns were visualized by using Coomassie-Blue staining system. Data for frequencies of the isolated fungi (dependent variables) and amounts of protein fractions (independent variables or predictors) were entered into a computerized stepwise multiple regression analysis. Using the predictors supplied by stepwise regression, 12 regression models were constructed to predict frequencies of the isolated fungi. Coefficient of determination (R^2) values of the models ranged from 62.72 to 100%. These results suggest that SDS-PAGE of proteins may provide a supplementary assay to microscopic examination to quantify fungal profiles of cotton seeds.

INTRODUCTION

The economic value of cottonseed is greatly influenced by the presence of fungi in the seed. Fungi or associated metabolites may reduce the vigor of planting seed (Halloin and Bourland, 1981; Davis, 1982), increase the amount of free fatty acid in the seed thereby reducing the quality of the oil (Rancadori *et al.*, 1971), or produce mycotoxins that render the seed unsuitable for consumption (Diener *et al.*, 1976). An understanding of the distribution and frequency of the mycoflora could lead to practical measures for control of the fungi, which devalue cotton seed (Klich, 1986).

Traditionally, mycologists and seed pathologists have assessed the fungal profiles of a seed lot by incubating a specific number of seeds on agar media or moist paper, then examining them for the presence of fungi (Wicklow, 1988).

Identification of fungi based on morphology is a difficult and tedious process. This is further complicated in that the morphology of spores is influenced to a great extent by cultural and environmental factors. In certain instances, some isolates form "mycelial type", without production of conidia. Also, classification based on morphology could be influenced by a personal bias (Synder and Toussoun, 1965).

Therefore, other reliable nonmorphological methods, either alternative or complementary to those based on morphology are required for quantification of fungi isolated from cottonseed.

Some published reports suggest that seed storage proteins or pathogenesis-related proteins may be important factors influencing susceptibility of seeds to fungi. For example, basic and acidic pathogenesis-related proteins accumulated in embryos of the maize cv. W64A in response to infection with *Fusarium moniliforme*. These proteins may play an important role in defense responses against fungal infection during seed germination (Raventos *et al.*, 1994).

Dormant cotton seeds contain proteins capable of inhibiting the activity of the proteolytic enzymes of the pathogen *Verticillium dahliae* (Mezhlum-yan *et al.*, 1994).

A protein designated hypogin, with a prominent suppressive action on the growth of the fungi *Mycosphaerella arachidicola, Fusarium oxysorpum*, and *Coprinus comatus*, was isolated from seeds of the ground nut *Arachis hypogaea*. (Ye and Ng, 2001).

A protein, with 23.8 KDa molecular mass and an acidic isoelectric point of 5.4, was isolated from corn seeds. This protein showed inhibitory activity against amylases from *Fusarium verticillioides* and *Aspergillus flavus* (Figueira *et al.*, 2003).

A protein designated mollisin, with 28 KDa molecular mass, was isolated from the seeds of the chestnut *Castanea mollissima*. The protein inhibited mycelial growth of *Fusarium oxysporum, Mycosphaerella arachidicola*, and *Physalospora pyricola*. The antifungal activity of mollisin was unaffected by incubation at 40°C for 10 minutes, underwent a decline after incubation at 60°C, and was completely abolished after treatment at 80°C (Chu and Ng, 2003).

The maize inbred Tex 6 has resistance to colonization and aflatoxin accumulation by *Aspergillus flavus*. A protein inhibiting the growth of *A. flavus* has been identified from aqueous extracts of mature Tex 6 seeds. (Moore *et al.*, 2004).

In the present study, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was employed to develop models to quantify frequencies of fungi isolated from cottonseeds. This approach has not been employed previously in the quantification of fungi isolated from cottonseeds.

MATERIALS AND METHODS

Fungal profiles of cotton cultivars:

Random 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 used in isolation.

Occurrernce of seedborne fungi was determined by the standard blotter method (ISTA, 1993). Ten nonsterilized 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.

Extraction of proteins from cottonseeds:

Protein extract was prepared according to Hussein (1992) in the following way: Seeds from healthy plants of cotton cultivars Giza 80, Giza 83 Giza 85, Giza 86, Giza 88, and Giza 89 were slightly ground and defatted by diethyl either or chloroform for 4 to 5 days. After drying at room temperature, ground seeds were suspended in a solution (1-3 ml/g seeds) consisting of 12.5 glucose and 1 g ascorbic acid dissolved in 100 ml phosphate buffer 8.3 and ground in liquid nitrogen to a fine powder. After thawing, the powder suspended in buffer was centrifuged at 19.000 rpm for 30 minutes at 0°C. The protein content in the supernatant was adjusted to a concentration of 3 to 4 mg/ml according to Bradford (1976) spectrophotometric method by using bovin serum albumin as a standard protein.

Electrophoresis of dissociated protein (SDS-PAGE):

For electrophoresis of dissociated protein, each supernatant was mixed with an equal volume of a solution consisted of (by volume) 64% buffer (0.15 M Tris-HCl, pH 6.8); 20% glycerol; 6% sodium dodecyl sulfate (SDS); 10% 2-6 mercaptoethanol, and 0.1% bromophenol blue, before boiling in water bath for 3 minutes. Twenty-microliter samples (40 µg of proteins) was subjected to electrophoresis in 15% polyacrylamide gel prepared in 0.1% SDS (Laemmli, 1970 and Latorre *et al.*, 1995) and stained with Brilliant Blue R-250 (Weeke, 1973).

Statistical analysis:

Gel was scanned for band R_f (position) and amount (%) by the gel documentation system AAB (Advanced American Biotechnology 1166). Stepwise regression technique with greatest increase in R^2 as the decision criterion was used to describe the effects of proteins (predictors or independent variables) on frequencies of the isolated fungi (dependent variables). Correlation and regression analyses were preformed with a computerized program.

RESULTS AND DISCUSSION

A total of 14 fungi were identified among the 6 cultivars that were tested (Table 1). No single cultivar yielded all the 14 fungi. Giza 88 and Giza 89 yielded the highest number of fungi (12 fungi), while Giza 86 yielded the lowest number (8 fungi). The other cultivars yielded a number of fungi ranged from 9 to 11. *Penicillium* spp., *Alternaria alternata, Aspergillus flavus, Aspergillus niger, Cephalosporium* sp., and *Fusarium moniforme* were isolated from all the tested cultivars.

Fig. 1 showed the phenogram constructed based on distances generated from cluster analysis of fungal profiles shown in Table 1. The smaller the distance, the more closely the cultivars were in their fungal profiles. Cluster analysis divided the cultivars into two distant groups.

Table 1. Frequencies (%) of fungi isolated from cottonseeds of eight cultivars.

Cultivar	Isolation frequency of													
	Rhizopus stolonifer	*Penicillium* spp.	*Alternaria alternata*	*Aspergillus flavus*	*Aspergillus niger*	*Fusarium* sp.	*Trichothecium roseum*	*Aspergillus* spp.	*Cephalosporium* sp.	*Cladosporium* sp.	*Fusarium oxysporum*	*Fusarium semitectum*	*Trichoderma* spp.	*Fusarium moniliforme*
	Y_1	Y_2	Y_3	Y_4	Y_5	Y_6	Y_7	Y_8	Y_9	Y_{10}	Y_{11}	Y_{12}	Y_{13}	Y_{14}
Giza 80	48	47	2	2	18	2	26	17	9	0.0	1	0.0	0.0	6
Giza 83	42	33	29	9	76	41	0.0	0.0	12	21	0.0	1	14	18
Giza 85	0.0	61	1	53	83	15	0.0	0.0	24	42	0.0	0.0	4	4
Giza 86	0.0	17	20	21	86	0.0	11	0.0	12	14	0.0	0.0	0.0	14
Giza 88	6	37	23	12	61	0.0	28	12	54	21	0.0	2	28	27
Giza 89	3	20	17	20	74	13	0.0	8	58	22	1	11	0.0	16

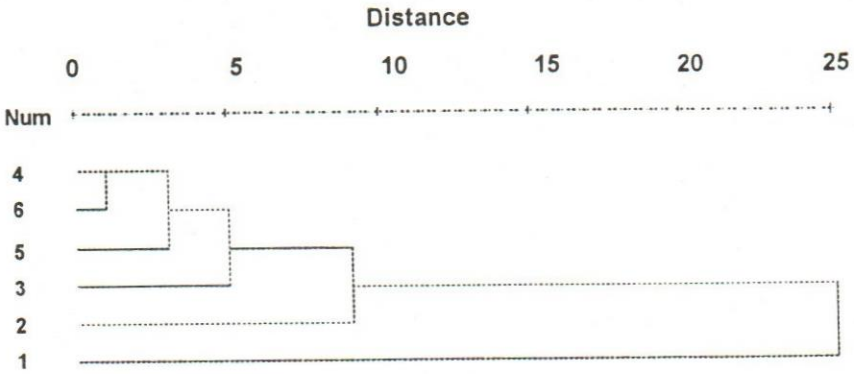

Fig. 1. Phenogram based on average linkage cluster analysis of susceptibility (%) of 6 cotton cultivars to 14 seedborne fungi. The tested cultivars were Giza 80 (1), Giza 83 (2), Giza 85 (3), Giza 86 (4), Giza 88 (5), and Giza 89 (6).

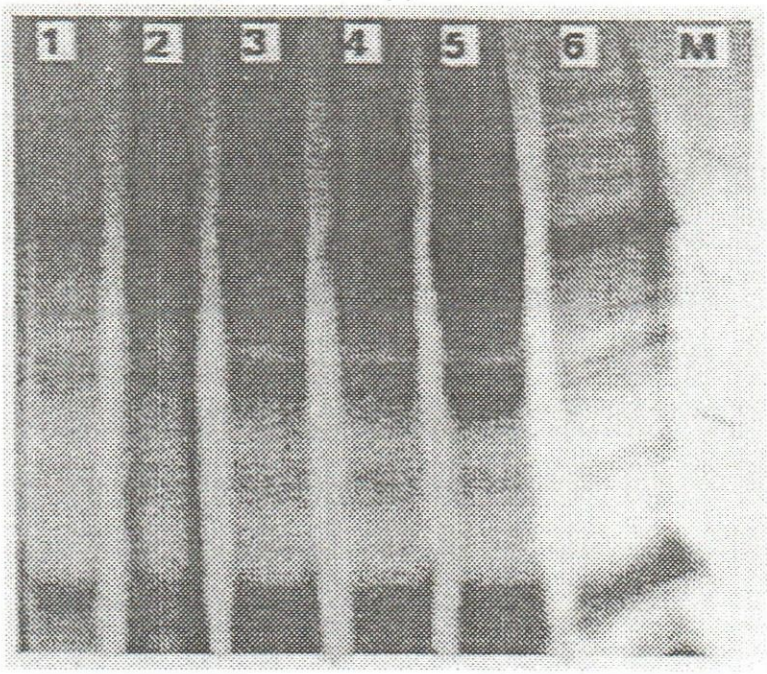

Fig. 2. Protein patterns obtained by SDS-PAGE from seeds of cotton cultivars Giza 80 (1), Giza 83 (2), Giza 85 (3), Giza 86 (4), Giza 88 (5), and Giza 89 (6). M was a protein marker.

One group included only culti Distance the other group included the remainder cultivars (Distance – 0.1). Within this group, cultivars associated strongly, whereas any of these cultivars associated weakly with Giza 80 (Table 2). The phenogram implied that Giza 80 had a unique fungal profile different from the profiles of the other cultivars. On the other hand, Giza 86 and Giza 89 showed very high similarity level in their fungal profiles (Distance = 0.7).

A total of 34 protein bands were identified among the 6 cultivars that were analyzed (Fig. 2 and Table 3). No single cultivar was stained for all the 34 bands. Similarly, no single band was common to all the cultivars. Giza 88 showed the least number of bands (7 bands), while the other cultivars showed a number of bands ranged from 8 to 9. Each cultivar was characterized by unique bands. For example, bands no_s. 6, 8, and 14 were unique to Giza 80.

Pearson correlation coefficient was calculated to measure the degree of association between frequencies of the isolated fungi and the amounts of the separated protein fractions (Table 4). However, few proteins were satisfactory correlated with frequencies of the isolated fungi. Thus, of the 476 correlation coefficients shown in Table 3, only 24 (5.04 %) were significant ($p < 0.05$) or highly significant ($p < 0.01$).

Data for isolation frequencies and amounts of protein fractions were entered into a computerized stepwise multiple regression analysis. The analysis constructed predictive models by adding predictors, in this case amounts of protein fractions, to the models 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 models only those variables that made a satisfactory significant contribution to the R^2 value of the model (Podleckis *et al.*, 1984). Using the predictors supplied by stepwise regression, 12 models were constructed to predict isolation frequencies (Table 5). R^2 values of the obtained models ranged from 62.72 to 100.00%. Of these models, 4 were one-variable models (Fig. 3).

The utility of the electrophoretic data depends on the method of statistical analysis. Multiple regression was a logical choice for construction of predictive models, but the complex nature of banding patterns warranted a method to eliminate bands with no predictive value. Stepwise regression is the best variable selection procedure because it eliminates from the model any variable whose contribution to predictive ability is statistically insignificant (Draper and Smith, 1981 and Podleckis *et al.*, 1984).

In the present study, satisfactory visualization of banding patterns were obtained by using the Coomassie Blue staining system for general proteins, and the stepwise regression models they generated proved effective in predicting isolation frequencies from banding patterns. Therefore, SDS-PAGE of proteins, such as that described herein, may provide a supplementary assay to microscopic examination to quantify fungal profiles of cottonseeds.

Table 2. Correlation among fungal profiles of six cotton cultivars.

Cultivar	Cultivar					
	Giza 80	Giza 83	Giza 85	Giza 86	Giza 88	Giza 89
Giza 80	----	0.327 [a]	0.179	0.088	0.198	0.015
Giza 83	0.327	----	0.595 *	0.707 **	0.426	0.577 *
Giza 85	0.179	0.595 *	----	0.771 **	0.580 *	0.516 **
Giza 86	0.088	0.707 **	0.771 **	----	0.705 **	0.801 **
Giza 88	0.198	0.426	0.580 *	0.705 **	----	0.786 **
Giza 89	0.015	0.577 *	0.716 **	0.801 **	0.786 **	----

[a] Pearson correlation coefficient (r) is significant at $p < 0.05$ (*) or $p < 0.01$ (**).

Table 3. Protein banding patterns for cotton cultivars obtained by sodium dodecyl sulfate-polyacylamide gel electrophoresis (SDS-PAGE) and stained with Comassie Brilliant Blue R-250.

Band			Cultivar					
No.	Position	MM[a]	Giza 80	Giza 83	Giza 85	Giza 86	Giza 88	Giza 89
1	0.0	115	0.00[b]	0.21	0.00	0.00	0.00	0.00
2	2	93	0.22	0.00	0.00	0.25	0.41	4.01
3	24	91	0.00	0.00	0.00	0.00	0.00	6.60
4	31	85	0.00	1.26	1.10	0.00	0.00	0.00
5	34	82	0.00	0.00	0.00	0.00	0.00	3.69
6	35	81	2.40	0.00	0.00	0.00	0.00	0.00
7	38	79	0.00	0.00	1.33	0.00	0.00	0.00
8	40	78	1.31	0.00	0.00	0.00	0.00	0.00
9	42	77	0.00	0.00	0.00	0.00	0.00	3.04
10	49	71	0.00	0.00	6.99	0.00	0.00	0.00
11	51	70	0.00	0.00	0.00	0.00	0.00	13.02
12	52	69	0.00	13.77	0.00	0.00	0.00	0.00
13	53	68	0.00	0.00	0.00	15.54	15.01	0.00
14	54	67	9.20	0.00	0.00	0.00	0.00	0.00
15	65	61	0.00	0.00	3.87	0.00	0.00	0.00
16	67	59	0.00	0.00	0.00	5.29	0.00	4.52
17	68	58	3.46	0.00	0.00	0.00	3.43	0.00

Table 3. Cont.

Band			Cultivar					
No.	Position	MM[a]	Giza 80	Giza 83	Giza 85	Giza 86	Giza 88	Giza 89
18	74	56	0.00	0.00	8.86	0.00	0.00	0.00
19	76	54	0.00	7.69	0.00	0.00	0.00	3.80
20	80	52	0.00	0.00	0.00	8.48	7.50	0.00
21	94	46	0.00	0.00	2.23	0.00	0.00	0.00
22	95	45	0.00	2.41	0.00	0.00	0.00	5.47
23	96	44	3.26	0.00	0.00	1.86	4.56	0.00
24	116	37	0.00	0.00	7.96	0.00	0.00	0.00
25	117	36	0.00	11.33	0.00	0.00	0.00	0.00
26	119	35	10.88	0.00	0.00	9.37	0.00	12.80
27	120	34	0.00	0.00	0.00	0.00	12.81	0.00
28	129	32	0.00	0.00	0.00	8.95	0.00	0.00
29	140	30	0.00	0.00	27.71	0.00	0.00	0.00
30	141	29	19.14	17.32	0.00	0.00	0.00	0.00
31	142	28	0.00	0.00	0.00	0.00	51.75	0.00
32	143	27	0.00	0.00	0.00	0.00	0.00	38.46
33	153	26	32.63	35.41	34.05	0.00	0.00	0.00
34	154	25	0.00	0.00	0.00	45.65	0.00	0.00

[a] Molecular mass in KDa.
[b] Amount (%) of the designated protein.

Table 4. Correlation between frequencies (Y_s) of fungi isolated from six cotton cultivars and protein content of seeds from these cultivars.

No. [a]	Y_1 [b]	Y_2	Y_3	Y_4	Y_5	Y_6	Y_7	Y_8	Y_9	Y_{10}	Y_{11}	Y_{12}	Y_{13}	Y_{14}
1	0.561[c]	-0.084	0.586	-0.288	0.188	0.908*	-0.401	-0.412	-0.357	0.036	-0.316	-0.151	0.274	0.224
2	-0.318	-0.501	0.090	-0.032	0.115	-0.043	-0.317	0.184	0.698	0.024	0.640	0.986**	-0.292	0.163
3	-0.297	-0.468	0.071	0.014	0.149	0.036	-0.401	0.123	0.658	0.072	0.633	0.983**	-0.331	0.107
4	0.205	0.476	0.042	0.432	0.396	0.836	-0.632	-0.649	-0.368	0.612	-0.498	-0.322	0.114	-0.248
5	-0.297	-0.468	0.071	0.014	0.149	0.036	-0.401	0.123	0.658	0.072	0.633	0.983**	-0.331	0.107
6	0.693	0.330	-0.571	-0.480	-0.939*	-0.306	0.561	0.724	-0.423	-0.720	0.633	-0.265	-0.331	-0.476
7	-0.363	0.744	-0.614	0.918*	0.324	0.099	-0.401	-0.412	-0.092	0.792	-0.316	-0.265	-0.158	-0.593
8	0.693	0.330	-0.571	-0.480	-0.939*	-0.306	0.561	0.724	-0.423	-0.720	0.633	-0.265	-0.331	-0.476
9	-0.297	-0.468	0.071	0.014	0.149	0.036	-0.401	0.123	0.658	0.072	0.633	0.983**	-0.331	0.107
10	-0.363	0.744	-0.614	0.918*	0.324	0.099	-0.401	-0.412	-0.092	0.792	-0.316	-0.265	-0.158	-0.593
11	-0.797	-0.468	0.071	0.014	0.149	0.036	-0.401	0.123	0.658	0.072	0.633	0.983**	-0.331	0.107
12	-0.571	-0.084	0.586	-0.288	0.188	0.908	-0.401	-0.412	-0.357	0.036	-0.316	-0.151	0.274	0.224
13	-0.471	-0.421	0.416	-0.127	0.227	-0.582	0.498	-0.029	0.156	-0.146	-0.500	-0.242	0.416	0.574
14	0.693	0.330	-0.571	-0.480	-0.939*	-0.306	0.561	0.724	-0.423	-0.720	0.633	-0.265	-0.331	-0.476
15	-0.363	0.744	-0.614	0.918*	0.324	0.099	-0.401	-0.412	-0.092	0.792	-0.316	-0.265	-0.158	-0.593
16	-0.523	-0.812	0.222	0.045	0.432	-0.286	-0.285	-0.261	0.175	-0.131	0.190	0.488	-0.521	0.069
17	0.368	0.289	-0.195	-0.543	-0.827	-0.533	0.945*	0.882*	0.113	-0.543	0.253	-0.240	0.428	0.211

Table 4. Cont.

No. [a]	Y_1 [b]	Y_2	Y_3	Y_4	Y_5	Y_6	Y_7	Y_8	Y_9	Y_{10}	Y_{11}	Y_{12}	Y_{13}	Y_{14}
18	-0.363	0.744	-0.614	0.918*	0.324	0.099	-0.401	-0.412	-0.092	0.792	-0.316	-0.265	-0.158	-0.593
19	0.183	-0.447	0.504	-0.207	0.265	0.719	-0.633	-0.213	0.269	0.086	0.278	0.691	-0.064	0.258
20	-0.475	-0.440	0.411	-0.118	0.243	-0.581	0.475	-0.056	0.123	-0.154	-0.499	-0.249	0.373	0.546
21	-0.363	0.744	-0.614	0.918*	0.324	0.099	-0.401	-0.412	-0.092	0.792	-0.316	-0.265	-0.158	-0.593
22	-0.049	-0.501	0.327	-0.112	0.230	0.432	-0.572	-0.059	0.497	0.087	0.489	0.908*	-0.209	0.204
23	0.110	0.041	0.002	-0.504	-0.587	-0.699	0.985**	0.701	0.116	-0.538	0.007	-0.318	0.480	0.383
24	-0.363	0.744	-0.614	0.918*	0.324	0.099	-0.401	-0.412	-0.092	0.792	-0.316	-0.265	-0.158	-0.593
25	0.561	-0.084	0.586	-0.288	0.188	0.908*	-0.401	-0.412	-0.357	0.036	-0.316	-0.151	0.274	0.224
26	0.021	-0.508	-0.227	-0.309	-0.313	-0.419	0.066	0.371	0.036	-0.593	0.801	0.485	-0.728	-0.258
27	-0.231	0.035	0.329	-0.206	-0.104	-0.368	0.635	0.390	0.570	0.036	-0.316	-0.038	0.878	0.748
28	-0.363	-0.557	0.200	0.041	0.382	-0.368	0.006	-0.412	-0.357	-0.216	-0.316	-0.265	-0.331	-0.010
29	-0.363	0.744	-0.614	0.918*	0.324	0.099	-0.401	-0.412	-0.092	0.792	-0.316	-0.265	-0.158	-0.593
30	0.995**	0.211	-0.035	-0.613	-0.637	0.427	0.165	0.291	-0.618	-0.570	0.287	-0.333	-0.069	-0.227
31	-0.231	0.035	0.329	-0.206	-0.104	-0.368	0.635	0.390	0.570	0.036	-0.316	-0.038	0.878	0.748
32	-0.297	-0.468	0.071	0.014	0.149	0.036	-0.401	0.123	0.658	0.072	0.633	0.983**	-0.331	0.107
33	0.659	0.725	-0.412	0.119	-0.284	0.558	-0.209	-0.109	-0.647	0.104	-0.029	-0.503	-0.142	-0.609
34	-0.363	-0.557	0.200	0.041	0.382	-0.368	0.006	-0.412	-0.357	-0.216	-0.316	-0.265	-0.331	-0.010

[a] Number of protein fraction.
[b] Identification of the isolated fungi is shown in Table 1.
[c] Pearson correlation coefficient (r), which measures the degrees of association between frequency of the isolated fungus and amount of the designated protein. Value of r is significant at $p < 0.01$ (**) or $p < 0.05$ (*).

Table 5. Stepwise regression models that describe the relationship between frequencies (Y^a) of fungi isolated from six cotton cultivars and protein content (X^b) of seeds from these cultivars.

Fungus	Stepwise linear regression model	Coefficient of determination (R^2)	F value[c]
Rhizopus stolonifer	$Y_1 = 0.97+2.42X30+0.39X27$	99.71[e]	511.79***
Penicillium spp.	$Y_2 = 38.99+4.18X16+16.55X7$	92.42[f]	18.30*
Alternaria alternata	[d]	. . . .	. . . .
Aspergillus flavus	$Y_4 = 12.8+30.23X7$	84.30	21.48**
Aspergillus niger	$Y_5 = 84.52–27.72X6–1.84X27–1.19X19$	99.83[g]	398.70***
Fusarium sp.	$Y_6 = 6.00+3.09X25$	82.40	18.73**
Trichothecium roseum	$Y_7 = -0.07+6.13X23+0.62X14$	100.00[h]	9523.23***
Aspergillus sp.	$Y_8 = 0.00+3.50X17+1.21X3+2.04X6$	100.00[i]	59652324.00***
Cephalosporium sp.	$Y_9 = 12.08+11.45X2+3.51X27+1.50X24–1.68X23$	100.00[j]	11430.80**
Cladosporium sp.	$Y_{10} = 15.6+11.84X21$	62.72	6.73x
Fusarium oxysporum	$Y_{11} = 0.004+0.08X26-0.09X28$	98.94[k]	140.02***
Fusarium semitectum	$Y_{12} = 0.73+3.07X2-0.16X26-0.55X7+0.02X30$	100.00[l]	50032.57***
Trichoderma spp/	$Y_{13} = 3.60+1.90X27$	77.12	13.48*
Fusarium moniliforme	. . . . .	. . . .	. . . .

[a] Dependent variables.
[b] Identification of the predictors (X_s) is shown in Table 3.
[c] F. value is significant at $P < 0.10$ (x), $P < 0.05$ (*), $P < 0.01$ (**), or $P < 0.005$ (***).
[d] No regression model could be constructed.
[e] Relative contributions of the predictors X30 and X27 to R^2 are 98.94, and 0.76%, respectively.
[f] Relative contributions of the predictors X16 and X7 to R^2 are 65.94, and 26.48%, respectively.
[g] Relative contributions of the predictors X6, X27 and X19 to R^2 are 88.12, 8.84, and 2.87%, respectively.
[h] Relative contributions of the predictors X23 and X14 to R^2 are 97.05, and 2.94%, respectively.
[i] Relative contributions of the predictors X17, X31, and X6 to R^2 are 77.70, 17.89, and 4.42%, respectively.
[j] Relative contributions of the predictors X2, X27, X24 and X23 to R^2 are 48.77, 44.01, 6.56, and 0.65%, respectively.
[k] Relative contributions of the predictors X26, and X28 to R^2 are 64.14, and 34.80%, respectively.
[l] Relative contributions of the predictors X2, X26, X7, and X30 to R^2 are 97.29, 2.08, 0.54 and 8.69%, respectively.

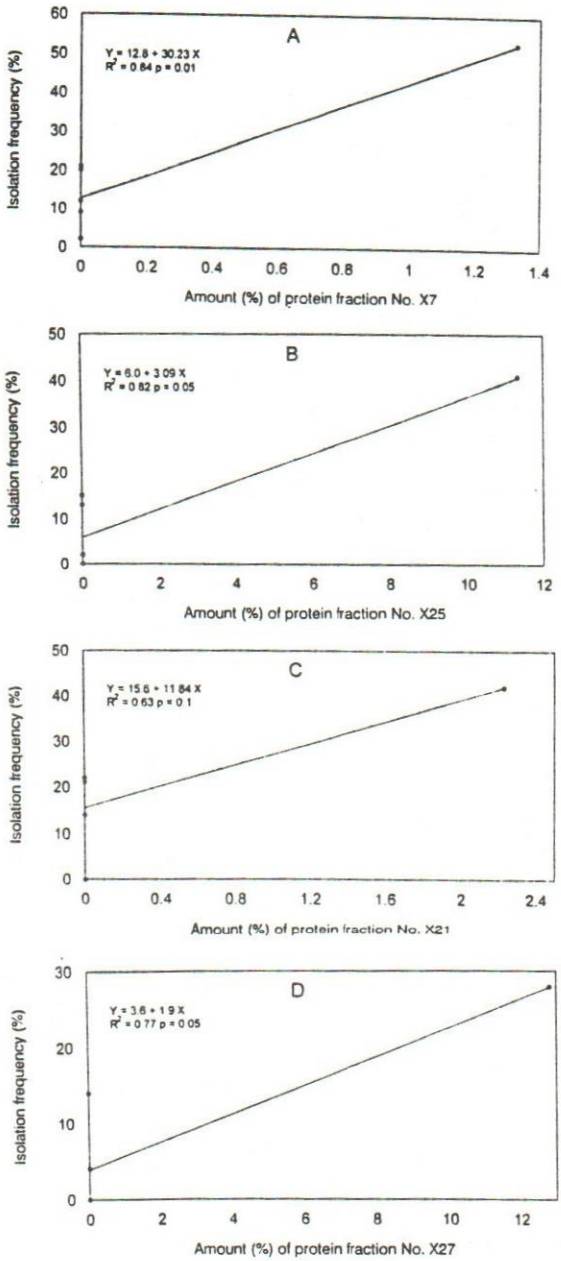

Fig. 3. Relationship between amounts of protein fractions and isolation frequencies of *Aspergillus flavus* (A), *Fusarium* sp. (B), *Cladosporium* sp. (C), and *Trichoderma* spp. (D).

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إستعمال التفريد الكهربى للبروتينات للتعبير الكمى عن فطريات بذرة القطن

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أمكن التعرف على 14 فطر من بذور القطن أصناف جيزة 80 وجيزة 83 وجيزة 85 وجيـزة86 وجيزة 88 وجيزة 89. لا يوجد صنف قطن أعطى جميع الفطريات (14 فطر) عند العزل منه. أكبر عـدد من الفطريات (12 فطر) أمكن عزله من صنفى القطن جيزة 88 وجيزة 89 ، أما أقل عدد من الفطريـات (8 فطريات) فقد أمكن عزله من جيزة 86. أعطت الأصناف الأخرى عدداً من الفطريات تراوح من 9 إلى 11. الفطريات بنيسيليوم والترناريا التراتـا وأسـبرجلس فلافـس وأسـبرجلس نيجـر وسيفالوسـبوريوم وفيوزاريوم مونيليفورمى أمكن عزلها من جميع الأصناف المختبرة . أظهر التحليل العنقودى للأصـناف – بناء على تكرارات الفطريات المعزولة منها – أن مجموعة الفطريات المعزولة من جيزة 80 هى مجموعـة متفردة فى تكرار عزلها من هذا الصنف مقارنة بتكرارات عزلها من باقى الأصناف. أظهر الصنفان جيـزة 86 وجيزة 89 مستوى عالى جداً من التشابه من حيث تكرار الفطريات المعزولة من كل منهما. إسـتعملت تقنية التفريد الكهربى لفصل بروتينات بذرة الأصناف ، وذلك بعد تفكيك هذه البروتينـات باسـتعمال مـادة صوديوم دوديسيل سلفيت. إستعملت مادة الكوماسى بلو لصبغ أنماط البروتينات المتحصل عليهـا. أمكـن – باستخدام أسلوب الإنحدار المتعدد المرحلى – التوصل إلى 12 نموذج إنحدار لوصف العلاقة بين تكـرارات الفطريات المعزولة (متغير تابع) وكميات البروتينات المفصولة (متغير مستقل) . تراوحت قيم معامل التحديد للنماذج المتحصل عليها من 62.72 إلى 100%. تدل نتائج الدراسة الحالية على أنه من الممكن إسـتخدام تقنية التفريد الكهربى للبروتينات كوسيلة مكملة للفحص الميكروسكوبى للتعبير الكمى عن الفطريات المعزولة من بذرة القطن.