

## MYCOLOGICAL EVALUATION OF SALTED *HYDROCYNUS FORSKALII* FISH IN ASSIUT GOVERNORATE

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### ABSTRACT

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The present study was performed to evaluate the mycological quality of 25 samples of commercially available salted fish (*Hydrocynus forskalii*) sold in retail outlets in Assiut Governorate, Egypt. Three isolation media [Dicloran Rose Bengal Chloramphenicol (DRBC); 10% NaCl malt extract agar and 20% NaCl malt extract agar] were used for counting and identification of fungi. Also, sensory quality, pH values and sodium chloride percentage were assessed. Sensory evaluation revealed that 12% of the samples were unacceptable while the remaining 88% samples were acceptable. Mean pH values were  $7.04 \pm 0.27$  and  $6.81 \pm 0.35$  for skin and muscular parts, respectively. Sodium chloride percentage ranged from 10.23 to 17.55% with a mean value of  $15.03 \pm 1.77$ . A total of 75 species in addition to some unidentified species of yeasts, dematiaceous hyphomycetes and pure mycelia were isolated from all samples on DRBC (61 species), 10% NaCl malt extract agar (46) and 20% NaCl malt extract agar (19). *Aspergillus*, *Petromyces*, *Penicillium*, *Eurotium*, *Cladosporium* and yeasts were the most common fungi recovered on the three media. Some of the isolated fungi are toxigenic and have the ability to produce mycotoxins which have potential hazards on human health.

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**Key words:** Fungi, Sensory, pH, Sodium chloride, Salted Fish

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### INTRODUCTION

Fish and fish products have traditionally been a popular part of the diet in many parts of the world, and in tropical areas including Africa and Far East where it constitutes a major part of the diet (FAO, 1981a and FAO, 1994). In poorest societies, fish, particularly as cured products, is often a significant source of high quality dietary protein, as well as being the least expensive source of animal protein available (Perisse *et al.*, 1974; Balachandran *et al.*, 1978; FAO, 1981a).

Fish constitute a part of protein in the diet of some people in Egypt. Salting is the most widespread and cheapest method for fish preservation, but the water activity of the salted fish is often not low enough to prevent mould spoilage. Fungi associated with salting fish differ according to the condition under which fish is stored (Ismail *et al.*, 1994). The salt curing may be done by unscientific methods under unhygienic conditions. As a result, the products are grossly contaminated with dirt, sand, microbes and insect

infestation and have only limited shelf life (Govindan, 1985).

Fungal contamination of fish is considered the main cause of spoilage which leads to off flavour and unpalatable taste and may constitute a public health hazard as well as severe economic losses (Karnop, 1980; Dorner, 1983; Ward and Baaj, 1988; Dimond and Kendall, 2011). Many strains of moulds isolated from different types of fish are able to produce toxic metabolites (mycotoxins) which have potential hazards on human health as they have carcinogenic effect. High mycotoxins levels could lead to liver cancer, whereas subacute levels are responsible for liver disease and organ damage (Pitt, 2000).

Since salted fish constitute an important part of the diet of great portion of consumers in Egypt and since it is subjected to many risks of contamination from various sources, therefore this study was performed to evaluate the mycological status of commercially available salted fish as well as estimation of pH value and NaCl%.

## MATERIALS and METHODS

### Collection of samples

Twenty five samples of salted fish (*Hydrocynus forskalii* Cuvier, 1819) were collected randomly from retail markets of different sanitation levels at Assiut city, Egypt, during the period from June 2010 to April 2011. The samples were transferred to the laboratory under aseptic condition without undue delay to be examined for their quality and fungal content.

### Preparation of samples (AOAC, 1995)

The fish heads, scales, tails, fins, guts and bones were removed and discarded. The fish were filleted to obtain all flesh and skin from head to tail and from top of back to belly on both sides. Some pieces of the fillet were selected randomly and kept separately at 4°C for sensory assessment. The other parts of the fillet were thoroughly homogenized in a sterile mortar and used for mycological and chemical analyses.

### Sensory evaluation (Ikeme, 1986)

Subjective evaluation of the salted fish quality was carried out by three taste panels from the Food Hygiene Department, Faculty of Veterinary Medicine, Assiut University, Egypt. Quality attributes studied included appearance, juiciness, saltiness, rancidity, flavor and general acceptability. Panel members scored all factors on a 5-point hedonic scale according to Ikeme (1986).

### Determination of pH

The pH value was measured using pH-meter instrument (Jenway 3505, UK) according to Lyhs *et al.* (1998).

### Estimation of sodium chloride percentage

Percentage of NaCl content was measured according to AOAC (1980).

### Mycological examination

Fish samples were prepared according to the technique recommended by American Public Health Association (1985). To 10 grams of muscle or skin sample, 90 ml of sterile saline solution (0.85% w/v) were added aseptically and thoroughly mixed for not more than 2.5 minutes using a sterile waring blender or homogenizer, to avoid mycelial fragment. Such homogenate represents the dilution of 10<sup>-1</sup>. The homogenated sample was mixed by shaking and 10 ml of the original dilutions was transferred into sterile flask containing 90 of sterile saline solution and mixed carefully by shaking. Several dilutions were done in a sequential manner by tenfold serial dilution to obtain suitable number of colonies which could be easily counted.

Three types of media were used for the isolation and enumeration of fungi: dicloran rose-bengal chloramphenicol agar medium (King *et al.*, 1979), malt extract medium + 10% NaCl and malt extract medium + 20% NaCl (Blakeslee, 1915). The inoculated media were incubated at 25°C for 5-20 days during which the developing colonies were counted, identified and the total mould count/ g were calculated. The identification of mould genera and species were carried out on the basis of their macroscopic and microscopic characteristics following the identification keys of Raper and Fennell (1965); Ellis (1971); Mossel (1977); Schipper (1978); Pitt (1979); Moubasher (1993); Samson *et al.* (2004); Leslie and Summerell (2006); Pitt and Hocking (2009).

## RESULTS

**Table 1:** Sensory evaluation of the examined retailed salted *Hydrocynus forskalii* fish samples.

General acceptability	Salted fish samples	
	No. +ve / 25	%
Excellent	0	0
Good	9	36
Medium	13	52
Bad	3	12

**Table 2:** Minimum, maximum and mean  $\pm$  SE of pH values and frequency distribution of the examined retailed salted fish samples\*.

	pH	Salted fish samples			
		Skin		Muscles	
		No. / 25	%	No. / 25	%
<b>Range</b>	> 5.5- 6	0	0	1	4
	> 6-6.5	1	4	4	16
	>6.5 -7	11	44	12	48
	> 7	13	52	8	32
	<b>Min.</b>	6.50		5.99	
	<b>Max.</b>	7.52		7.32	
	<b>Mean <math>\pm</math> SE</b>	7.04 $\pm$ 0.27		6.81 $\pm$ 0.35	

\* Egyptian standard (EOSQC, 2005): pH 6-6.5

**Table 3:** Minimum, maximum and mean  $\pm$  SE of NaCl percentage and frequency distribution of the examined retailed salted fish samples\*.

	NaCl	Salted fish samples	
		No. / 25	%
<b>Range</b>	> 6-9	0	0
	> 9-12	2	8
	> 12-15	8	32
	>15-18	15	60
	<b>Min.</b>	10.23	
	<b>Max.</b>	17.55	
	<b>Mean <math>\pm</math> SE</b>	15.03 $\pm$ 1.77	

\* Egyptian standard (EOSQC, 2005): NaCl % not less than 6 %.

**Table 4:** Mean counts of fungal genera and species recovered from skin and muscle samples of retail salted fish on DRBC, 10% NaCl malt extract agar and 20% NaCl malt extract agar at 25°C. The results are calculated as colony forming units (CFUs/g) in all samples\*.

Fungal genera and species	Skin												Muscle															
	DRBC				MSA 10%				MSA 20%				DRBC				MSA 10%				MSA 20%							
	TC	% TC	NCI	OR	TC	% TC	NCI	OR	TC	% TC	NCI	OR	TC	% TC	NCI	OR	TC	% TC	NCI	OR	TC	% TC	NCI	OR	TC	% TC	NCI	OR
<i>Acremonium</i>	1380	5.94	5	L	2	0.05	1	R					220	0.82	7	M					2	0.52	1	R				
<i>A. hyalinulum</i>	1180	5.08	2	R									100	0.37	2	R												
<i>A. strictum</i>	160	0.69	2	R	2	0.05	1	R					40	0.15	2	R					2	0.52	1	R				
<i>Acremonium</i> sp.	40	0.17	2	R									80	0.3	4	L												
<i>Alternaria</i>	280	1.21	7	M	18	0.47	6	L					520	1.93	10	M	12	0.62	4	L								
<i>A. alternata</i>	240	1.03	6	L	14	0.37	5	L					480	1.78	9	M	12	0.62	4	L								
<i>A. chlamyospora</i>	40	0.17	1	R	2	0.05	1	R					20	0.07	1	R												
<i>Alternaria</i> sp.					2	0.05	1	R																				
<i>Ascotricha</i> sp.													20	0.07	1	R												
<i>Aspergillus</i>	2780	11.97	18	H	1426	37.5	16	H	66	23.4	5	L	2260	8.39	19	H	324	16.72	14	H	36	9.38	8	M				
<i>A. candidus</i>	120	0.52	1	R					2	0.71	1	R	20	0.07	1	R	20	1.03	1	R								
<i>A. japonicus</i>													20	0.07	1	R												
<i>A. niger</i>	2420	10.42	7	M	270	7.1	16	L	40	14.18	1	R	1760	6.53	16	H	188	9.7	9	M								
<i>A. ochraceus</i>	40	0.17	2	R	10	0.26	4	L	4	1.42	1	R	180	0.67	7	M	12	0.62	5	L	2	0.52	1	R				
<i>A. oryzae</i>																	2	0.1	1	R								
<i>A. sydowii</i>	20	0.09	1	R	6	0.16	2	R	12	4.26	3	L	140	0.52	3	L	10	0.516	4	L	14	3.65	4	L				
<i>A. tamarii</i>	80	0.34	1	R	40	1.05	1	R									4	0.21	1	R								
<i>A. terreus</i>	60	0.26	3	L	16	0.42	2	R	8	2.84	2	R	40	0.15	2	R	88	4.54	4	L	8	2.08	2	R				
<i>A. ustus</i>													20	0.07	1	R												
<i>A. versicolor</i>	20	0.09	1	R	1082	28.46	1	H					40	0.15	2	R					6	1.56	2	R				
<i>A. wentii</i>					2	0.05	16	H																				
<i>Aspergillus</i> sp.	20	0.09	1	R									40	0.15	1	R					6	1.56	2	R				
<i>Botryotrichum atrogriseum</i>	20	0.09	1	R																								
<i>Byssoschlamys spectabilis</i>													200	0.74	3	L												
<i>Cladosporium</i>	3360	14.46	16	H	372	9.78	13	H	30	10.64	6	L	1960	7.27	10	M	196	10.11	13	H	30	7.81	7	M				
<i>C. cladosporioides</i>	2660	11.45	11	M	328	8.63	12	M	30	10.64	6	L	1660	6.16	8	M	150	7.74	10	M	30	7.81	7	M				
Fungal genera and species	Skin												Muscle															
	DRBC				MSA 10%				MSA 20%				DRBC				MSA 10%				MSA 20%							
	TC	% TC	NCI	OR	TC	% TC	NCI	OR	TC	% TC	NCI	OR	TC	% TC	NCI	OR	TC	% TC	NCI	OR	TC	% TC	NCI	OR	TC	% TC	NCI	OR
<i>C. herbarum</i>	180	0.77	5	L	44	1.16	5	L					40	0.15	2	R	38	1.96	2	R								
<i>C. sphaerospermum</i>	20	0.09	1	R	4	0.11	2	R					40	0.15	2	R												
<i>Cladosporium</i> sp.	500	2.15	2	R									220	0.82	2	R	8	0.41	2	R								
<i>Cochliobolus</i>	220	0.947	5	L	4	0.11	2	R					240	0.89	5	L												
<i>C. lunatus</i>	220	0.947	5	L	2	0.05	1	R																				
<i>C. specifer</i>					2	0.05	1	R									4	0.2	1	R								
<i>Emericella</i>	160	0.69	5	L					2	0.71	1	R	160	0.59	5	L	6	0.31	2	R	2	0.52	1	R				
<i>E. nidulans</i>	160	0.69	5	L					2	0.71	1	R	100	0.37	4	L	2	0.1	1	R	2	0.52	1	R				
<i>E. varicolor</i>																	2	0.1	1	R								
<i>Emericella</i> sp.													60	0.22	1	R	2	0.1	1	R								
<i>Eurotium</i>	360	1.55	3	L	426	11.2	14	H	28	9.93	7	M					368	18.99	13	H	170	44.27	11	M				
<i>E. amstelodami</i>					112	2.95	4	L									140	7.22	3	L								
<i>E. chevalieri</i>	20	0.09	1	R	206	5.42	10	M	26	9.22	7	M					186	9.6	11	M	170	44.27	11	M				
<i>E. repens</i>																	20	1.032	1	R								
<i>E. rubrum</i>	20	0.09	1	R	6	0.16	3	L																				
<i>Eurotium</i> sp.	320	1.38	1	R	102	2.68	2	R	2	0.71	1	R	60	0.22	1	R	22	1.14	2	R								
<i>Fennellia flavipes</i>					2	0.05	1	R																				
<i>Fusarium</i>	740	3.19	7	M	4	0.1	2	R					720	2.67	8	M	10	0.52	4	L								
<i>F. acuminatum</i>													20	0.07	1	R	2	0.1	1	R								
<i>F. oxysporum</i>	40	0.17	1	R																								
<i>F. semitectum</i>	180	0.77	4	L	4	0.1	2	R					360	1.34	4	L	2	0.1	1	R								
<i>F. solani</i>													20	0.07	1	R												
<i>F. verticillioides</i>	60	0.26	2	R																								
<i>Fusarium</i> sp.	460	1.98	1	R									320	1.19	4	L	6	0.31	2	R								
<i>Graphium</i> sp.													20	0.07	1	R												
<i>Neosartorya fumigata</i>	40	0.17	1	R	8	0.21	2	R	32	11.35	3	L					2	0.1	1	R	8	2.08	1	R				
<i>Nigrospora oryzae</i>	20	0.09	1	R	2	0.05	1	R					40	0.15	2	R	4	0.21	2	R								

Table 4: continued

Fungal genera and species	Skin												Muscle																				
	DRBC				MSA 10%				MSA 20%				DRBC				MSA 10%				MSA 20%												
	TC	%	TC	NCI	OR	TC	%	TC	NCI	OR	TC	%	TC	NCI	OR	TC	%	TC	NCI	OR	TC	%	TC	NCI	OR	TC	%	TC	NCI				
<i>Penicillium</i>	1900	8.18	15	H	76	1.3	10	M	42	14.89	7	M	1000	3.71	16	H	112	5.78	7	M	96	25	4	L									
<i>P. aurantiogriseum</i>	20	0.09	1	R	20	0.53	1	R	12	4.26	2	R	60	0.22	2	R																	
<i>P. chrysogenum</i>	120	0.52	3	L	2	0.05	1	R					220	0.82	5	L																	
<i>P. citrinum</i>	20	0.09	1	R																													
<i>P. corylophilum</i>									8	2.84	2	R									2	0.52	1	R									
<i>P. duclauxii</i>	60	0.26	2	R									60	0.22	2	R																	
<i>P. expansum</i>	20	0.09	1	R																													
<i>P. oxalicum</i>	1420	6.11	6	L	32	0.84	5	L					300	1.11	5	L	94	4.85	2	R													
<i>P. pinophilum</i>	40	0.17	2	R									20	0.07	1	R																	
<i>P. purpurogenum</i>													20	0.07	1	R																	
<i>Penicillium</i> sp.	200	0.86	5	L	22	0.58	3	L	22	7.8	3	L	320	1.19	7	M	18	0.93	7	M	94	24.48	3	L									
<i>Petromyces flavus</i>	6280	27.03	17	H	588	15.47	11	M	52	18.44	4	L	3500	12.99	17	H	676	34.88	12	M	10	2.6	2	R									
<i>Phoma epicoccina</i>	60	0.26	1	R																													
<i>Pseudoallescheria boydii</i>													20	0.07	1	R																	
<i>Rhizopus</i>	40	0.17	1	R	20	0.53	1	R									4	0.21	1	R													
<i>R. oryzae</i>	40	0.17	1	R																													
<i>R. stolonifer</i>					20	0.53	1	R									4	0.21	1	R													
<i>Scolecobasidium variabilis</i>					2	0.05	1	R																									
<i>Scopulariopsis</i>	60	0.26	3	L									80	0.3	4	L																	
<i>S. brevicaulis</i>	20	0.09	1	R																													
<i>S. brumptii</i>													40	0.15	2	R																	
<i>S. halophilica</i>					2	0.05	1	R	20	7.09	1	R									22	5.73	2	R									
<i>S. japonicus</i>	40	0.17	2	R									20	0.07	1	R																	
<i>Scopulariopsis</i> sp.													20	0.07	1	R																	
<i>Setosphaeria rostrata</i>	40	0.17	2	R	4	0.1	2	R					40	0.15	2	R																	
<i>Sporothrix schenckii</i>																	2	0.1	1	R													
<i>Stachybotrys</i>	220	0.95	4	L	2	0.1	1	R					20	0.07	1	R																	
Fungal genera and species	Skin												Muscle																				
	DRBC				MSA 10%				MSA 20%				DRBC				MSA 10%				MSA 20%												
	TC	%	TC	NC	OR	TC	%	TC	NCI	OR	TC	%	TC	NCI	OR	TC	%	TC	NCI	OR	TC	%	TC	NCI	OR	TC	%	TC	NCI				
<i>S. chartarum</i>	100	0.43	4	L	2	0.1	1	R																									
<i>Stachybotrys</i> sp.	120	0.52	1	R									20	0.07	1	R																	
<i>Stemphylium botryosum</i>	60	0.26	2	R	20	0.53	1	R																									
<i>Syncephalastrum racemosum</i>					10	0.26	1	R					170	0.63	1	R	4	0.21	1	R													
<i>Trichothecium roseum</i>													40	0.15	2	R																	
<i>Ulocladium</i> sp.	60	0.26	3	L																													
<i>Wallemia sebi</i>									2	0.71	1	R																					
<i>Unidentified mycelia (dark &amp; white)</i>	80	0.34	4	L	4	0.11	2	R					100	0.37	3	L	26	1.34	2	R													
<b>Yeasts</b>	5070	21.83	17	H	804	21.15	9	M	8	2.87	2	R	15620	57.96	11	M	186	9.6	5	L	4	1.04	2	R									
Black													20	0.07	1	R																	
Orange-red	120	0.52	4	L	18	0.47	3	L	2	0.71	1	R	4160	15.44	3	L	2	0.1	1	R	2	0.52	1	R									
White	4950	21.31	14	H	784	20.62	8	M	6	2.13	2	R	11440	42.45	10	M	184	9.49	5	L	2	0.52	1	R									
<b>Total (fungi and yeasts)</b>	23230	100			3802	100			282	100			26950	100			1938	100			384	100											
<b>No. of genera: 30</b>	<b>20</b>				<b>18</b>				<b>9</b>				<b>20</b>				<b>15</b>				<b>9</b>												
<b>No. of species: 75</b>	<b>47</b>				<b>35</b>				<b>16</b>				<b>46</b>				<b>31</b>				<b>14</b>												

\*TC: Total counts, %TC: Percentage total count (calculated per total counts of all fungi), NCI: number of cases of isolation, OR: Occurrence remarks; H= high (13-25), M= moderate (7-12), L= low (3-6), R= rare (1-2).

## DISCUSSION

The results of sensory evaluation (Table 1) indicated that most salted fish samples (88%) were organoleptically accepted where 9 (36%) and 13 (52%) out of 25 samples were categorized as of good and medium quality, respectively. Only 3 (12%) samples were of bad quality. Our results are nearly similar to those obtained by Ahmed (1976), Essa (1998) and Sayed (2008) who revealed that most salted fish samples they examined were physically normal. On the other hand different results were obtained by El-Morshdy *et al.* (1981) and Nayel (2007) who detected higher percentages of bad quality salted fish.

It is evident from the results recorded in Table (2) that the pH values of the samples varied from 6.5 to 7.52 with a mean value of  $7.04 \pm 0.27$  on skin part of fish, whereas, on muscle part they varied from 5.99 to 7.32 with a mean value of  $6.81 \pm 0.35$ . Out of 25 samples of skin part of fish 11 and 13 were in the pH range of  $>6.5-7.0$  and  $>7$ , respectively. Only one sample achieved pH value of 6.5. On muscular part of fish 4, 12 and 8 of the examined samples were in the pH range of  $>6-6.5$ ,  $>6.5-7.0$  and  $>7$ , respectively. However, only one sample achieved more acidic pH value of 5.99.

From the summarized results in Table (2) it can be concluded that 96%, and 80% of the examined samples of skin and muscular parts had pH values exceeded those of the Egyptian standards for salted fish (pH 6.0- 6.5) (EOSQC, 2005), only 4% and 20% of samples, respectively, had values complying with the Egyptian standards. The pH values of the analyzed samples slightly differed from those previously obtained by Ahmed (1976), Abd El-Rahman *et al.* (1988) and El-Sheshnagui (2006). However, they were generally higher than those reported by NRCT (1981-1982), Sakai *et al.* (1983), Chang *et al.* (1991), Yatsunami and Echigo (1991), Silla-Santos (1996), Hernandez-Herrero *et al.* (1999a,b), Majumdar *et al.* (2006), Yung-Hsiang *et al.* (2006), Sayed (2008) for a variety of salted fish products. On the other hand, the obtained values were lower than those recorded by Steinkraus (1983), Surono and Hosono (1994) and Anihouvi *et al.* (2006).

For sodium chloride contents, the percentages were varied from 10.23 - 17.55 % with a mean value of  $15.03 \pm 1.77$ . Out of the 25 examined salted fish samples 2, 8 and 15 had NaCl in the range of  $>9 - 12\%$ ,  $>12-15\%$  and  $>15-18\%$ , respectively (Table 3). From these results, it is evident that all examined samples had NaCl content more than 6 % which comply with the established Egyptian standards (EOSQC, 2005) for salted fish. Our results were nearly similar to those recorded by NRCT (1981-

1982) for pla-ra, Abd El-Rahman *et al.* (1988) for meloha, and Majumdar *et al.* (2006) for Iona ilish. On the other hand they were slightly different from those registered by Shahine (1956), Ahmed (1976), Jennie and Muchtadi (1978), El-Morshdy *et al.* (1981), Chang *et al.* (1991), Surono and Hosono (1994), Essa (1998) and Sayed (2008). Lower values were reported by Sakai *et al.* (1983), Anihouvi *et al.* (2006) and El-Sheshnagui (2006), while Steinkraus (1983), Sanni *et al.* (2002) and Yung-Hsiang *et al.* (2006) detected higher percentage.

It was observed that the fungal propagules recovered on DRBC were higher than those recovered on malt extract agar amended with either 10% or 20% NaCl with the lowest count being recorded on 20% NaCl malt extract agar (Table 4). This may be attributed to the effect of high concentration of salt which inhibits the growth of many species of fungi as reported by Atapattu and Samarajeewa (1990), Ismail *et al.* (1994), Essa (1998) and Ahmed *et al.* (2005). Addition of sodium chloride at high rate to the medium gives the opportunity to halophilic moulds (which probably found in salted fish samples) to appear in such media. The low mould counts recorded on 20% NaCl malt extract agar is in agreement with those obtained by Abdel-Rahman *et al.* (1988). However, higher mould counts were recorded by Ismail *et al.* (1994). This variation in mould counts in salted fish samples may be due to different levels of sanitary measures adopted during handling, manufacturing and storage.

It is noteworthy that many fungi were isolated on the three media and these were *Acremonium strictum*, *Aspergillus niger*, *A. ochraceus*, *A. sydowii*, *A. terreus*, *A. versicolor*, *Cladosporium cladosporioides*, *Emericella nidulans*, *Eurotium chevalieri*, *Eurotium* sp., *Neosartorya fumigata*, *Penicillium chrysogenum*, *Petromyces flavus*, *Scopulariopsis* sp. and orange-red and white yeasts.

On the other hand, some were recorded only on one medium such as *Acremonium hyalinulum*, *Acremonium* sp., *Ascotricha* sp., *Aspergillus japonicus*, *A. ustus*, *Botryotrichum atrogriseum*, *Byssochlamys spectabilis*, *Setosphaeria rostrata*, *Fusarium oxysporum*, *F. solani*, *F. verticillioides*, *Graphium* sp., *Penicillium citrinum*, *P. duclauxii*, *P. expansum*, *P. pinophilum*, *P. purpurogenum*, *Phoma epicoccina*, *Pseudoallescheria boydii*, *Rhizopus oryzae*, *Scopulariopsis brevicaulis*, *S. brumptii*, *S. japonicum*, *Setosphaeria rostrata*, *Stachybotrys chartarum*, *Trichothecium roseum* *Ulocladium* sp. and black yeasts on only DRBC; *Alternaria* sp., *Aspergillus oryzae*, *A. wentii*, *Cochliobolus specifer*, *Emericella varicolor*, *Eurotium repens*, *Fennellia flavipes*, *Rhizopus stolonifer*, *Scolecobasidium variabile*, and *Sporothrix schenkii* on 10% NaCl MSA medium only; and *Penicillium corylophilum*

and *Wallemia sebi* on 20% NaCl MSA medium only (Table 4). Many of these fungi were isolated from salted fish in Egypt as reported by (Abdel-Rahman *et al.*, 1988, Ismail *et al.*, 1994, Essa 1998, Youssef *et al.*, 2003 and Ahmed *et al.*, 2005). Also, in other countries such as Sri Lanka (Atapattu and Samarajeewa 1990 and Wheeler and Hocking 1993), Japan (Hitokoto *et al.*, 1976), Indonesia (Wheeler *et al.*, 1986) and in Ghana (Lu *et al.*, 1988).

It is noteworthy also that many fungi were isolated from only skin parts and these were *Alternaria* sp., *Botryotrichum atrogriseum*, *Fusarium oxysporum*, *F. verticillioides*, *Penicillium citrinum*, *P. expansum*, *Phoma epicoccina*, *Rhizopus oryzae*, *Scolecobasidium variabile*, *Scopulariopsis brevicaulis*, *Setosphaeria rostrata*, *Stachybotrys chartarum*, *Stemphylium botryosum*, *Ulocladium* sp. and *Wallemia sebi*. On the other hand, others were isolated only from muscular parts such as *Ascotricha* sp., *Byssochlamys spectabilis*, *Emericella* sp., *Fusarium acuminatum*, *F. solani*, *Graphium* sp., *Penicillium purpurogenum*, *Pseudoallescheria boydii*, *Scopulariopsis brumptii*, *Scopulariopsis* sp., *Sporothrix schenkii*, *Trichothecium roseum*, and black yeasts.

It was observed that *Eurotium* was isolated in high frequency from 14 skin and 13 muscular samples out of 25 samples examined on 10% NaCl malt extract agar as this genus is considered as halophilic fungus. *Eurotium* amounted 11.2% and 18.99% of the total fungal propagules on skin and muscular parts, respectively. *E. chevalieri* and *E. amstelodami* were the most prevalent species.

*Cladosporium* was frequently isolated on DRBC from 16 samples yielding 14.46% of the total propagules on skin part and from 10 samples yielding 7.72% of the propagules on muscular part. While on 10% NaCl malt agar this genus was recovered in high frequency from 13 samples from both skin and muscular parts, accounting for 9.78% and 10.11% of the total propagules, respectively. *C. cladosporioides* and *C. herbarum* were the most prevalent species.

Both *Aspergillus* and *Penicillium* species were recovered in relatively high counts on dicloran rose-bengal agar, in percentages of 11.97% and 8.18% of skin and 8.39% & 3.71% of muscular parts of the total propagules, respectively. Both species were isolated on 10% salt malt extract agar, in percentages of 37.5% and 1.3 of skin and 16.72% and 5.78% of muscular parts of the total propagules, respectively. On 20% salt malt extract media they were recovered in percentages of 23.40% and 14.89% of skin and 9.38% and 25% of muscular parts of the total propagules, respectively. The total counts of genus *Aspergillus* and *Penicillium* were sharply decreased on 20% NaCl malt extract medium as affected by the

presence of sodium chloride (Table 4). In Egypt, Youssef *et al.* (2003) isolated both *Aspergillus* and *Penicillium* species in percentages of 72.9%, 3.8%, respectively; Essa (1998) isolated both *Aspergillus* and *Penicillium* species in percentage of 93.46% and 0.42% of the total propagules, respectively. *Aspergillus* (53.3%) and *Penicillium* (44.4%) species were also isolated by Ismail *et al.* (1994) from salted fish samples. Also, Abdel-Rahman *et al.* (1988) could isolate both *Aspergillus* and *Penicillium* species which accounted for 26.3% and 38.5% of the total propagules from salted fish samples, respectively. Several authors such as Watson (1993), McMahon (1994) and Hassan (1995) reported the importance of *Aspergillus* and *Penicillium* species in production of mycotoxins which have toxic and carcinogenic effects on public health.

*Petromyces flavus* (the aflatoxigenic *Aspergillus* species) followed by yeasts, *Aspergillus*, *Cladosporium*, *Penicillium* and *Fusarium* predominated on skin parts, while on muscular parts yeasts predominated over *Petromyces flavus*, *Aspergillus*, *Cladosporium*, *Penicillium*, *Fusarium* in the number of propagules.

From the current results, it could conclude that some of the examined salted fish are highly contaminated with moulds due to neglected sanitary measures. Furthermore, mishandling of such types of fish resulted in presence of a variety of fungi in high counts.

The incidence of moulds could be attributed to improper sanitation during catching, handling, processing, salting storage, transportation, distribution and marketing of fish (Novotny *et al.*, 2004). Contaminations with a variety of mould species resulted in undesirable changes of fish and rendering it unfit for marketing and increase the risk of infection with respective disease to consumers as a probable result of aflatoxins production by some fungal strains (Ward and Baaj, 1988; Dimond and Kendall, 2011).

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## تقييم الحالة الفطرية لأسماك كلب السمك المملح في محافظة أسيوط

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أجريت هذه الدراسة علي ٢٥ عينة من اسماك الملوحة (كلب السمك المملح) تم تجميعها عشوائيا من منافذ بيع الأسماك المملحة في محافظة أسيوط بغرض تقييم الحالة الفطرية لها باستخدام ثلاث بيئات مختلفة وهي ديكلوران روز بنجال كلورامفينيكول، مستخلص المالت المضاف إليها ١٠% ملح طعام، ومستخلص المالت المضاف إليها ٢٠% ملح طعام. كما تم تقييم الأسماك حسيا بالأضافة الي قياس الأيون الهيدروجيني وكوريد الصوديوم. أظهرت نتائج التقييم الحسي أن ١٢% من العينات كانت غير مقبولة ظاهريا بينما ٨٨% كانت مقبولة. قيم متوسطات تركيز أيون الهيدروجين كانت  $7.04 \pm 0.27$  و  $6.81 \pm 0.35$  في جلد وعضلات الأسماك المملحة علي التوالي، أما بالنسبة لقيم ملح الطعام فقد تراوحت بين  $10.23 - 17.55$  بمتوسط  $15.02 \pm 1.77$ %. بلغ إجمالي ما تم عزله و تصنيفه من الفطريات ٧٥ نوع، كان منها ٦١ نوع باستخدام البيئة الأولى و ٤٦ نوع باستخدام البيئة الثانية، و ١٩ نوع باستخدام البيئة الثالثة، هذا بالإضافة إلى بعض الأنواع الغير مصنفة من الخمائر التي تم عزلها على الثلاث بيئات المستخدمة. كان من أكثر الأنواع التي تم عزلها على البيئة الأولى تنتمي إلى أجناس الاسبرجلس، بيتروميسس، بنسليوم، الخمائر ، كلادوسبوريوم بينما كانت أكثر الأنواع عزلاً على البيئة الثانية هي التابعة للاسبرجلس، كلادوسبوريوم وإيورتيوم أما في حالة البيئة الثالثة فقد سجلت الأنواع التابعة لأجناس إيورتيوم ، بنسليوم ، كلادوسبوريوم، اسبرجلس معدلات تعداد متوسطة من الأسماك التي تم فحصها في هذه الدراسة. هذا وقد تم مناقشة المخاطر الصحية لبعض الفطريات التي تم عزلها علي صحة المستهلك.

الكلمات الكاشفة: فطريات، تقييم حسي، كلوريد الصوديوم ، الأيون الهيدروجيني ، الأسماك المملحة.