

Biodiversity of Seedborne Mycobiota Associated with *Sesamum indicum* L

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Sesame (*Sesamum indicum* L.) is one of the most important and widely distributed oil seed crop in Egypt and Saudi Arabia. The comprehensive outcome recommended that agar plate method was more conformable than moist blotter method in yielding more seedborne fungal flora of *S. indicum*. Nearly 38.9% of the investigated seed samples were contaminated with ochratoxin A. Seventy-three fungal species belonging to 12 genera were recorded on 18 seed samples of *S. indicum* collected from different locations in Egypt (Bani Swief, Ismailia, Sohag) and Saudi Arabia (Jizan, Makkah). The prevalent genera were *Aspergillus*, *Fusarium* and *Penicillium*. Altogether seventeen fungal isolates belonging to *Aspergillus*, *Penicillium* genera were able to produce ochratoxin A mycotoxin *in vitro*. The chromatographic analysis indicated that nine fatty acids namely caproic; palmitic; margaric; stearic; oleic; linoleic; álinolenic; arachidic and eicosatrienoic were common in mycelia of all ochratoxin A producing isolates. For identifying and establishing taxonomic relationship between fungal flora, fatty acid analysis is an important tool. In conclusion, this study detected many ochratoxin A producing fungi associated with sesame seeds in both Egypt and Saudi Arabia. It is also concluded that cellular fatty acid markers are powerful tool for analyzing the diversity of the fungal isolates belonging to similar or different species.

Key words: *Sesamum indicum*, Biodiversity, OTA, Moisture content, Lipid content, Egypt, SA.

Sesame (*Sesamum indicum* L.) is one of the most important and widely distributed oil seed crop in many arid and semiarid countries including Egypt and Saudi Arabia (SA). It is considered as a drought tolerant crop and is confined to sandy soil. A sesame seed has a high nutritive value (calcium, crude fiber, protein) and rich source of edible oil¹ hence used in baking products and oil extraction². Sesame oil is thermostable and has high degree of resistance to rancidity, hence it is used in paints and soap industry and also has great

medicinal properties. In Egypt, the production areas are confined to sandy soil of Ismailia, Sharkia, Giza Beni-Suef and Sohag governorates, however in SA, the production areas are located in Jizan and holy Makkah governorates. Natural contamination of many seeds with seedborne fungi is one of the most important biotic stress affecting sowing, maintenance and storage of seeds, which ultimately leads to deterioration of seed quality³. The contaminations with mycotoxins have phytotoxic and carcinogenic effects on humans and animals. Kakde *et al.*⁴ reported that seedborne fungi plays significant role in infection, altering quality and longevity of sesame seeds during the storage.

Ochratoxin A (OTA), is a secondary metabolite produced by some seedborne fungi

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belonging to *Aspergillus* and *Penicillium* genera⁵. OTA is one of the most important mycotoxin that has dragged the attention of scientists worldwide due to its hazardous behavior towards plants and humans^{6, 7}. The dissimilarity in mycotoxin production by the same fungal species has also been reported^{3, 8} and hence significant correlation coefficients were observed among frequencies of fungal species⁹. Although the standard morphological methods for fungal identification¹⁰ are used as the basic method and chemotaxonomy is also employed as additional tool. Chemotaxonomical approach includes analysis of mycelial fatty acid composition, has been used routinely to characterize, differentiate, and identify genera, species, and strains of fungi^{3, 11, 12}. Recently, developed programs of multivariate discriminate analysis have helped to increase the value of fungal fatty acid data in routine taxonomy and identification work¹².

In the present study, we report, seedborne fungi of *S. indicum* associated with sesame seed samples collected from different governorates in both Egypt and SA. Also, the biodiversity of OTA producing isolates was carried out according to fatty acids profile of their mycelia.

MATERIALS AND METHODS

Seed sample collection

Samples (18) of *Sesamum indicum* L. seeds were collected from different governorates in Egypt (Bani Swief, 6 samples; Ismailia, 2 samples; Sohag, 4 samples) and SA (Jizan, 4 samples; Makkah, 2 samples). The samples were collected in sterile cellophane bags at 2°C.

Determination of seed moisture content

The moisture content was determined by weighing the sample and then dried the same sample in an oven for 24 h at 105°C, then cooled in desiccators and reweighed. The moisture content is expressed as percentage of the wet weight.

Enumeration of fungal flora

The samples were disinfected with Na-hypochlorite followed with washing with sterile distilled water. The disinfected seeds were placed aseptically on either blotter or agar plate (PDA, Difco Laboratories, Detroit, MI, USA) containing Rose Bengal (33 µg ml⁻¹, w/v) and streptomycin (30 µg ml⁻¹, w/v) and incubated at 28°C+1. Seven

seeds were placed in each petri-dish (9 cm in diameter). The fungal colonies developing around the seeds incubated on both agar plates and blotter papers were examined, and the moulds were identified microscopically according to Domsch *et al.*¹⁰.

Survey for OTA production by seedborne fungal isolates

Seventy-four seedborne fungal isolates were screened for production of OTA on glucose-ammonium nitrate salt broth³. The culture broth medium was incubated for 10 days at 28°C+1 under static conditions in dark. At the end of the incubation period, the cultures were filtered and culture filtrates were used for estimation of OTA.

Extraction, chromatographic separation, and quantitative estimation of OTA

All the solvents used in the present study were fine grade of Sigma Chemical Co. (St. Louis, MO, USA). OTA standard was the product of Merck Chemical Co. (Darmstadt, Germany). Silica gel DG (Kieselgel-DG, Riedel-De Haen, Seelze-Hannover, Germany) was used for thin layer chromatography. OTA was extracted (from culture filtrates of each fungal isolate) by chloroform⁽¹³⁾ and cleaned up according to Scott¹⁴. The chromatographic separation of OTA was carried out using thin layer chromatography (TLC) plates (20 × 20 cm) coated with 0.3 mm-thick silica gel DG (Kieselgel-DG, Riedel-De Haen, Seelze-Hannover, Germany) and developed with toluene:ethyl acetate:formic acid (5:4:1, v/v/v). The plates were examined under long ultraviolet light illumination (366 nm), and standard OTA was used as reference. The elution of OTA spot was carried out using benzene:acetonitrile (98:2, v/v). The eluted extract was filtered (Millex-HV 0.45 µm 25 mm; Millipore Corporation, Bedford, USA) and a volume of 5.0 µl was analysed by High Performance Liquid chromatography [HPLC] (Shimadzu Corporation, Japan). A mobile phase consisting of acetonitrile:water:acetic acid (99:99:2, v/v/v) was used for the resolution of OTA on a C₁₈ Hypersil column as described by Aboul-Enein *et al.*¹⁵. The excitation and emission wavelengths were set to 385 and 435 nm, respectively¹⁶. OTA was quantified by comparison with a calibration curve of OTA standards using the Shimadzu CLASS-VP software ver. 6.14 SP2 (Shimadzu Corporation, Japan) according to Valero *et al.*¹⁷.

Fatty acids analysis

Total lipid contents were extracted from fresh mycelial growth by using chloroform:methanol (2:1, v/v) as described by Fölsh *et al.*¹⁸. 0.05% (w/v) of butylated hydroxytoluence (BHT; 2,6 di-tert-butyl-p-cresol) was added to all solvents to prevent lipid peroxidation. Fatty acid methyl esters (ME) were prepared by methanolysis in H₂SO₄-MeOH¹⁹ analyzed by gas liquid chromatography (GLC) (Perkin-Elmer Model 910, Perkin Elmer, Shelton, CT, USA) equipped with a flame ionization detector²⁰. Qualitative and quantitative analysis of peak fatty acid methyl ester was carried via comparing their retention times with those of an authentic methyl standard (Sigma Co., St. Louis, USA).

Statistical analysis

All data were subjected to statistical analysis. Treatments were compared using Fisher's Least Significant Difference (LSD) analysis. Cluster analysis was performed using SPSS 20 software package.

RESULTS AND DISCUSSION

Eighteen seed samples of *S. indicum* were examined for their natural contamination with OTA in relation to seed moisture contents (Table 1). The results indicated that the total contamination percent of the Egyptian seed samples were significantly lesser (16.7%) than those of the Saudi samples (83.3%) (Table 1). This may be due to the reason that Egyptian farmers have many years of experience in agricultural practices and handling the quality and production of sesame crop as compared to farmers in Saudi Arabia. Prigojin *et al.*²¹ also reported that the years of experience are more important than the education of the farmers to produce good quality of agricultural commodities. Only 38.9% of the total examined seed samples were contaminated (between 21.0 to 93.7 (ig/kg) with OTA and is directly proportional to their moisture contents. Our results corroborates with the findings of Alqarawi *et al.*⁽³⁾ who reported that moisture content plays an important role in natural contamination of seeds with mycotoxins. Arnold *et al.*²² and Moslem *et al.*²³ demonstrated significant correlation between the location of seed production as well as collection and seed contamination with mycotoxins.

Table 1. Number, moisture content (%) and natural contamination of seed samples of *S. indicum* (out of 18 samples collected from different agricultural areas in Egypt and Saudi Arabia)

	Location (Governorate) samples	Number of collected samples	Number of contaminated	Ochratoxin A (ig/Kg)	Moisture content (%) of collected samples
Egypt	Bani Swief	6	1	87.9	7.0; 5.2; 5.1; 6.2; 5.8; 6.1 (5.9) ^b
	Ismailia	2	ND	ND	5.1; 6.4 (5.75) ^b
	Sohag	4	1	58.7	7.3; 6.3; 5.2; 5.8 (6.15) ^b
Sub total; Mean Saudi Arabia	12	2	146.6; 73.30 ^c	(5.93) ^b	
	Jizan	4	4	41.4; 32.7; 21.5; 21.0	8.6; 5.6; 7.8; 7.2 (7.3) ^b
	Holy Mecca	2	1	93.7	12.6; 13.4 (13.0) ^b
Sub total; Mean Total	6 18	5 7	210.3; 42.06 ^c 50.98 ^c	(10.35) ^b (7.04) ^b	

a: Total number of samples. b: Mean moisture content (%). c: Mean contamination of OTA (ig/Kg).

ND: Not detected under the experimental conditions.

The comprehensive outcome of our results in Table 2 recommended that agar plate method was more reliable as compared to moist blotter paper method in yielding more fungal flora. Our results are in concurrence with those of Alqarawi et al.³ who reported that agar plate

Table 2. Incidence (%) and occurrence remarks (out of 17 *S. indicum* seed samples) of seedborne fungal isolation of *S. indicum* seeds following incubation on agar plates and blotters

Seedborne fungal flora	Incidence (%) and occurrence remarks (out of 17 seed samples) of seedborne fungal isolation of <i>S. indicum</i> following incubation on agar plates and blotters			
	Agar Plate	Blotter test	Average	^a Occurrence remarks
<i>Alternaria alternata</i>	ND	0.4	0.2	L
<i>A. brassicae</i>	0.41	1.23	0.82	R
<i>A. solani</i>	0.27	0.82	0.545	R
<i>Aspergillus caespitosus</i>	1.0	2.15	1.575	R
<i>A. nidulans</i>	3.57	4.82	5.56	L
<i>A. ochraceus</i>	6.3	3.47	4.205	H
<i>A. flavus</i>	4.32	2.48	3.4	R
<i>A. nidulans</i>	0.86	ND	0.43	R
<i>A. niger</i>	4.94	2.14	2.855	L
<i>A. oryzae</i>	ND	0.4	0.2	R
<i>A. parasiticus</i>	4.35	1.7	3.025	M
<i>A. carbonarius</i>	12.36	7.2	9.78	H
<i>A. terreus</i>	0.8	ND	0.4	M
<i>A. tetrazonus</i>	0.13	ND	0.065	R
<i>Chaetomium elatum</i>	0.37	0.14	0.255	R
<i>C. globosum</i>	0.12	ND	0.06	R
<i>C. spirale</i>	2.4	ND	1.2	R
<i>Cladosporium cladosporioides</i>	0.17	ND	0.085	L
<i>Drechslera ellisii</i>	0.22	0.14	0.18	M
<i>Emericella nidulans</i>	ND	0.13	0.065	R
<i>E. quadrilineata</i>	0.12	ND	0.06	R
<i>Fusarium oxysporum</i>	1.27	2.3	7.5	M
<i>F. chlamydosporum</i>	2.4	ND	1.2	L
<i>F. dimerum</i>	0.17	ND	0.085	R
<i>F. equiseti</i>	ND	0.14	0.07	M
<i>F. semitectum</i>	ND	1.2	0.6	L
<i>F. verticillioides</i>	ND	0.17	0.085	R
<i>Penicillium citrinum</i>	2.1	0.9	1.5	M
<i>P. chrysogenum</i>	0.12	ND	0.06	R
<i>P. nordicum</i>	0.12	0.2	0.16	R
<i>P. oxalicum</i>	0.62	ND	0.31	R
<i>P. purpurogenum</i>	1.07	ND	5.35	R
<i>P. verrucosum</i>	0.12	0.5	0.31	L
<i>P. viridicatum</i>	1.21	1.3	1.255	R
<i>Phoma sp.</i>	ND	0.19	0.095	R
<i>P. herbarum</i>	0.18	ND	0.09	R
<i>Rhizoctonia solani</i>	0.14	0.11	0.125	R
<i>Pythium ultimum</i>	ND	0.13	0.065	R
<i>Sclerotium bataticola</i>	0.17	0.12	0.145	R
Sterile mycelia (hyaline and dark)	0.12	ND	0.06	R

^a Occurrence (out of 57 samples): H = High occurrence, >24 cases. M = Moderate occurrence, 12-24 cases.

L = Low occurrence, 6-11 cases. R = Rare occurrence, <6 cases.

ND: Not detected under the experimental conditions

method were superior to yield higher number of seedborne fungi in *Ephedra alata* and *Ephedra aphylla*. Thirty eight fungal species belonging to twelve genera were associated with seed samples of *S. indicum* in both Egypt and SA (Table 2). The genus *Aspergillus* (11 species, ~ 28.9% of the total fungi) was the most prevalent genus. The most prevalent species were *A. carbonarius*, *A. ochraceus* and *A. niger*. The other species (*A. flavus*, *A. nidulans*, *A. caespitosus*, *A. oryzae*, *A. terreus*, *A. tetrazonus*, *A. parasiticus*) were ranged between low to rare incidence. The genus *Aspergillus* was followed by *Penicillium* (~18.4%); *Fusarium* (~15.8%) and *Chaetomium* (~7.9%) as well as *Alternaria* (~7.9%). Our results corroborate with the findings of other researchers^{24, 25} who reported that *Aspergillus* was followed by *Penicillium* and *Fusarium* and were the most predominant seedborne fungi associated with sesame seeds in SA, Pakistan, Egypt, India and Nigeria. The natural contamination of oil seeds with seedborne fungi plays a vital role in deteriorating the quality of seeds and oil, hence has a critical influence on the production of sesame seeds²⁶.

Seedborne fungal isolates (174 isolates) were collected from seeds of *S. indicum* by both agar plate and blotter paper methods. All the isolates

were screened based on their potentiality to produce OTA *in vitro*. Altogether 17 fungal isolates belonging to *Aspergillus* and *Penicillium* could produce OTA in variable amounts ranged between 7.2 to 147.5 µg/100 ml culture medium (Table 3). Our findings are in harmony with the reports of Fungaro et al.²⁷; Moslem et al.²³ and Vega et al.²⁸ who also reported the production of OTA by *Aspergillus* and *penicillium* in coffee beans respectively. In our results, no obvious relationship between productivity of OTA by the screened isolates and their geographic origin was found (Table 3). The findings corroborated with the results of Arnold et al.²²; Duarte-Escalante et al.²⁹; Mikhail et al.³⁰ and Alqarawi et al.³, who also reported the relation between the fungal diversity and their geographic origin is still unknown.

Numerous identification techniques have been developed to identify fungal species, including morphological examination, nuclear staining, anastomosis testing, pectic zymogram testing, various molecular techniques and chemotaxonomical approach. The fatty acid signatures as chemotaxonomical approach has been used as routine taxonomy and in identification work^{31, 32, 33}. In present study, the mycelial fatty acid profiles of OTA-producing

Table 3. Ochratoxin A production (µg/100 ml culture medium) by different seedborne fungal isolates

Isolate code	Orginal location	Seedborne fungal isolates	Ochratoxin A production (µg/100 ml culture medium)
1	Bani Swief	<i>A. ochraceus</i>	122.4
2	Bani Swief	<i>A. ochraceus</i>	118.6
3	Bani Swief	<i>A. ochraceus</i>	120.7
4	Jizan	<i>P. viridicatum</i>	24.8
5	Bani Swief	<i>P. nordicum</i>	112.4
6	Holy Makkah	<i>P. verrucosum</i>	27.3
7	Ismailia	<i>A. niger</i>	14.5
8	Ismailia	<i>A. niger</i>	13.4
9	Jizan	<i>P. crustosum</i>	12.3
10	Sohag	<i>P. brevicompactum</i>	17.6
11	Sohag	<i>P. brevicompactum</i>	17.5
12	Sohag	<i>A. carbonarius</i>	147.5
13	Holy Makkah	<i>A. niger</i>	7.2
14	Jizan	<i>A. niger</i>	32.6
15	Holy Makkah	<i>A. ochraceus</i>	37.5
16	Jizan	<i>A. ochraceus</i>	36.4
17	Ismailia	<i>A. ochraceus</i>	10.4

Table 4. Cellular fatty acid profile of different ochratoxin A-producing seedborne isolates associated with of *S. indicum*.

IN	Cellular fatty acid profile (%)																	
	C ₆	C ₈	C ₁₀	C ₁₂	C ₁₄	C _{14:1}	C ₁₆	C _{16:1}	C ₁₇	C ₁₈	C _{18:1}	C _{18:2}	C _{18:3}	C ₂₀	C _{20:1}	C _{20:3}	C _{20:4}	TU
1	1.04	0.00	2.01	0.47	0.33	0.37	7.25	0.92	8.99	16.37	4.19	30.19	4.94	14.12	0.00	1.77	7.04	49.42
2	4.64	0.00	1.22	1.14	2.01	0.34	7.69	0.96	9.01	16.47	4.78	28.47	5.01	15.55	0.00	1.58	1.13	42.27
3	4.67	0.00	0.77	1.21	2.34	0.37	8.01	1.04	9.24	15.92	4.51	24.65	4.56	14.35	0.00	1.24	7.12	43.49
4	3.28	2.27	1.43	0.47	0.00	0.00	3.12	2.07	7.01	12.43	5.67	27.61	1.12	13.98	0.00	7.16	12.38	56.01
5	1.03	2.07	0.00	0.94	0.00	0.00	3.58	2.14	6.87	12.67	5.12	32.20	1.36	12.64	0.12	7.12	12.14	60.20
6	3.24	3.54	0.00	0.95	0.41	0.00	1.45	0.00	8.39	8.12	5.64	36.37	4.25	17.26	0.52	2.41	7.45	56.64
7	4.52	1.29	4.09	2.41	0.88	0.00	1.25	0.00	8.47	13.92	6.12	33.17	4.78	14.09	0.00	5.01	0.00	49.08
8	0.87	0.24	4.07	2.42	0.81	0.00	1.36	0.00	7.93	18.06	6.37	32.59	4.12	15.84	0.00	5.32	0.00	48.40
9	1.48	2.46	4.09	2.47	0.87	0.00	4.01	0.12	5.36	18.37	1.64	37.47	4.67	15.14	0.00	5.41	6.44	55.75
10	1.24	3.58	2.14	1.58	0.00	0.00	4.25	0.17	5.78	18.01	1.59	28.89	0.00	20.84	1.78	2.13	8.02	42.58
11	1.79	1.17	2.57	1.59	0.00	0.00	7.97	0.16	5.66	18.21	1.62	25.42	0.00	17.86	1.48	2.07	12.43	43.18
12	2.21	1.69	2.36	1.51	0.00	0.85	1.07	0.00	5.78	11.03	14.24	36.07	2.97	16.78	1.36	2.08	0.00	57.57
13	2.67	0.88	0.78	0.63	0.31	0.87	1.36	0.00	5.04	10.04	13.98	40.17	2.89	17.01	0.00	3.37	0.00	61.28
14	1.58	1.09	0.81	0.64	0.37	0.86	1.48	0.42	12.04	3.45	14.08	40.04	2.36	17.64	0.00	3.14	0.00	60.90
15	2.88	1.08	0.92	0.68	0.34	0.84	2.11	0.38	13.40	4.01	14.33	27.04	0.00	21.34	0.00	3.47	7.18	53.24
16	2.58	1.08	2.17	0.00	0.57	0.87	3.31	0.31	12.96	3.98	4.97	28.31	0.00	22.07	0.00	3.08	13.74	51.28
17	2.78	1.07	2.12	0.00	0.52	0.81	3.07	0.42	12.48	3.78	4.88	27.68	4.01	21.89	0.00	1.33	13.16	52.29

IN: Isolate number;

C₆: Caproic; C₈: Caprylic; C₁₀: Capric; C₁₂: Lauric; C₁₄: Myristic; C_{14:1}: Myristoleic; C₁₆: Palmitic; C_{16:1}: Palmitoleic; C₁₇: Margaric; C₁₈: Stearic; C_{18:1}: Oleic; C_{18:2}: Linoleic; C_{18:3}: α Linolenic; C₂₀: Arachidic; C_{20:1}: Cis-11 Eicosenoic; C_{20:3}: Eicosatrienoic; C_{20:4}: Arachidonic.

TU: Total Unsaturated fatty acids.

Table 5. Similarity matrix of of different OTA producing seventeen isolates belong to *Aspergillus* and *Penicillium* genera

Fungal isolates	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	1.00																
2	1.00	1.00															
3	1.00	1.00	1.00														
4	0.81	0.81	0.81	1.00													
5	0.71	0.71	0.71	0.87	1.00												
6	0.71	0.71	0.71	0.75	0.87	1.00											
7	0.75	0.75	0.75	0.80	0.69	0.80	1.00										
8	0.75	0.75	0.75	0.80	0.69	0.80	1.00	1.00									
9	0.88	0.88	0.88	0.93	0.81	0.81	0.87	0.87	1.00								
10	0.71	0.71	0.71	0.87	0.87	0.75	0.69	0.69	0.81	1.00							
11	0.71	0.71	0.71	0.87	0.87	0.75	0.69	0.69	0.81	1.00	1.00						
12	0.71	0.71	0.71	0.75	0.75	0.75	0.80	0.80	0.71	0.75	0.75	1.00					
13	0.81	0.81	0.81	0.75	0.65	0.75	0.93	0.93	0.81	0.65	0.65	0.87	1.00				
14	0.88	0.88	0.88	0.81	0.71	0.71	0.87	0.87	0.88	0.71	0.71	0.81	0.93	1.00			
15	0.88	0.88	0.88	0.81	0.71	0.71	0.75	0.75	0.88	0.81	0.81	0.71	0.81	0.88	1.00		
16	0.81	0.81	0.81	0.75	0.65	0.65	0.69	0.69	0.81	0.75	0.75	0.65	0.75	0.81	0.93	1.00	
17	0.88	0.88	0.88	0.81	0.71	0.71	0.75	0.75	0.88	0.71	0.71	0.71	0.81	0.88	0.88	0.93	1.00

isolates were shown in table 4. Nine fatty acids namely caproic, C_6 ; palmitic, C_{16} ; margaric, C_{17} ; stearic, C_{18} ; oleic, $C_{18:1}$; linoleic, $C_{18:2}$; α linolenic, $C_{18:3}$; arachidic, C_{20} and eicosatrienoic, $C_{20:3}$ were common among mycelia of all OTA producing *Aspergillus* and *Penicillium* isolates (Table 4). Such fatty acids have been reported previously as common fatty acids in many fungi belong to *Aspergillus*¹² and *Penicillium*³¹. They are also common precursors as well as intermediates in biosynthesis and metabolism of other fatty acids³⁴. The present dendrogram showed that the 17 fungal isolates could be divided into two major groups and seven subgroups on the basis of their cellular fatty acids (Fig. 1). Cluster analysis of fatty acid pattern of OTA producing isolates clearly separated all the isolates into two distinct main groups at ~0.75 similarities (Fig. 1). The main cluster has divided the isolates between *Aspergillus* and *Penicillium* genus which included 64.7% and 35.3% of these isolates respectively. The first cluster (11 isolates) was divided into two

subclusters between *A. ochraceus* (included 6 isolates) and the black *Aspergillus* species (included 5 isolates). The Egyptian isolates of *A. ochraceus* were collected from Bani Swief (isolates no., 1; 2 & 3) and Ismailia (isolate no. 17) however the Saudi isolates collected from Jizan (isolate no., 16) and Holy Mecca (isolate no., 15). The second subcluster of black *Aspergillus* species was divided between *A. niger* (4 isolates) and *A. carbonarius* (one isolate). *A. niger* isolates were collected from Ismailia governorate (isolates no., 7 & 8); Holy Mecca (isolate, 13) and Jizan (isolate, 14). The second main subcluster of *Penicillium* isolates (six isolates) was divided into two subgroups with great diversity at ~0.84 (Fig. 1). The first subgroup (4 isolates) was divided between *P. brevicompactum* (isolates no., 10 & 11) from Sohag, Egypt and both *P. viridicatum* (one isolate no. 4) and *P. crustosum* (one isolate no. 9), which were from Jizan, SA. The second subgroup included two isolates belongs to *P. verrucosum* (isolate no., 6) and *P. nordicum* (isolate no., 5) which were from

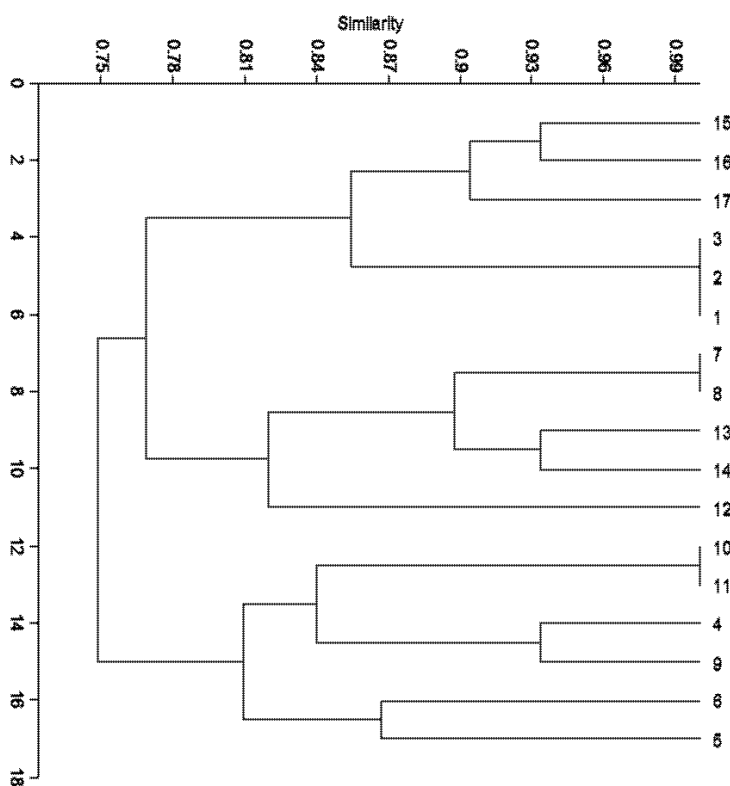


Fig. 1. Chemotypes tree (Dendrogram) based on fatty acids pattern of OTA producing seventeen isolates belong to *Aspergillus* and *Penicillium* genera

holy Mecca (SA) and Bani Swief (Egypt), respectively. Similarity matrix (Table 5) constructed on the basis of Nei and Li's index was generated on fatty acid pattern of different OTA producing isolates. It reveals similarities of the chemotypes varied from 1.0 % to 0.64 % illustrating four different clustered groups: the *A. ochraceus* cluster (one); the black *Aspergillus* species cluster (two) and the two uniseriate clusters of *Penicillium* species. The cellular fatty acid analysis is a useful tool for identifying and establishing taxonomic relationships between fungal flora^{12, 33} and great diversity were reported by many authors among OTA producing *Aspergillus* species^{23, 35} and *Penicillium* species^{36, 37}. No obvious relationship was observed between fatty acids pattern and geographic origin of the isolates in our results. In this context, it was reported that the relation between the fungal diversity and their geographic origin remain largely unknown^{3, 22, 29, 30}.

CONCLUSIONS

In conclusion, we have detected many OTA producing fungi associated with sesame seeds in both Egypt and SA. It is also concluded that cellular fatty acid markers are powerful tool for analyzing the diversity of the fungal isolates belonging to similar or different species.

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