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MOLDS CONTAMINATING SHELLS AND CONTENTS OF COMMERCIAL HENS AND DUCKS' EGGS IN ASSIUT CITY

(With 4 Tables)

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الفطريات الملوثة لقشر ومحتويات بيض الفراخ والبط التجاري في مدينة أسيوط

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أجريت هذه الدراسة على عدد ٢٢٥ بيضة تمثل ٤٥ عينة عشوائية من الأنواع المختلفة من البيض وهى 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 الميكولوجى أن بيض مزارع الفلاحين أكثر الأنواع تلوثا بالفطريات بينما الأنواع البلدية أقلها تلوثا. وجد أن التعداد الكلى للفطريات على السطح الخارجي(لكل٥بيضة) وكذلك المحتوى الداخلي(لكل٥بيضة) للبيض البلدي وبيض المزارع وبيض البط هو ٥٧٠٠ ، ٣١٠٠ - ١٣١٠٠ ، ٨٤٥٠ و ٨٥٠٠ ، ٣٣٥٠ /قشروملى 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 أسبيرجيللس, بنسيليوم وكلادوسبوريوم تليها الأنواع التابعة لجنسي أكريمونيوم وريزوبس 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 Sivancsan (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 exmined shells were contaminated by mold and yeast (100%) with an average count of 380, 873 and 567 colonies/shell. While, 93.33, 93.33 and100% of the examined egg content of different types were positive for existance 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 8500and 3350/5 shells and ml of content in native breeds, farms and ducks' eggs, respectively. The results declared that, the outer surface of egg hells 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 (11and 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).

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

elevated circulating urea on the vascular walls. Increased urea level was possibly the cause of the widespread degenerative changes in the various parenchymatous tissues. Similar results were reported by Kim *et al.* (1982), Chandra *et al.* (1984a,b) and Javed *et al.* (1995).

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