Mycobiology 31(3): 166-172 (2003)

Studies on Mycological Status of Salted Fish "Moloha" in Upper Egypt

M. S. Youssef*, N. F. Abo-Dahab and R. M. Farghaly

Botany Department, Faculty of Science, Sohag, South Valley University, Egypt
Botany Department, Faculty of Science, Assiut, Al-Azhar University, Egypt
Dept. Food Hygiene, Fac. Veterinary Medicine, South Valley Univ., Qena, Egypt

(Received June 9, 2003)

Chemical analysis of salted fish was analyzed in 60 samples collected from various moloha markets in Sohag, Qena and Aswan Governorates, Upper Egypt. Moloha contained 52.9% water content, while organic matter content represented 71.79% of dry weight and 33.81% (338.12±8.64 mg g^{-1}) of fresh weight. Total salts and soluble salts represented 13.29% and 10.19% (132.88±7.65 and 101.93±5.76 mg g^{-1} of fresh weight), respectively. pH values were more or less neutral. Mycological investigation of examined samples revealed that fifty-five fungal species and one variety belonging to 11 genera were identified. The fungal genera of highest occurrence and their respective number of species were *Aspergillus* (*A. flavus*, *A. niger*, *A. fumigatus*, *A. montevidensis*, *A. ficuum*, *A. parasiticus* and *A. mangini*) and *Penicillium* (*P. citrinum*, *P. puberulum*, *P. aurantiogriseum* and *P. roquefortii*). On the other hand, yeast represented 18.2% and 3.0% of total counts of fungi on Czapeks-dextrose agar and 15%NaCl-Czapeks-dextrose agar media, respectively. Samples were assayed for potential presence of mycotoxins. Ten out of 60 samples (16.7%) were proved to be toxic. It is the first record of mycotoxins contamination of salted fish in Egypt. The ability of 340 isolates of recovered fungi was screened for production of mycotoxins and extracellular enzymes.

KEYWORDS: Extracellular enzymes, Fungi, Moloha, Mycotoxins, Salted fish

Fish and their products are an important source of animal protein with high biological value. They comprise many essential amino acids, vitamins, polysaturated fatty acids, essential minerals as well as appreciable amounts of trace elements (Krause, 1966). Moloha is fermented salted fish that constitute a part of popular diet in Egypt. It is manufactured by mixing fish with sodium chloride (4:1 to 9:1 ratio) and allowing the mixture to be fermented naturally for 150~250 days.

Moloha is a type of salted fish favorable for many people in Upper Egypt especially poor due to cheap cost. Two known small size species of fresh water fish are used in manufacture of Saedy moloha, and these are *Leptocypris niloticus* (Joannis) commonly named Bebee-Morgan Nili and *Alestes baremoze* (Joannis) known as pebbly fish. After fish had catched, the harvest dried in open air for at least 15 days, which became liable for air, soil and unhygienic handling contamination. Numerous pathogenic bacteria and fungi isolated from different types of fish were able to grow and produce their toxic secondary metabolites, which are retained in fish flesh even after salting and storage periods. These toxic substances caused serious public health hazards (Bullerman, 1979; El-Sayed, 1995; Swaminathan and Sparling, 1998). In Egypt, considerable quantities of moloha are consumed particularly in spring and breakfast feast. Several cases of human gastroenteritis, severe diarrhea and food poisoning outbreaks were recorded after moloha consumption.

In Egypt, there are many researches focused on bacterial and fungal pathogens of fresh water fish (Salem *et al.*, 1989; Badran *et al.*, 1994; Abo-El-Alla and Bastawrows, 1999). In contrast, there is very little work have been done on microflora of fresh water salted fish (Abdel-Rahman *et al.*, 1988). However to the best of my knowledge there is no previous research have been carried out on mycotoxins that contaminated Saedy moloha and their public health hazards. So, the present investigation was designed to study fungal flora contamination of moloha with special reference to their toxins production. Also, chemical analysis of samples and screening the ability of recovered fungal isolates to produce extracellular enzymes were carried out.

Materials and Methods

Collection of samples. A total of sixty random samples of ready to eat Saedy moloha were collected from different moloha markets and shops in Sohag, Qena and Aswan Governorates, Upper Egypt (20 samples, each). Each sample (100~150 g) was placed in a sterile bag and transferred to the laboratory and kept at 4±1°C till chemical, mycological, mycotoxins and enzymatical investigations.

Chemical analysis of samples. Moloha samples were analyzed chemically for estimation of dry weight (85°C for 24 h), total soluble salts (Allen, 1989; using electric

*Corresponding author <E-mail: fpyoussef@yahoo.com>

conductivity WPA 3BD), pH value (pH meter, Beckman), organic matter (Vogel, 1974) and elements including sodium, potassium, calcium, magnesium, manganese, copper, cadmium, nickel and zinc (atomic absorption spectrophotometer, Perkin Elmer 2380). The operational conditions for atomic absorption were as follows; acetylene, 2.4 ml^{-1}, air, 6.3 $ml\ min^{-1}$, lamp current, 10 mA; wave length, 196.0 nm; slit width, 1.0 nm (Gajan and Larry, 1972).

Determination of mycoflora. The dilution-plate method as described by Johnson and Curl (1972) was used for isolation of fungi. Modified Czapek's-dextrose agar alone and with 15% NaCl were used for isolation of glucophilic and halophilic or/and halotolerant fungi, respectively. Chloramphenicol (0.5 mg ml^{-1}) and rose bengal (30 ppm) were added to the media as bacteriostatic agents (Al-Doory, 1980). Twelve plates, six for each medium, were used for each sample. The plates were incubated at 28±2°C for 7~15 days. The developing fungi were identified, counted and calculated per g of fresh weight fish. The following references were used for identification of fungi: Ellis (1971, 1976), Gilman (1975), Raper and Fennel (1977), Pitt (1979, 1991), Domsch *et al.* (1980), Ramirez (1982), Sivanesan (1984), Kozakiewicz (1989), Moubasher (1993) and Samson *et al.* (1995).

Sample preparation for mycotoxin analysis. Fifty g of each sample were defatted with cyclohexane (150 m*l*) for 10 h using Soxhlet type extractor. The defatted residue was extracted with ethyl acetate (three times, 50 m*l* each). The extracts were combined, dried over anhydrous sodium sulphate, filtered and then concentrated under vacuum to near dryness, transferred into brown glass vial and evaporated under nitrogen stream. For cleaning up the crude extracts: 25 mg of crude extracts was suspended in 1 m*l* chloroform and applied to 14×0.8 cm column containing 2.5 g kiesel gel 60, 70/230 silica gel. The washing and eluting solvents (8 m*l*, each) were carried out according to AOAC (1984).

Mycotoxins production by fungal isolates. A number of 340 fungal isolates (represented 26 species + 1 variety appertaining to 6 genera) were firstly grown on potato-dextrose agar slants at 28°C for 7~10 days. For each isolate an inoculum (10^6~10^7 spores/m*l*) was cultivated on Czapeks medium fortified by 2 g yeast extract and 10 g peptone per liter and incubated at 28°C for 12 days. After the incubation period had finished, the culture in each flask (medium + mycelium) homogenized for 5 min in a high-speed blender (1,600 rpm) with chloroform (three times, 75 m*l*, each). The combined extracts were dried over anhydrous sodium sulphate, filtered and then concentrated under vacuum and transferred into a dram vial with a small amount of chloroform.

Bioassay of toxin. Brine shrimps (*Artemia salina* L.) larvae were used for mycotoxin bioassay test according to Korpinen (1974).

Thin layer chromatography (TLC). For qualitative detection of mycotoxins, thin layer chromatography technique was employed using precoated silica gel 60 plates (E. Merck, Germany). Aflatoxins B_1, B_2, G_1 & G_2, ochratoxins A & B, sterigmatocystin, citrinin, $T\text{-}_2$ toxin, diacetoxyscirpenol, patulin, fumigillin, terrin, gliotoxin, rubratoxin B, roquefortin and zeralenone were applied as standard references. The developing solvent system was ethyl acetate-hexane (v/v, 30:70) and the developed plates were viewed under UV irradiation (AOAC, 1980 and Dorner, 1998).

High performance liquid chromatography (HPLC). HPLC analysis was done using spherisorb 5 sil column (250×4.6 mm). Mobile phase was chloroform-methanol (v/v, 97:3) with flow rate 1.2 m*l* min^{-1} for 20 min. The quantitative determination of mycotoxins was carried out compared with standard mycotoxins (Sigma).

Screening for extracellular enzymes production by isolated fungi. A solid casein hydrolysis medium was used for testing of fungal proteolytic activity (Paterson and Bridge, 1994). The appearance of a clear zone around fungal colony indicates casein hydrolysis.

The lipolytic activity of moloha fungi was detected in cultures grown on Ultman and Blasins (1974) agar medium which contained Tween 80. Production of lipolytic enzymes by a colony was seen either as visible precipitate due to formation of crystals of calcium salt of oleic

Table 1. Chemical analysis of salted fish "Saedy moloha" samples*

Parameters[a]	Average±SD[b]
MC%	52.9±1.27
OM mg g^{-1}	338.12±8.64
pH	7.003±0.57
TS mg g^{-1} fresh weight	132.88±7.65
T SS mg g^{-1} fresh weight	101.93±5.76
Soluble elements (mg g^{-1})	
Na	32.38±8.07
K	1.47±0.31
Ca	0.04±0.01
Mg	0.26±0.18
Mn	0.004±0.0005
Cu	0.018±0.004
Cd	0.003±0.001
Ni	0.004±0.002
Zn	0.003±0.001

[a]MC%: Moisture content percentage, OM: Organic matter, TS: Total salts, TSS: Total soluble salts, *average of 60 samples.
[b]Standard deviation.

acid or as opaque zone surrounding the colony.

Results and Discussion

Moloha samples contained 33.81% (338.12±8.64 mg g^{-1}) of fresh weight and 71.79% of dry weight organic matter content. The pH value was 7.003±0.57, most of the samples were slightly alkaline (38 samples). Total salts and soluble salts were relatively high (132.88±7.65 and 101.93 ±5.76 mg g^{-1} fresh weight), respectively. Sodium content (32.38±8.07 mg g^{-1}) was generally proportional related to the total soluble salts. Potassium, calcium and magnesium contents were detected at 1.47±0.31, 0.04±0.01 and 0.26±0.18 mg g^{-1}, respectively. Five micro-elements including manganese (0.004±0.0005 mg g^{-1}), copper (0.018±0.004 mg g^{-1}), cadmium (0.003±0.001 mg g^{-1}), nickel (0.004±

Table 2. Total counts (TC, calculated per g fresh sample), number of cases of isolation (NCI, out of 60 samples) and occurrence remarks (OR) of fungal genera and species isolated from salted fish (moloha) on Czapek's-dextrose and 15% NaCl-Czapek's-dextrose agar media at 28°C using dilution-plate method

Genera and species	Glycophilic fungi				Halophilic & Halotolerant fungi			
	TC	TC%	NCI	OR	TC	TC%	NCI	OR
***Aspergillus* (total count)**	65740	72.9	60	H	18240	63.1	60	H
A. flavus Link	20500	22.7	54	H	7472	27.8	52	H
A. niger Van Tieghem	9600	10.6	44	H	3416	12.7	37	H
A. fumigatus Fresenius	1336	1.5	33	H	–	–	–	–
A. flavus var. *columnaris* Raper & Fennel	12880	14.3	30	M	–	–	–	–
A. ficuum (Reich.) Hennings	4088	4.5	30	M	3192	11.9	24	M
A. parasiticus Speare	8872	9.8	29	M	2040	7.6	20	M
A. carbonarius (Bainier) Thom	3120	3.5	14	L	520	1.9	11	L
A. awamori Nakazawa	1392	1.5	14	L	200	0.7	10	L
A. duricaulis Raper & Fennel	920	1.0	11	L	–	–	–	–
A. terreus Thom	480	0.5	10	L	–	–	–	–
A. candidus Link	352	0.4	9	L	–	–	–	–
A. heteromorphus Batista & Maia	296	0.3	7	R	176	0.7	5	R
A. brunneo-uniseriatus Singh & Bakshi	480	0.5	6	R	–	–	–	–
A. pulverulentus (McAlpine) Thom	280	0.3	6	R	–	–	–	–
A. phoenicis (Cda.) Thom	272	0.3	5	R	304	1.1	9	L
A. sydowi (Bain. & Sart.) Thom & Church	200	0.2	4	R	192	0.7	7	R
A. aculeatus Iizuka	160	0.2	4	R	–	–	–	–
A. brevipes Smith	160	0.2	3	R	–	–	–	–
A. niveus Blochwitz	160	0.2	2	R	20	0.07	1	R
A. aureolatus Munt.-Cvet. & Bata	80	0.09	2	R	–	–	–	–
A. alutaceus Berk & Curt	56	0.06	1	R	348	1.3	18	M
A. sulphureus (Fres.) Thom & Church	40	0.04	1	R	–	–	–	–
A. zonatus Kwon & Fennell	16	0.02	1	R	–	–	–	–
A. oryzae (Ahlb.) Cohn	–	–	–	–	336	1.2	9	L
A. versicolor (Vuillemin) Tiraboschi	–	–	–	–	24	0.09	1	R
***Penicillium* (total count)**	3464	3.8	40	H	3160	11.7	26	M
P. citrinum Thom	1016	1.1	28	M	876	3.3	22	M
P. puberulum Bainier	920	1.02	10	L	–	–	–	–
P. brevicompactum Dierckx	488	0.5	10	L	56	0.2	3	R
P. verruculosum Peyronel	392	0.4	9	L	24	0.09	1	R
P. purpurogenum Stoll	320	0.4	5	R	–	–	–	–
P. chrysogenum Thom	96	0.1	2	R	–	–	–	–
P. duclauxii Delacroix	80	0.09	2	R	–	–	–	–
P. expansum Link ex Gray	72	0.08	2	R	280	1.0	8	L
P. rugulosum Thom	48	0.05	1	R	–	–	–	–
P. aurantiogriseum Dierckx	32	0.04	1	R	628	2.3	10	L
P. roquefortii Thom	–	–	–	–	1256	4.7	11	L
P. waksmanii Zaleski	–	–	–	–	40	0.15	1	R
***Mucor* (total count)**	1488	1.6	23	M	–	–	–	–
M. racemosus Fresenius	832	0.9	22	M	–	–	–	–
M. circinelloides Van Tieghem	640	0.7	6	R	–	–	–	–
M. hiemalis Wehmer	16	0.02	1	R	–	–	–	–

Table 2. Continued

Genera and species	Glycophilic fungi				Halophilic & Halotolerant fungi			
	TC	TC%	NCI	OR	TC	TC%	NCI	OR
***Scopulariopsis* (total count)**	1008	1.1	12	L	109	0.4	6	R
S. brevicaulis (Sacc.) Bainier	408	0.5	10	L	48	0.18	4	R
S. constantini Bainier	600	0.7	6	R	61	0.23	6	R
***Eurotium* (total count)**	1188	1.3	8	L	4512	18.8	41	H
E. montevidensis Talice & Mackinnon	–	–	–	–	1292	4.8	32	H
E. chevalieri Mangin	48	0.05	1	R	1200	4.5	20	M
E. herbariorum Mangin	656	0.7	8	L	660	2.5	18	M
E. repens De Bary	484	0.5	4	R	630	2.3	6	R
E. amstelodami Mangin	–	–	–	–	600	2.2	5	R
E. halophilicus Christ., Papav. & Benjam.	–	–	–	–	60	0.2	2	R
E. rubrum Konig, Spieckerman & Bremer	–	–	–	–	38	0.14	2	R
E. verruculosum Vuillemin	–	–	–	–	36	0.13	1	R
***Rhizopus stolonifer* (Ehrenberg) Lind**	496	0.5	8	L	–	–	–	–
***Spicaria violacea* Abbott**	120	0.1	4	R	–	–	–	–
***Cladosporium cladosporioides* (Fr.) de Vries**	120	0.1	2	R	–	–	–	–
***Alternaria alternata* (Fr.) Keissler**	80	0.09	2	R	–	–	–	–
***Gymnoascus reesii* Baranetzky**	80	0.09	2	R	40	0.15	1	R
***Apiocera chrysosperma* (Bulliard) Fries**	–	–	–	–	48	0.18	2	R
***Trichoderma viride* Pers. Ex S. F. Gray**	32	0.4	1	R	–	–	–	–
Yeast	16400	18.2	20	M	800	3.0	12	L
Total count	**90216**				**26909**			
Number of genera (12)	**11**				**6**			
Number of species (56 + 1var.)	**46 + 1 var.**				**32**			

OR: Occurrence remarks. H: High occurrence; more than 30 cases out of 60 tested. M: Moderate occurrence; between 16~30 cases. L: Low occurrence; between 8~15 cases. R: Rare occurrence; less than 8 cases.

0.002) and zinc (0.004±0.002 mg g^{-1}) were detected in low concentrations in different samples tested (Table 1). These results are in agreement with those obtained by El-Sawi *et al.* (1994).

Mycological analysis of tested samples based on dilution-plating method using Czapek's-dextrose and 15%NaCl-Czapek's-dextrose agar media at 28°C revealed that 55 fungal species and one variety belonging to 11 genera were identified (Table 2). The gross total viable counts as well as number of fungal genera and species of glucophilic fungi (90216 colonies/g fresh sample, 45 species + 1 var. and 10 genera) were more than halotolerant and/or halophilic fungi (26909, 32 species and 5 genera). This indicated that numerous fungi could not tolerate NaCl concentrations. The genera of highest occurrence on the two media were *Aspergillus* (25 species + 1 variety and 21 species) and *Penicillium* (10 and 7 species), respectively. *A. flavus*, *A. niger*, *A. fumigatus*, *A. montevidensis* (Telomorph: *E. montevidensis*), *A. ficuum*, *A. parasiticus* and *A. mangini* (Telo. *E. herbariorum*); *P. citrinum*, *P. puberulum*, *P. aurantiogriseum* and *P. roquefortii* were the most common species. The remaining fungal genera and species were less frequent on the two isolation media. Most of the recorded fungal species in this study had identified before from salted, smoked and fresh water fish (Abdel-Rahman *et al.*, 1988; Nagla Abdel-Monem, 1994; El-Sayed, 1995; Edris, 1996). It is worth-mentioning that members of *Aspergillus glaucus* group (*Eurotium* spp.) are halophilic or halotolerant and osmophilic or osmotolerant in nature (El-Kady *et al.*, 1986; Youssef *et al.*, 2000).

On the other hand, yeast represented 18.2% and 3.0% of total count of fungi on the two media, respectively. Shaheen (1986) isolated some aspergilli, penicilli and yeasts from skin, gills, liver and kidneys of apparently health fresh water fish. Also, Solntseva *et al.* (1987) isolated 13 genera of yeasts from seabream and roach.

The toxicity test using brine shrimp larvae (*Artemia salina* L.) revealed that the ethyl acetate extracts of ten (16.7%) out of 60 moloha samples proved to be toxic. Based on TLC and HPLC analyses, aflatoxin B_1 was detected in four samples with concentrations ranging from 540 to 980 μg/kg of fish. While aflatoxins B_1 and B_2 were detected in two samples (340~780 μg/kg) whereas, aflatoxins B_1, B_2, G_1 and G_2 were recorded in one sample (480~750 μg/kg). These toxic samples were heavily contaminated with many members of *Aspergillus flavus* group

Table 3. Sample number (SN), sample source, biological assay (Brine shrimp), natural occurring of mycotoxins identified and common mycotoxin-producing fungi associated with the toxic salted fish samples

SN	Sample source	Dead larvae (%)	Mycotoxins identified (μg/Kg)	Mycotoxin producing-fungi
2	Sohag	100	Aflatoxin B_1 (960 μg/kg)	*A. flavus* and *A. parasiticus*
5		85	Sterigmatocystin (620 μg/kg)	*A. versicolor*, *E. montevidensis*, *E. repens*, *E. herbariorum* and *E. amstelodami*
8		95	Aflatoxins B_1 & B_2 (780 & 640 μg/kg)	*A. flavus*, *A. parasiticus* and *A. flavus* var. *columnaris*
22	Qena	88	Aflatoxin B_1 (540 μg/kg), Sterigmatocystin (420 μg/kg)	*A. flavus*, *A. parasiticus* and *A. flavus* var. *columnaris*, *E. chevalieri*, *E. montevidensis*, *E. herbariorum*, *E. amstelodami* and *E. repens*
24		78	Citrinin (525 μg/kg)	*A. candidus*, *P. citrinum* and *P. expansum*
29		100	Aflatoxin B_1 (980 μg/kg)	*A. flavus*, *A. parasiticus* and *A. flavus* var. *columnaris*
36		85	Sterigmatocystin (650 μg/kg)	*E. chevalieri*, *E. montevidensis*, *E. herbariorum*, *E. repens* and *E. amstelodami*
46	Aswan	100	Aflatoxins B_1, B_2, G_1, G_2 (480~750 μg/kg)	*A. flavus*
52		93	Aflatoxins B_1 & B_2 (660 & 340 μg/kg)	*A. flavus*, *A. parasiticus* and *A. flavus* var. *columnaris*
57		90	Aflatoxin B_1 (540 μg/kg) Sterigmatocystin (220 μg/kg)	*A. flavus*, *A. parasiticus*, *A. flavus* var. *columnaris* and *A. oryzae*, *E. chevalieri*, *E. montevidensis*, *E. herbariorum* and *E. repens*

(*A. flavus*, *A. flavus* var. *columnaris*, *A. parasiticus* and *A. oryzae*). On the other hand, four samples were contaminated with sterigmatocystin (220~650 μg/kg) and these samples were heavily contaminated with *Aspergillus glaucus* group (*Eurotium chevalieri*, *E. montevidensis*, *E. mangini*, *E. repens* and *E. amstelodami*). Also, one sample was contaminated with citrinin (525 μg/kg) and rich with citrinin-producing fungi (*A. candidus*, *P. citrinum* and *P. expansum*) as recorded in (Table 3). The obtained results are in harmony to those recorded by Jansyn and Lahai (1992), Munimbazi and Bullerman (1996).

It is clear that these toxins have been produced by infecting toxigenic fungi during long drying period of fish before but not after salting and fermenting procedures. Shih and Marth (1972) and El-Kady *et al.* (1991) reported that high content of NaCl inhibited aflatoxin production by toxigenic aspergilli, while low level of NaCl stimulated toxin production, which in general agreed with the present results. Also, trace elements have stimulatory or inhibitory effect on mycotoxin biosynthesis. Based on obtained results, Ca, Mg, Mn, Cu, Cd, Ni and Zn were detected in toxic samples at 0.02 to 0.03, 0.09 to 0.19, 0.002 to 0.003, 0.008 to 0.012, 0.002 to 0.003, 0.001 to 0.003 and 0.001 to 0.003 mg g^{-1}, respectively (Table 1). In this respect, Marsh *et al.* (1976), El-Kady *et al.* (1991) stated that Zn, Fe, Mg and Ca increased aflatoxin production at low concentration (2~50 mg g^{-1}), whereas, Cu, Ni and Cd depressed the production.

On the other hand, ochratoxin A, $T\text{-}_2$ toxin, diacetoxyscirpenol, patulin, terrin, gliotoxin, rubratoxin B, roquefortin and zeralenone could not be detected in any moloha sample tested. It is the first record of mycotoxins in salted fish in Egypt.

Testing the ability of 340 fungal isolates (represented 26 species + 1 variety appertaining to 5 genera) to produce mycotoxins revealed that 127 isolates (37.4%) proved to be mycotoxin-producers (Table 4). The ability of toxin production as well as the amount of released toxin differs not only among the fungal species but also among the different isolates of the same species. The obtained results are in harmony with those obtained from fresh water and smoked fish by Nagla Abdel-Monem (1994), El-Sayed, (1995), Munimbazi and Bullerman, (1996).

Since enzymes are important chemical weapons aiding the fungal pathogen to invade host tissue, it was essential to shed some light on the ability of moloha fungi to produce these secondary metabolites. Proteolytic, lipolytic and urease enzymes were respectively detected in 82.3%,

Table 4. Production of secondary metabolites by dominant fungi isolated from salted fish in Upper Egypt

Genera and species[a]	TIT[b]	Number of isolates able to produce				Mycotoxins producting isolates (ug/50 ml medium)
		Protease	Lipase	Urease	Count	Mycotoxins identified
Total isolates	**340**	**280**	**284**	**309**	**127**	
(%)		**82.3**	**83.5**	**90.9**	**37.4**	
***Aspergillus* (total count)**	**207**	**170**	**175**	**192**	**74**	
A. flavus Link	30	27	27	29	15	Aflatoxins B_1 (250~390)
					8	Aflatoxins B_1, B_2, G_1, G_2 (200~350)
A. niger Van Tieghem	22	20	20	21	–	-ve
A. fumigatus Fresenius	20	17	18	18	12	Fumagillin (220~340)
A. flavus var. *columnaris* Raper & Fennel	18	14	14	18		-ve
A. ficuum (Reich.) Hennings	15	12	13	14	–	-ve
A. parasiticus Speare	28	24	25	27	5	Aflatoxins B_1 (220~360)
					3	Aflatoxins B_1, B_2 (180~340)
					12	Aflatoxins B_1, B_2, G_1, G_2 (225~380)
A. carbonarius (Bainier) Thom	12	10	10	10	–	-ve
A. awamori Nakazawa	10	8	8	8	–	-ve
A. duricaulis Raper & Fennel	9	7	7	9	2	Fumagillin & Gliotoxin (165~230)
A. terreus Thom	10	7	8	10	6	Citrinin & terrein (100~180)
A. candidus Link	6	4	4	5	3	Citrinin (120~170)
A. phoenicis (Cda.) Thom	9	7	7	8	–	-ve
A. alutaceus Berk & Curt	12	10	11	11	8	Ochratoxin A (160~220)
A. oryzae (Ahlb.) Cohn	4	3	3	4	–	-ve
***Penicillium* (total count)**	**71**	**60**	**61**	**67**	**32**	
P. citrinum Thom	20	17	18	19	11	Citrinin (120~180)
P. puberulum Bainier	8	7	7	8	4	Rubratoxin B & Penicillic acid (250~425)
P. brevicompactum Dierckx	10	8	8	9	–	-ve
P. verruculosum Peyronel	7	6	6	6	–	-ve
P. expansum Link ex Gray	8	7	7	8	5	Citrinin (150~200)
P. aurantiogriseum Dierckx	8	6	7	8	5	Penicillic acid (140~280)
P. roquefortii Thom	10	9	8	9	7	Roquefortin (200~360)
***Mucor racemosus* Fresenius**	**8**	**5**	**–**	**–**	**–**	**-ve**
***Scopulariopsis brevicaulis* (Sacc.) Bainier**	**10**	**7**	**8**	**8**	**–**	**-ve**
***Eurotium* (total count)**	**38**	**33**	**34**	**36**	**21**	
E. montevidensis Talice & Mackinnon	16	14	15	15	10	Sterigmatocystin (480~540)
E. chevalieri Mangin	12	11	10	12	9	Sterigmatocystin (340~450)
E. herbariorum Mangin	10	8	9	9	2	Sterigmatocystin (420~540)
***Rhizopus stolonifer* (Ehrenberg) Lind**	**6**	**5**	**6**	**6**	**–**	**-ve**

[a]Rare fungi were omitted from the table.
[b]TIT: Total isolates tested.

83.5% and 90.9% of tested fungal cultures (Table 4). Moharram and El-Zyat (1989) recorded somewhat similar results by testing the ability of fungi isolated from scale samples of *Tilapia nilotica* to produce protease and lipase enzymes. Fungal enzymes could cause tissue damage such as invasive aspergillosis and other fish diseases (Nagla Abdel-Monem, 1994).

In conclusion, it is clearly evident that Saedy moloha is considered a vehicle for numerous fungal pathogens contamination. As a result of mycological contamination and the risk of mycotoxins as carcinogenic and hepatotoxic agents should be taken into consideration. So, for human public health, salted fish must be subjected to quality control. Moloha samples must be examined chemically, microbiologically, microboial transformation and secondary metabolite production at regular times.

References

Abdel-Rahman, H., El-Khatib, T. and Refai, R. S. 1988. Microbiological studies on the Egyptian salted fish "Moloha". *Assiut. Vet. Med. J.* Assiut Univ. Egypt **38**: 91-97.

Abo-El-Alla, A. A. and Bastawrows, A. F. 1999. Occurrence and significance of some aerobic pathogens in *Oreochromis niloticus* and *Labeo niloticus* fishes in Assiut. *Vet. Med. J.* Assiut Univ. Egypt **42**(83): 174-190.

AL-Doory, Y. 1980. Laboratory medical mycology. Lea and

Febiger, Philadelphia Kimpton Publishers, London, pp. 410.

Allen, S. E. 1989. Chemical analysis of ecological materials. Blackwell Scientific Publisher, London, UK.

AOAC. 1980. Association of Official Analytical Chemists, Official methods of analysis. 13th Ed. Washington, DC, USA.

_____. 1984. Association of Official Analytical Chemists, Official methods of analysis. 14th Ed. Washington, DC, USA.

Badran, A. F., Eissa, I. A. M. and El-Attar, A. A. 1994. Some microbial problems in fresh water fish farms with domestic waste water pollution. *J. Egypt. Vet. Med. Ass.* **54**(4): 303-311.

Bullerman, L. B. 1979. Significance of mycotoxins to food safety and human health. *J. Food Prot.* **42**: 65.

Domsch, K. W., Gams, W. and Anderson, T. H. 1980. Compendium of soil fungi. Academic Press, London, pp. 859.

Dorner, J. W. 1998. Chromatographic analysis of mycotoxins. In: Chromatographic of environmental and food toxicants. T. Shibamato (ed.). pp. 113-168, Marcel Dekker, Inc. New Yourk, Hong Kong.

Edris, A. M. 1996. Microbial evaluation of some marketed smoked fish. *Zagazig Vet. Med. J.*, Egypt **24**(1): 76- 81.

El-Kady, I. A., El-Maghraby, O. M. O. and Sabah Saber, M. 1986. Halophilic or halotolerant fungi of four seeds from Egypt. *Crypt. Mycol.* **7**: 289-293.

_____, _____ and _____. 1991. Effect of different ions and sodium chloride concentrations on production of aflatoxins. *Bull. Fac. Sci.*, Assiut Univ. Egypt 123-135.

Ellis, M. B. 1971. Dematiaceous Hyphomycetes. Common wealth Mycological Institute, Kew, Surrey, UK, pp. 608.

_____. 1976. More Dematiaceous Hyphomycetes. Common wealth Myco-logical Institute, Kew, Surrey, UK, pp. 507.

El-Sawi, M. Nagwa, El-Maghraby, O. M. O., Mohran, H. S. and Abo-Gharbia, M. A. 1994. Abnormal contamination of cottage cheese in Egypt. *J. Appl. Anim. Res.* **(6)**: 81-90.

El-Sayed, Y. S. A. 1995. Mycological studies on locally produced smoked fish with special reference to toxigenic strains. Ph. D. thesis, Food Control Dept. Vet. Med., Zagazig Univ., Egypt.

Gajan, R. J. and Larry, D. 1972. Determination of lead in fish by atomic absorption spectrophotometry and by polarography. I-Development of method. *J. Assoc. Off. Anal. Chem.* **55**: 127.

Gilman, J. C. 1975. A manual of soil fungi. 2nd Indian Reprint, Published by Oxford & IBH Publishing Co, 66 Janpath, New Delhi, India, pp. 450.

Jansyn, F. E. and Lahai, G. P. 1992. Mycotoxic flora and mycotoxins in smoked dried fish from Sierraleone. Nahrung **36**(5): 485-489.

Johnson, L. F. and Curl, E. A. 1972. Methods for research on ecology of soil-borne pathogens. Burgess Publishing Co., Minneapolis, MN, USA.

Korpinen, E. L. 1974. Studies on *Stachybotrys alternans.* I- Comparison of rabbit skin, mouse fibroblast culture and brine shrimp tests as indicators of *Stachybotrys* toxins, *Acta Pathol. Microbiol. Scan. Sect. Bull.* **82**: 465-469.

Kozakiewicz, Z. 1989. *Aspergillus* species on stored products (Mycological paper No. 161). C.A.B. International Mycological Institute, Kew, Surrey, UK.

Krause, M. V. 1966. Food nutrition and diet therapy. W.B. Saunders Company, Philadelphia.

Marsh, P. B., Simpson, M. E. and Trucksess, M. W. 1976. Effect of trace metals on the production of aflatoxins by *Aspergillus parasiticus. Appl. Microbiol.* **30**: 52-57.

Moharram, A. M. and El-Zayat, S. A. 1989. Lipase and protease production by fungi isolated from scales of *Tilapia nilotica. Bull. Fac. Sci.* Assiut Univ. **18**(1-D): 109-117.

Moubasher, A. H. 1993. Soil fungi in Qatar and other Arab counteries. The center of Scientific and Applied Research, University of Qatar, Doha. Qatar.

Munimbazi, C. and Bullerman, L. 1996. Moulds and mycotoxins in food from Brondi, *J. Food Protection* **59**(8): 869-875.

Nagla Abdel-Monem, A. M. 1994. Some studies on mycoflora of fresh water fish with special reference to aspergillosis. Ph. D. thesis, Animal medicine Dept., Fac.Vet. Med. Assiut, Assiut Univ., Egypt.

Paterson, R. R. M. and Bridge, P. D. 1994. Biochemical techniques for filamentous fungi. Int. Mycol. Instit. CAB, Surrey, p. 21, UK.

Pitt, J. I. 1979. The genus *Penicillium* and its teleomorphic states *Eupenicillium* and *Talaromyces*. Commonwealth Scientific and Industrial Research Organization, Division of Food Research, North Ryde, N.S.W. Australia, Academic Press, INC. Ltd. London. pp. 634.

_____. 1991. A laboratory guide to common *Penicillium* species. (2nd ed.), Commonwealth Scientific and Industrial Research Organization, Division of Food Processing, North Ryde, N.S.W. Australia, Academic Press, INC. Ltd. London. pp. 187.

Ramirez, C. 1982. Manual and atlas of penicillia. Elsevier Biomedical Press, Ams-terdam, The Netherlands. pp. 874.

Raper, K. B. and Fennel, D. I. 1977. The genus *Aspergillus*. R. E. Krieger Publishing Company, Huntington, NY, USA. pp. 686.

Salem, A. A., Refai, M. K., Elissa, I. A. M., Marzouk, M. S., Moustafa, M. and Manal, A. 1989. Mycological investigations on cultured *Tilapia nilotica* in Egypt. *Alex. J. Vet. Sci.*, Egypt **5**(2): 625-636.

Samson, R. A., Hoekstra, E. S., Frisvad, J. S. and Filtenborg, O. 1995. Introduction to food-borne fungi (4th ed.). Centraalbureau voor Schimmelcultures Baarn Delft. The Netherlands.

Shaheen, A. A. M. 1986. Mycoflora of some fresh water fishes. M.Vet. Sc. Thesis, Fac. Vet. Zagazig, Zagazig Univ., Egypt.

Shih, C. N. and Marth, E. H. 1972. Experimental production of aflatoxin on bricks cheese. *J. Milk Food Technol.* **35**: 585-587.

Sivanesan, A. 1984. The Bitunicate Ascomycetes and their anamorphs. Strauss and Cramer Gmbh, Hirschberg ISBN, Germany, pp. 701.

Solntseva, I. O., Vinogradova, G. I., Nagornaya, S. S. and Kvasnikov, E. I. 1987. Yeast flora of fishes of water reservoirs of the Upper and Middle Volga. *Mikrobiologicheskii Zhurnal* **49**(3): 38-41.

Swaminathan, B. and Sparling, P. H. 1998. The bacteriology of food excluding dairy products. In Topły and Wilson. Collier, L., Balows, A. and Sussman, M., Microbiology and microbial infection. 9th Ed., Vol. 2 Edward Arnold Publishers Ltd. London, pp. 407-408.

Ultman, V. and Blasins, G. 1974. A simple medium for the detection of different lipolytic activity of microorganisms. *Zentrabl. Bakteriol. Hyg. II. Abt. A.* **(2)**: 264-267.

Vogel, A. I. 1974. A textbook of practical organic chemistry including qualitative organic analysis. 5th Ed. Longman, London.

Youssef, M. S., Abo-Dahab, N. F. and Abu-Seidah, A. A. 2000. Mycobiota and mycotoxins contamination of dried raisins in Egypt. *The African Journal of Mycology and Biotechnology*, Egypt **8**(3): 69-86.