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Effect of Amino Acid Content on the Level of Cottonseed Colonization by Mycoflora

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ABSTRACT

Non-sterilized seeds from 12 cotton (*Gossypium barbadense* L.) genotypes were examined for qualitative and quantitative estimates of seedborne fungi. The observed fungi were *Alternaria alternata*, *Aspergillus flavus*, *A. niger*, *Aspergillus* sp. *Chaetomium* sp. *Cladosporium* sp. *F. oxysporum*, *Penicillium* sp. *Rhizopus stolonifer* and *Stemphylium* sp. The quantitative estimates of the fungi showed that *R. stolonifer* (39.7%), *A. niger* (33.5%) and *Penicillium* sp. (23.3%) were the most predominant fungi isolated from the seeds. Other fungi occurred at frequencies ranged from 0.3 to 17.7%. The HPLC analysis of amino acid composition of cottonseed revealed the presence of 17 amino acids but the occurrence of each in the seeds varied with the genotype. Data for frequencies of the isolated fungi (dependent variables) and contents of amino acids (predictors or independent variables) were entered into a computerized stepwise multiple regression analysis. Using the predictors supplied by stepwise regression, eight models were constructed. R^2 values of the models ranged from 57.74 to 99.41%. Thus, it was possible to group the isolated fungi into four distinct categories based on their sensitivity to the amino acids (the size of R^2 value). The first category included the insensitive fungi *A. alternata* and *Chaetomium* sp. where no regression models could be constructed. The second category included the moderately sensitive fungi *F. oxysporum* and *R. stolonifer*, where R^2 values were 65.72 and 57.74%, respectively. The third category included the sensitive fungi *A. flavus*, *A. niger*, *Penicillium* sp. and *Aspergillus* sp., where R^2 values were 86.35, 90.84, 90.00 and 82.62%, respectively. The fourth category included the highly sensitive fungi *Cladosporium* sp. and *Stemphylium* sp., where R^2 values were 99.41 and 98.76, respectively. The results of the present study suggest that certain amino acids regulate colonization of cottonseed by mycoflora and that control of these fungi may be possible by modifying amino acid content of the seed.

Key Words: *Gossypium barbadense*; Amino acids; Seeds mycobiota

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 & Bourland, 1981; Davis, 1982), increase the amount of free fatty acids in the seed thereby reducing the quality of the oil (Roncadori *et al.,* 1971), or produce mycotoxins that render the seed unsuitable for consumption (Diener *et al.,* 1976).

Under Egyptian conditions, the fungi involved in cottonseed deterioration include *Alternaria* spp. *Aspergillus* spp. *Cephalosporium* spp. *Cladosporium* spp. *Curvularia* spp. *Fusarium moniliforme*, *F. oxysporum*, *F. semitectum*, *F. solani*, *Helminthosporium* spp. *Nigrospora* spp. *Pythium* spp. *Rhizoctonia* spp. *Trichothecium* spp. *Epicoccum* spp. *Penicillium* spp. *Chaetomium* spp. *Diplodia gossypii*, *Rhizopus* spp. and others (El-Helaly *et al.,* 1966; Waked *et al.,* 1981; Amer, 1986; Mohamed, 1999).

A considerable body of literature has shown a strong relationship between amino acids and physiological activities associated with pathogenicity of phytopathogenic fungi like germination of spores, elongation of germ tubes and production and efficiency of enzymes. For instance, Dube and Bordia (1982) found that all seven amino acids tested against *Helminthosporium sacchari* in Richard's medium containing 1% sodium polypectate completely inhibited sporulation but did not significantly affect growth. Alanine, asparagine, glycine and tryptophan completely suppressed pectic acid lyase (PAL) synthesis; others showed modulating effect on substrate preference, depolymerizing capacity and pH optima. Arginine and glutamic acid supported some PAL activity; phenylalanine repressed it and induced a hydrolytic enzyme (polygalacturonase)

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activity on sodium polypectate. Ansari *et al.* (1989) studied the nutritional requirements of *Alternaria brassicae* for growth and sporulation. Of nine amino acids tested, DL-threonine induced the greatest mycelia growth and minimal growth occurred on L-tyrosine. Sporulation was good on DL-threonine, DL-valine, L-isoleucine and poor on DL-aspartic acid, glycine and L-tyrosine. Mehta *et al.* (1991) tested amino acids for the secretion of pectotyltic and cellulolytic enzymes. They found complete inhibition of polygalacturonase (PG) synthesis with leucine and phenylalanine in *F. oxysporum* and *F. moniliforme*, respectively. Leucine showed complete inhibition of pectinmethylgalacturonase (PMG) synthesis in *F. oxysporum*. In *F. moniliforme*, cysteine and phenylalanine also inhibited PMG synthesis. Total inhibition of cellulase production was found in *F. moniliforme* by leucine, phenylalanine and tryptophan, whereas none could control that total cellulase synthesis in *F. oxysporum*.

In order to examine some possible effects of plant root exudates on *Metarhizium anisopoliae*, Li and Holdom (1995) examined the effects of a range of amino acids on colony formation, mycelia growth and sporulation of two isolates (EF 25 & EF 55). L-glutamine, L-serine, L-asparagine and L-alanine were best for growth and L-cysteine, L-aspartic acid and L-threonine were the worst. L-lysine and L-threonine were best for sporulation. Neither isolate sporulated on L-cysteine, L-glutamine or L-leucine nor did little or no sporulation occurred on L-alanine and L-serine. Guha-Roy and Samaddar (1997) examined the effects of different amino acids on the formation and germination of sporangia of *Phytophthora nicotianae* var. *nicotianae* by flooding oat meal agar grown mycelia discs of the organism with different concentrations of the tested amino acids. The results indicated that some amino acids such as glycine and glutamic acid were highly stimulatory to production and germination of sporangia of *P. nicotianae* var. *nicotianae*. valine, histidine and lysine caused moderate to fair production and germination of the pathogen sporangia. Kalaimani (1997) investigated the nutrition of six isolates of *Colletotrichum falcatum* collected from sugarcane from various sites in Tamil Nadu, India. Of the amino acids tested, valine induced maximal mycelia growth in all the six isolates. Threonine and tyrosine inhibited mycelial growth of all the six isolates. Aspartic acid gave maximal sporulation in isolates I and II, and valine gave good sporulation in the four other isolates. Elson *et al.* (1998) evaluated the influence of various amino acids on conidial germination, colony diameter and conidiation of *H. solani* grown on solid-phase basal salts media. Total conidia production was improved by use of tyrosine or arginine as the sole nitrogen source. Use of leucine, lysine, methionine, phenylalanine or threonine severely inhibited *H. solani* conidia production. Use of a nitrogen source containing a mixture of amino acids resulted in a defined medium that permitted conidiation and growth of *H. solani* that was similar to or better than that obtained with standard V8 Juice medium. Growth (colony diameter & biomass production) and sporulation of *Pestalotia psidii*, *Colletotrichum gloeosporioides* and *Botryodiplodia theobromae*, the foliar pathogens of guava, were tested in Czapek's dox medium supplemented with the amino acids arginine and cysteine. The two amino acids supported the growth and sporulation of all the pathogens, with cysteine at 500 ppm being the most suitable (Mishra & Sitansu, 2007).

Amino acids have also been shown to be correlated with resistant or susceptibility to plant diseases. For example, El-Hamalawy and Menge (1995) found that total free amino acid content of the avocado bark tissue was highly correlated with canker size on stem ($r = 0.89$) caused by *P. citricola*. Through correlations and path coefficient analysis of a field trial with peas cv. Rachana (resistant) and T163 (susceptible), Bhattacharya and Shukla (1996) concluded that severity of powdery mildew (*Erysiphe polygoni*) on field pea was substantially increased by the accumulation of free amino acids. Omokolo *et al.* (2002) found a significant negative correlation ($r = - 0.65$, $P < 0.05$) between the level of amino acids in cacao (*Theobroma cacao* L.) pods and the lesion size caused by *P. megakarya*. Omokolo and Boudjeko (2005) investigated the putative role of amino acids in susceptibility of *Xanthosoma sagittifolium* to *Pythium myriotylum* root rot disease by comparing the alteration of amino acids in the roots of the white (susceptible), red (tolerant) and yellow (resistant) cultivars. At day 2 after inoculation, total amino acid content increased in the three cultivars. However, this increase was negatively correlated to the disease only in the yellow cultivars ($r = - 0.94$, $P < 0.05$).

Little is known about the constituents of cottonseed that affect susceptibility to mycoflora. However, one report demonstrated that dormant cottonseeds contained proteins capable of inhibiting the activity of the proteolytic enzymes of the pathogen *Verticillium dahliae* (Mezhlum-Yan *et al.*, 1994). The considerable body of work with amino acids has provided a convincing evidence that amino acids may play on important role in regulating the interaction of mycoflora with cottonseed. Therefore, the main objective of the present study was to evaluate the role of amino acids in determining the level of cottonseed colonization by mycoflora. An understanding of such a potential role could lead to practical methods for control of seed-borne fungi, which devalue cottonseed.

MATERIALS AND METHODS

Fungal profiles of cotton genotypes: Random samples of cotton (*Gossypium barbadense* L.) genotypes were obtained from Cotton Research Institute, Agriculture Research Center, Giza, Egypt. Random subsamples of 100 seeds for each genotype were used in isolation.

Occurrence of seed-borne fungi was determined by the standard botter method (ISTA, 1993). Ten non-sterilized seeds for each genotype, 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-h light and 120h darkness at 20±2°C for seven days. After incubation, each colony was examined microscopically for identification to genus or species level according to Gilman (1966), Booth (1971), or Barnett 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.

Analysis of cottonseed amino acid composition: The amino acid composition of seed samples was determined according to High-Performance Liquid Chromatography-Pico-Tag method (Cohen *et al.,* 1989), which performed as follows:

Extraction of total amino acids: A sample corresponding to one mg protein was weighted into 25×150 mm hydrolyzed tube, aliquot (7.50 mL) of 6 N HCl was added, purged with nitrogen for 60 sec and the tube was capped immediately. The tube was placed in 110°C oven for 24 h, removed and allowed to cool. The contents of the tube were quantitatively transferred to 25 mL volumetric flask and completed to volume with High-Performance Liquid Chromatography (HPLC) grade water. One mL of the solution was filtered through 0.45 µm Millipore membrane filter.

Derivatization of amino acids: Ten microliters of the filtered sample in 6 x 50 mm tube was placed into drying vial and dried in a freeze-dryer workstation for 10-15 min. Aliquot (30 µL) of redried solution (consisted of a mixture of 200 µL methanol, 200 µL 0.2 N sodium acetate & 100 µL triethylamine) was added to the sample tubes and redried again in the workstation.

Aliquot (30 µL) of the freshly prepared derivatization agent (a mixture of 350 µL methanol & 50 µL phenylisothiocyanate) was added to the tube contents and allowed to react for 20 min. dried in the workstation for 15 min.

Thirty µL methanol was added and dried again, 250 µL of sample diluents (Waters, USA) were added to the dried tube, vortexed and transferred to injection vials. The standard amino acid (Sigma, USA) solution was treated the same as the sample.

Separation of total amino acids derivatives by using HPLC: Separation of total amino acid derivatives was performed on a stainless steel Pico-Tag amino acid column (150 × 3.9 mm). Spectra Physics P2000 variable wavelength detector was adjusted to 254 nm. The amino acids were quantified by comparison of peak area with those corresponding amino acid standard solutions using the Spectra Physics Data System program.

Statistical analysis: Linear correlation coefficient (r) was calculated to evaluate the degree of association between frequencies of the isolated fungi and the percentage of each amino acid. Stepwise regression technique with greatest increase in R^2 as the decision criteria was used to describe the effect of amino acids on frequencies of the isolated fungi. Correlation and regression analyses were performed with a computerized program (SPSS).

RESULTS

The mean percentage of fungal recovery from cottonseeds (Table I) showed that *R. stolonifer* (39.7%), *A. niger* (33.5%), and *Penicillium* sp. (23.3%) were the most predominant fungi isolated from the non-sterilized cottonseeds. The other fungi occurred at frequencies ranged from 0.3 to 17.7%. A total of 10 fungi were identified among the 12 genotypes that were tested (Table I). No single genotype yielded all the 10 fungi. The genotype 488/2000 yielded the highest number of fungi (8 fungi), while each of 514/2000 and Giza 74 yielded the lowest number (4 fungi). The other genotypes yielded a number of fungi ranged from 5 to 7. *R. stolonifer* was the only fungus, which was isolated from all the tested genotypes.

The HPLC analysis of amino acid composition of cottonseeds revealed the presence of 17 amino acids but the occurrence of each in the seeds varied with the genotype (Table II).

Of the significant 12 r values shown in Table III, six were positive and six were negative. Isolation frequency of *A. alternata* (Y_1), *Chaetomium* sp. (Y_4) and *R. stolonifer* (Y_8) was not significantly correlated with the content of any amino acid, while the isolation frequency of each of the other fungi was significantly correlated with the content of one or two amino acids.

Content of each of glutamic, glycine, arginine, theroinine, valine, cysteine and luecine was not significantly correlated with the frequency of any of the isolated fungi, while content of each of the other amino acids was significantly correlated with the isolation frequency of one or two fungi.

Data for frequencies of the isolated fungi and contents of amino acids were entered into a computerized stepwise multiple regression analysis. The analysis constructed a predictive model by adding predictors, in this case amino acids, to the model in order of their contribution to R^2. The analysis was effective in eliminating those factors with little or no predictive value by incorporating into the model only those factors that made a statistically significant contribution to the R^2 value of the model (Podleckis *et al.,* 1984). Using the predictors supplied by stepwise regression, eight models were constructed (Table IV & V). R^2 values of the regression models ranged from 57.74 to 99.41%. Each of serine, alanine and isoleucine was included in 4 models, while each of histidine, tyrosine and methionine were included in 3 models. Each of the remaining amino acids were included in one or two models. However, arginine and valine were notable exceptions, because neither of them was included in any regression model.

Table I: Frequencies (%) of fungi isolated from seeds of 12 cotton genotypes

Genotype	Isolation frequency [a] (%) of									
	Alternaria alternata	*Aspergillus flavus*	*A. niger*	*Chaetomium* sp.	*Cladosporum* sp.	*Fusarium oxysporum*	*Penicillium* sp.	*Rhizopus stolonifer*	*Stemphylium* sp.	*Aspergillus* sp.
	(Y_1)	(Y_2)	(Y_3)	(Y_4)	(Y_5)	(Y_6)	(Y_7)	(Y_8)	(Y_9)	(Y_{10})
427/2002	0.0	8.0	50.0	0.0	0.0	2.0	20.0	66.0	24.0	0.0
405/2002	0.0	20.0	70.0	0.0	0.0	0.0	22.0	30.0	14.0	0.0
423/2002	0.0	2.0	62.0	12.0	0.0	0.0	32.0	22.0	34.0	0.0
514/2002	0.0	0.0	0.0	2.0	0.0	0.0	2.0	24.0	2.0	0.0
501/2002	0.0	6.0	22.0	4.0	0.0	0.0	2.0	27.0	6.0	0.0
490/2002	0.0	0.0	14.0	0.0	0.0	0.0	12.0	26.0	50.0	0.0
449/2002	6.0	52.0	8.0	0.0	0.0	0.0	54.0	43.0	6.0	4.0
488/2002	4.0	12.0	8.0	0.0	4.0	0.0	40.0	25.0	24.0	12.0
812/2002	0.0	6.0	46.0	0.0	0.0	0.0	22.0	31.0	28.0	16.0
491/2002	3.0	0.0	0.0	0.0	4.0	0.0	46.0	80.0	6.0	0.0
507/2002	0.0	2.0	22.0	0.0	18.0	0.0	28.0	24.0	18.0	0.0
Giza 74	0.0	86.0	100.0	0.0	0.0	2.0	0.0	78.0	0.0	0.0
Mean	1.1	16.2	33.5	1.5	2.2	0.3	23.3	39.7	17.7	2.7

[a] Frequency (%) of fungi isolated from 100 nonsterilized seeds from each genotype by the standard blotter method and examined 7 day from incubation at 20°C±2 and alternative cycle of cool white light/darkness

Table II: Amino acid composition (%, w/w) of cottonseeds

Amino acid	Genotype											
	427/2002	405/2002	423/2002	514/2002	501/2002	490/2002	449/2002	488/2002	812/2002	491/2002	507/2002	Giza 74
Aspartic	0.031	0.025	0.017	0.120	0.020	0.027	0.116	0.008	0.038	0.031	0.022	0.052
Glutamic	0.208	0.099	0.215	0.183	0.137	0.027	0.072	0.024	0.229	0.125	0.157	0.153
Serine	0.027	0.018	0.024	0.043	0.020	0.027	0.037	0.029	0.030	0.027	0.021	0.069
Glycine	0.053	0.051	0.057	0.049	0.063	0.040	0.037	0.020	0.058	0.074	0.068	0.064
Histidine	0.079	0.014	0.077	0.016	0.059	0.029	0.077	0.022	0.049	0.102	0.065	0.040
Arginine	0.022	0.162	0.028	0.050	0.038	0.039	0.079	0.068	0.163	0.031	0.030	0.054
Thereonine	0.042	0.088	0.036	0.101	0.038	0.065	0.059	0.035	0.099	0.076	0.034	0.087
Alanine	0.371	0.240	0.339	0.199	0.322	0.372	0.312	0.158	0.255	0.217	0.235	0.372
Proline	0.038	0.065	0.042	0.063	0.039	0.081	0.035	0.061	0.061	0.074	0.027	0.026
Tyrosine	0.043	0.071	0.038	0.009	0.054	0.075	0.068	0.068	0.042	0.073	0.090	0.050
Valine	0.040	0.037	0.039	0.064	0.040	0.052	0.044	0.043	0.032	0.036	0.052	0.045
Methionine	0.022	0.044	0.025	0.053	0.030	0.067	0.037	0.059	0.031	0.045	0.082	0.036
Cysteine	0.027	0.032	0.019	0.027	0.040	0.075	0.052	0.039	0.009	0.020	0.012	0.010
Isoleucine	0.015	0.022	0.013	0.040	0.022	0.051	0.037	0.023	0.017	0.034	0.025	0.025
Leucine	0.060	0.043	0.057	0.077	0.055	0.070	0.061	0.054	0.068	0.041	0.045	0.063
Phenylalanine	0.073	0.084	0.077	0.116	0.092	0.086	0.052	0.063	0.056	0.077	0.070	0.087
Lysine	0.038	0.047	0.050	0.020	0.017	0.018	0.017	0.018	0.008	0.051	0.016	0.027

Table III: Correlation between frequencies (Y_s) of fungi isolated from 12 cotton genotypes and amino acid composition of seeds from these genotypes

Amino acid	Y_1 [a]	Y_2	Y_3	Y_4	Y_5	Y_6	Y_7	Y_8	Y_9	Y_{10}
Aspartic	0.186[b]	0.333	-0.261	-0.165	-0.257	-0.010	-0.007	0.068	-0.497[x]	-0.082
Glutamic	-0.421	-0.127	0.392	0.390	-0.027	0.301	-0.282	0.125	-0.136	-0.025
Serine	-0.009	0.785[**]	0.354	-0.193	-0.256	0.573[x]	-0.326	0.494	-0.438	-0.019
Glycine	-0.122	-0.037	0.282	0.143	0.264	0.177	-0.242	0.409	-0.327	-0.415
Histidine	0.472	-0.074	-0.118	0.224	0.184	0.116	0.504[x]	0.480	-0.089	-0.186
Arginine	-0.103	0.136	0.280	-0.290	-0.254	-0.243	0.056	-0.216	-0.012	0.554
Therionine	-0.046	0.229	0.167	-0.348	-0.393	0.021	-0.302	0.214	-0.289	0.135
Alanine	-0.364	0.354	0.493	0.230	-0.379	0.557[x]	-0.329	0.278	0.255	-0.405
Proline	0.220	-0.511[x]	-0.411	-0.183	-0.280	-0.476	0.072	-0.156	0.377	0.192
Tyrosine	0.355	0.038	-0.144	-0.391	0.565[x]	-0.219	0.464	0.046	0.181	-0.043
Valine	-0.226	-0.049	-0.367	-0.088	0.232	-0.062	-0.365	-0.289	-0.098	-0.366
Methionine	0.015	-0.226	-0.440	-0.381	0.710[**]	-0.389	0.042	-0.364	0.176	-0.054
Cysteine	0.141	-0.109	-0.447	-0.135	-0.300	-0.282	0.670	-0.292	0.385	-0.125
Isoleucine	0.283	-0.006	-0.567[x]	-0.369	-0.037	-0.289	-0.031	-0.027	0.028	-0.246
Leucine	-0.378	0.093	-0.046	0.040	-0.493	0.155	-0.476	-0.192	0.178	0.194
Phenylalanine	-0.405	-0.139	0.002	0.185	-0.203	0.061	-0.740[**]	-0.072	-0.259	-0.602*
Lysine	0.193	-0.077	0.313	0.370	-0.166	0.162	0.225	0.396	-0.045	-0.504[x]

[a] Identification of the isolated fungi is shown in Table I

[b] Pearson's correlation coefficient (r), which measures the degree of association between frequency of the isolated fungus and the content of the designated amino acid. Value of r is significant at $P < 0.01$ (**), $P < 0.05$ (*), or $P < 0.10$ (x)

Table IV: Stepwise regression models that describe the effect of amino acid content (X_s[a]) of seeds from 12 cotton genotypes on frequencies (Y_s[b]) of fungi isolated from these genotypes

Fungus	Stepwise linear regression model	Coefficient of determination (R^2) %	F. value[C]
Alternaria alternata	[d]……	…..	…..
Aspergillus flavus	$Y = -54.05 + 1806.75X_3 + 726.58X_{10} - 613.10X_{12}$	86.35	16.87^{***}
A. niger	$Y = -48.353 - 567.37X_{14} + 366.92X_8 + 962.68X_7 + 850.30X_{12} - 176.78X_2$	90.84	11.90^{**}
Chaetomium sp.	[d]…..	…..	…..
Cladosporium sp.	$Y = 12.09 + 354.67X_{12} 275.27X_{14} + 75.54X_5 + 20.07X_1 + 46.61X_4 - 23.75X_9$	99.41	140.53^{***}
Fusarium oxysporum	$Y = -1.16 + 30.66X_3 + 4.41X_8 - 26.07X_{14}$	65.72	5.11
Penicillium sp.	$Y = 88.03 - 945.83X_{16} + 580.88X_{17} - 81.30X_8 + 592.23X_{14}$	90.00	15.74^{***}
Rhizopus stolonifer	$Y = -13.46 + 950.64X_3 + 451.29X_5$	57.74	6.15^{*}
Stemphylium sp.	$Y = -151.36 - 193.40X_1 + 2789.35X_{15} - 783.70X_3 + 861.27X_{10} + 742.05X_{17} - 446.30X_{13} - 292.61X_4$	98.76	45.59^{***}
Aspergillus sp.	$Y = 44.72 - 317.29X_{16} - 82.99X_5 - 118.64X_{10} - 22.30X_8$	82.62	8.35^{**}

[a] Identification of the predictors (X_s) and their relative contributions are shown in Table V

[b] Dependent variables

[c] Value is significant at $P < 0.005$ (***), $P < 0.05$ (*), $P < 0.01$ (**), or $P < 0.05$ (*)

[d] Regression model could be constructed

DISCUSSION

The predominance of *A. niger* relative to the other fungi isolated from cottonseeds, except *R. stolonifer*, is consistent with the findings of Simpson *et al.* (1973) who found that *A. niger* was a predominant fungus at several locations in their study, infecting up to 23% of the seeds. *Rhizopus stolonifer* and *Penicillium* sp. are among the fungi involved in cotton boll rot and may cause deterioration in fiber quality under favorable environmental conditions (Abd El-Rehim *et al.*, 1993). *Cladosporium* sp. is involved in sooty mold of cotton (Zayed, 1997). *Alternaria* has been reported as a predominant 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 of Simpson *et al.* (1973). Klich (1986) found *A. alternata* in more than 10% of the seed. In the present study, *A. alternata* was found in 1.1% of the seed. Generally, fusaria were major components of the fungal flora in earlier studies (Roncadori *et al.*, 1971; Simpson *et al.*, 1973). In the present study, *F. oxysporum* was found in 0.3% of the seeds.

In the present study, associations among amino acids and fungi isolated from cottonseeds were identified and the relative strength of these associations was measured by calculating Pearson's correlation coefficient (r). However, one should keep in mind that the significant r value should be interpreted with caution (Gomez & Gomez, 1984), because the existence of a process may not be proved by the existence of a pattern (Nelson & Campbell, 1992) i.e., significant positive or negative r value does not necessarily prove that amino acids are beneficial or detrimental to fungi. Thus, the primary utility of the correlation technique was to identify the potentially interactive pairs of amino acids and fungi. The interpretations of the nature of such interactions required information about the metabolic pathways of fungi and biological activities of amino acids. In spite of these limitations, certain general conclusions could be drawn. The positive r value may indicate that amino acids possessed stimulatory effects on growth and sporulation of cottonseed mycoflora. On the other hand, the negative r value could be attributed to the inhibitory activities of amino acids. These causal relationships between amino acids and seedborne fungi are consistent with biological expectations as previously mentioned in the introduction.

Table V: Identification of the predictors included in stepwise regression models shown in Table 4 and their relative contributions to the total variation in frequencies of the isolated fungi

Fungus and predictor	Number	Relative Contribution (%)
Aspergillus flavus		
Serine	X_3	61.60
Tyrosine	X_{10}	11.96
Methionine	X_{12}	12.80
A. niger		
Isoleucine	X_{14}	32.09
Alanine	X_8	22.86
Therionine	X_7	18.89
Methionine	X_{12}	9.90
Glutamic	X_2	7.11
Cladosporium sp.		
Methionine	X_{12}	50.42
Isoleucine	X_{14}	24.19
Histidine	X_5	20.85
Aspartic	X_1	2.20
Glycine	X_4	1.39
Proline	X_9	0.36
Fusarium oxysporum		
Serine	X_3	32.78
Alanine	X_8	19.15
Isoleucine	X_{14}	13.79
Penicillium sp.		
Phenylalanine	X_{16}	54.80
Lysine	X_{17}	11.76
Alanine	X_8	11.84
Isoluecine	X_{14}	11.59
Rhizopus stolonifer		
Serine	X_3	24.41
Histedine	X_5	33.33
Stemphylium sp.		
Aspartic	X_1	24.71
Leucine	X_{15}	28.16
Serine	X_3	15.06
Tyrosine	X_{10}	9.91
Lysine	X_{17}	12.16
Cysteine	X_{13}	4.21
Isoleucine	X_4	4.56
Aspergillus sp.		
Phenylalanine	X_{16}	36.18
Histidine	X_5	22.53
Tyrosine	X_{10}	15.59
Alanine	X_8	8.31

The R^2 values of the obtained regression models ranged from 57.74 to 99.41%. Obviously, the larger the R^2 value is, the more sensitive the fungus is in its response to biological activities of the amino acids. Thus, it was possible to group the isolated fungi into four distinct categories based on their sensitivity to the amino acids. The first category included the insensitive fungi *A. alternata* and *Chaetomium* sp., where no regression models could be constructed. The second category included the moderately sensitive fungi *F. oxysporum* and *R. stolonifer*, where R^2 values were 65.72 and 57.79%, respectively. The third category included the sensitive fungi *A. flavus*, *A. niger*, *Penicillium* sp. and *Aspergillus* sp., where R^2 values were 86.35, 90.84, 90.00 and 82.62%, respectively. The fourth category included the highly sensitive fungi *Cladosporium* sp. and *Stemphylium* sp., where R^2 values were 99.41 and 983.76%, respectively.

In conclusion, the results of the present study suggest that certain amino acids regulate colonization of cottonseed by mycoflora and that control of these fungi may be possible by modifying amino acid content of the seed.

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