

Animal Health Research Institute  
Assiut Regional Laboratory

**MOLDS CONTAMINATING SHELLS AND  
CONTENTS OF COMMERCIAL HENS  
AND DUCKS' EGGS IN ASSIUT CITY**  
(With 4 Tables)

By

**AMAL ALI ABDEL-HALEEM  
and A.A. EL-SHANAWANY\***

\*Botany and Microbiology Department, Faculty of Science, AL-Azhar University,

**الفطريات الملوثة لقشر ومحتويات بيض الفراخ والبط التجاري  
في مدينة أسيوط**

**آمال على عبد الحليم , عبد الرحيم أحمد الشنواني**

أجريت هذه الدراسة على عدد ٢٢٥ بيضة تمثل ٤٥ عينة عشوائية من الأنواع المختلفة من البيض وهي الأنواع البلدية وبيض المزارع وبيض البط (١٥ عينة لكل نوع) وذلك لفحصها ميكولوجياً لمعرفة مدى تلوثها بالفطريات والخمائر. وقد تمت الدراسة باستخدام وسط العزل Malt extract وال تحضين عند درجة ٢٨°م. ولقد تبين من الفحص الميكولوجي أن بيض مزارع الفلاحين أكثر الأنواع تلوثاً بالفطريات بينما الأنواع البلدية أقلها تلوثاً. وجد أن التعداد الكلي للفطريات على السطح الخارجي (لكل بيضة) وكذلك المحتوى الداخلي (لكل بيضة) للبيض البلدي وبيض المزارع وبيض البط هو ٥٧٠٠ ، ٣١٠٠ - ١٣١٠٠ ، ٨٤٥٠ و ٨٥٠٠ ، ٣٣٥٠ /قشروملي على الترتيب. تم عزل وتعريف ٢٨ نوعاً فطرياً تنتمي إلى ١٢ جنساً وذلك من الأنواع الثلاثة تحت الدراسة كانت أكثر الفطريات تعداداً وانتشار تلك الأنواع التي تتبع أجناس أسبيرجيلس، بنسيليوم وكلايدوسبوريوم تليها الأنواع التابعة لجنسي أكريمونيوم وريزويس بالإضافة إلى عدد من الخمائر الغير معرفة.

### SUMMARY

The molds inhabiting the outer surfaces of the shell as well as the content of the eggs were studied on malt extract agar at 28°C. Forty-five samples of native breeds, farms and duck's eggs (15 samples each) were employed. The results indicated that the farm eggs were more contaminated than the other types. Whereas the native breeds eggs were less polluted. The total counts of fungi on egg shells were 5700, 13100 & 8500 colonies 5 shells (calculated per 5 eggs) while in eggs content

were 3100, 8450 & 3350 colonies/ml (calculated per 5 eggs) of the native breeds, farms and ducks' eggs, respectively. From the three substrates 28 species representing 12 genera could be identified. Species of *Penicillium*, *Aspergillus* and *Cladosporium* were the most prevalent followed by *Acremonium* and *Rhizopus* species. Also, Some non-filamentous fungi (yeasts) were isolated from the three types of egg with high or moderate incidence.

**Key words:** *Molds, shells, contents, ducks, hens eggs.*

## INTRODUCTION

Moulds as undesirable microorganisms are widely distributed in nature. Unfortunately eggs are susceptible to fungal contamination at different stages till consumption (Fajardo *et al.*, 1995 and Bastawrows *et al.*, 2001). Contamination of eggs with mould will cause off-flavors "musty odors" and tastes spoilage and fungal rotting of eggs rendering it unfit for marketing and consumption (Frazier and Westhoff 1986 and Zaki *et al.*, 1997). The hyphae of the mould may weaken the yolk membrane enough to cause its rupture, after which the growth of the mould is stimulated greatly by the food released from the yolk (Aleksandrov, 1974; Frazier and Westhoff, 1986 and Jordan, 1990).

Also, certain moulds are capable of producing toxic metabolite or mycotoxins, which could be regarded as potential health hazard (Cole and Cox, 1981). Furthermore, great economic losses, reducing embryonic mortalities and lowering the hatchability takes place as a result of fungal infection of eggs (Osteminko, 1981; Matthes, 1984; Hamet *et al.*, 1991; Aly *et al.* 1993 and Zaki *et al.*, 1997).

The aim of the present work was designed to isolate and identify various fungi, which contaminate native breed, farms and ducks' eggs. Also, to correlate between the occurrence of fungi on the outer surface of shells and in the content of the eggs.

## MATERIAL and METHOD

### Collection of samples:

A total of 225 eggs (45 samples) of commercial eggs, including native breeds, farms and ducks' eggs (15 each), were collected from poultry farms, farmers' houses and supermarkets in Assiut city. Each group (5 eggs) represents one sample, was placed in a sterile plastic bag and dispatched to the laboratory with a minimum of delay. The eggs were prepared and examined for the presence of different molds.

### **Preparation of samples:**

#### **Egg shells:**

Egg shells of each group were tested by surface rinse technique as described by Moats (1979).

#### **Egg contents:**

The egg contents were prepared according to Speck (1976).

#### **Dilution:**

Ten-fold serial dilutions up to  $10^6$  were aseptically prepared from the rinse solution as well as from the homogenized egg contents using sterile 0.1% peptone water. Molds count was determined using Malt extract agar according to Harrigan and Margarete (1976).

#### **Identification of isolated strains:**

Suspected mold strains were identified on Glucose-Czapeck' agar medium according to Raper and Fennell (1965); Ellis (1971); Pitt and Hocking (1985) and Sivanesan (1984).

## **RESULTS**

The obtained results are recorded in Tables 1, 2 and 3.

## **DISCUSSION**

In the current study 45 samples of native breeds, farms and ducks' eggs (15 samples each) were employed to determine the molds inhabiting the outer surface of the shell as well as the content of eggs.

The data presented in Table 1 reveal that all of the examined shells were contaminated by mold and yeast (100%) with an average count of 380, 873 and 567 colonies/shell. While, 93.33, 93.33 and 100% of the examined egg content of different types were positive for existence of mold and yeast, with an average count of 207, 563, and 223 colonies/ ml, respectively. Higher counts were obtained by Ahmed et al. (1987) and El-Prince (1988) in the examined commercial hens' eggs. However, El-Prince recorded lower incidence of mold and yeast in the examined samples.

Tables 2, 3 and 4 showed that the total count of fungi on egg shell and in content was 5700 and 3100; 13100 and 8450 and 8500 and 3350/5 shells and ml of content in native breeds, farms and ducks' eggs, respectively. The results declared that, the outer surface of egg shells was

more contaminated than the content of eggs. Our results were in agreement with the finding of EL-Prince and Hemida (1997). They isolated fungal species from hen's egg shell and content with a percentage of 84.44% and 48.88 %, respectively. Also the average total of fungi isolated by Bastawrows *et al.* (2001) was 320/shell and 70/ml egg content.

Twenty-eight species belonging to 12 genera were recovered in the present study from all egg samples used on Malt extract agar at 28C°. The highest number of genera and species (11 and 20) was recorded in the egg shell of native bread eggs, while the lowest number was recorded in egg contents of farms' eggs (4 and 11).

*Penicillium* was the most common genus on either surface of egg shell and content of egg of all the three types used. From egg shell it was marked that 73.3%, 100% and 100% of the samples of egg shells constituting 50.9%, 77.5 % and 54.1% of the total fungi with native breeds, farms and ducks' eggs, respectively.

In egg content, *Penicillium* emerged 60 %, 93.3 % and 93.3 % of the samples matching 74.2 %, 66.9 % and 68.7 % of the total count of fungi with the three types of eggs used, respectively. From this genus 12 species were isolated of which *P. chrysogenum* and *P. corylophilum* were the most common species in egg shell and content of all types of egg.

*P. nigricans* was recovered in high incidence with 60 % of farm egg content, while *P. islandicam* was recovered in moderate frequency (33 % of the samples) from farm egg shells. The remaining *Penicillium* species were recovered in low and rare frequencies of occurrence (Tables 1,2 and 3). Moursy *et al.* (1982) isolated *penicillium oxalicum*, *P. cyclopium*, and *P. chrysogenum* from hens' eggs. Also, Morgan *et al.* (1983) recorded that *Penicillium* was the most genus in imported hens' eggs. The same Findings were obtained by El-Prince and Hemida (1997), Bastawros *et al.* (2001) and Frazier and Westhoff (1986).

*Aspergillus* (4 species) ranked the second most prevalent genus. It was recovered from 26.7 -73 % of the samples comprising 450- 1300 of total fungi of egg shell. In egg content, it occupied 26.7-80 % of the samples matching 150 – 1800 of the total fungi in all egg types used. *A.niger* was the most prevalent species in farm egg content (46.76%). The remaining *Aspergillus* species were of low incidence (Tables 1, 2 and 3). This result was in agreement with those obtained by Hofstad *et al.* (1984) mentioned that, in the breeding farms when the hatchery, litter and feed were heavily contaminated with spores of *Aspergillus may*

result in infection of birds and also, could be transmitted to eggs. Also, EL-Badry and Sokkar (1998) isolated *A. fumigatus* (21 isolates); *A. flavus* (14) *A. niger* (23) and *A. flavipes* (2) from feeds, water, egg shell, hatcheries and broilers room. Nearly similar results were reported by Aleksandrov (1974), Moursy *et al.* (1982), EL-Prince and Hemida (1997) and Bastawrows *et al.* (2001).

*Cladosporium* came third in frequency of occurrence. It is represented by *C. cladosporioides*, *C. herbarum* and *C. sphaerospermum*. It ranged from 60-73.3 % of egg shell samples constituting 11.1-28.1 % of total fungi, while in egg content it ranged from 20-53.3 % of egg samples matching 6.5-23.9 % of total fungi. Also, this genus was isolated from egg shell and egg content with different incidences by Eckman and Jones (1979); Morgan *et al.* (1983); Aman *et al.* (1993); EL-Prince and Hemida (1997) and Bastawrows *et al.* (2001).

*Acromonium strictum* and *Rhizopus stolonifer* were isolated in moderate incidence (each from 33.3 % of the samples) from egg shell of duck and native breeds. This result is compatible with those reported by EL-Badry and Sokkar (1988), Moursy *et al.* (1982) and EL-Prince and Hemida (1997).

The remaining genera and species were isolated in low and rare frequencies of occurrence (Tables 2, 3 and 4). Eighteen fungal species were isolated only from the outer surface of egg shells but not isolated from the content of eggs of the three types of eggs. On the other hand 3 fungal species were isolated only from the content of duck eggs only (Tables 1, 2 and 3). These results are in agreement with those recorded by Sambyal *et al.* (1981); Matthes (1984); Aly *et al.* (1993) and Das *et al.* (1994).

A yeast non-filamentous fungus was isolated from the three types of eggs with incidence ranged from 20–100% of the sample isolates. Moursy *et al.* (1982) isolated yeast (*candida albicans*) from the hen eggs. Aman *et al.* (1993) showed that, yeasts were isolated from 20% of examined shells of farm and balady eggs while; it could not be isolated from the contents.

It could be noticed that the number of species and genera recovered from the shell samples are higher than those from contents, as the shell is more liable to be contaminated. Therefore, proper farm hygiene, handling and storage of eggs are necessary for obtaining eggs of good quality and to safe guard consumers from being infected.

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**Table 1:** Statistical analytical results of molds recovered from commercial eggs

Samples	Egg Shells					Egg contents				
	+ve samples		Maximum	Minimum	Average	+ve samples		Maximum	Minimum	Average
	No./15	%				No./15	%			
Native breeds' eggs	15	100	1350 Colonies /shell	100 Colonies /shell	380 /shell	14	93.33	1400 Colonies/ml	50 Colonies /ml	221/ml
Farms' eggs	15	100	1650 Colonies /shell	200 Colonies /shell	873 /shell	14	93.33	1550 Colonies/ml	50 Colonies /ml	604/ml
Ducks' eggs	15	100	1200 Colonies /shell	100 Colonies /shell	567 /shell	15	100	350 Colonies/ml	100 Colonies /ml	223/ml



**Table 2:** Total counts (Tc, calculated per 5 eggs), percentage of frequency (F %) of fungal genera and species isolated from shell surface and content of native breeds' eggs.

Genera and species	Rinsed shell		Content	
	Tc	F %	Tc	F %
<i>Acremonium strictum</i>	100	13.3	-	-
<i>Alternaria alternata</i>	100	13.3	50	6.7
<i>Aspergillus</i>	450	26.7	450	33.3
<i>A. flavus</i>	200	13.3	350	20
<i>A. niger</i>	100	13.3	50	6.7
<i>A. terreus</i>	150	13.3	50	6.7
<i>Cladosporium</i>	1600	73.3	200	20
<i>C. cladosporioides</i>	450	33.3	100	13.3
<i>C. herbarum</i>	50	6.7	-	-
<i>C. sphaerospermum</i>	1100	53.3	100	6.7
<i>Fusarium solani</i>	50	6.7	-	-
<i>Gliocladium roseum</i>	50	6.7	-	-
<i>Penicillium</i>	2900	73.3	2300	60
<i>P. aurantiogriseum</i>	300	26.7	150	6.7
<i>P. chrysogenum</i>	1400	50	900	46.7
<i>P. corylophilum</i>	100	13.3	600	33.3
<i>P. funiculosum</i>	200	6.7	50	6.7
<i>P. janthellum</i>	350	20	-	-
<i>P. nigricans</i>	550	13.3	600	6.7
<i>Phoma glomerata</i>	50	6.7	50	6.7
<i>Rhizopus stolonifer</i>	300	33.3	50	6.7
<i>Scopulariopsis brevicaulis</i>	50	6.7	-	-
<i>Trichothecium roseum</i>	50	6.7	-	-
Yeasts	4000	93.3	1650	73.3
<i>Total counts</i>	5700	100	3100	93.3
<i>Number of genera = 11</i>	11		6	
<i>Number of species = 20</i>	20		13	

**Table 3:** Total counts (Tc, calculate per 5 eggs), percentage of frequency (F %) of fungal genera and species isolated from shell surface and content of farmers' eggs.

Genera and species	Rinsed shell		Content	
	Tc	F %	Tc	F %
Aspergillus	1300	40	1800	80
A. flavus	1000	26.7	1150	33.3
A. niger	300	13.3	650	46.7
Cladosporium	1450	60	850	40
C. cladosporioides	1100	33.3	250	26.7
C. herbarum	150	13.3	150	13.3
C. sphaerospermum	200	20	450	20
Fusarium solani	100	6.7	-	-
Penicillium	10150	100	5650	93.3
P. aurantiogriseum	250	20	100	6.7
P. chrysogenum	3150	80	2350	66.7
P. corylophilum	2450	53.3	500	26.7
P. isdandicum	900	33.3	550	20
P. lanosum	50	6.7	-	-
P. nigricans	1100	27.7	2150	60
P. requeforti	2250	33.3	-	-
Rhizopus stolonifer	100	13.3	150	13.3
Yeasts	1100	40	800	26.7
<i>Total counts</i>	13100	100	8450	93.3
<i>Number of genera = 5</i>	5		4	
<i>Number of species = 14</i>	14		11	

**Table 4:** Total counts (Tc, calculated per 5 eggs), percentage of frequency (F %) of fungal genera and species isolated from shell surface and content of ducks' eggs.

Genera and species	Rinsed shell		Content	
	Tc	F %	Tc	F %
<i>Acremonium strictum</i>	700	33.3	-	-
<i>Alternaria alternata</i>	100	13.3	-	-
<i>Aspergillus</i>	950	73.3	150	26.7
<i>A. flavus</i>	350	26.7	50	6.7
<i>A. niger</i>	350	33.3	100	13.3
<i>A. ochraceus</i>	100	6.7	-	-
<i>A. terreus</i>	150	6.7	-	-
<i>Cladosporium</i>	1300	73.3	800	53.3
<i>C. cladosporioides</i>	750	60	500	40
<i>C. herbarum</i>	300	33.3	100	13.3
<i>C. sphaerospermum</i>	250	20	200	13.3
<i>Penicillium</i>	4600	100	2300	93.3
<i>P. aurantiogriseum</i>	500	20	-	-
<i>P. chrysogenum</i>	3100	93.3	1550	80
<i>P. citrinum</i>	300	13.3	-	-
<i>P. corylophilum</i>	450	33.3	350	26.7
<i>P. islandicum</i>	50	6.7	100	26.7
<i>P. janthenillum</i>	-	-	50	6.7
<i>P. lilacinus</i>	-	-	50	6.7
<i>P. nigricans</i>	150	20	200	20
<i>P. purpogenum</i>	50	6.7	-	-
<i>Rhizopus stolonifer</i>	200	26.7	50	6.7
<i>Scopulariopsis brevicaulis</i>	650	13.3	-	-
<i>Trichothecium roseum</i>	-	-	50	6.7
Yeasts	2000	53.3	700	20
<i>Total counts</i>	8500	100	3350	100
<i>Number of genera</i>	7		4	
<i>Number of species</i>	18		13	