Middle East Journal of Applied Sciences
ISSN 2077-4613

Volume : 05 | Issue : 04 | Oct.-Dec.| 2015
Pages: 1044-1054

Mycoflora and Mycotoxin Contaminated Chicken and Fish Feeds

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ABSTRACT

Information about fungi associated with food and feeds is important in assessing risk of mycotoxin contamination. Mould contamination not only causes deterioration of food and feeds, but also can adversely affect the health of humans and animals, once they may produce toxic metabolites. Moulds are capable of reducing the nutritional value of feedstuff as well as elaborating several mycotoxins. Mycotoxin-contaminated feed has adverse effects on chicken, fish and animal health and productivity. In point prevalence study feedstuff used for chicken and fish nutrition in Egypt was analyzed for fungal flora and natural incidence of selected my cotoxins. Analyzed fungal flora of chicken and fish feed samples which collected from three different localities i.e. Cairo, Qalubiya and Sharkiya governorates in Egypt yielded 885 fungal isolates. Fish feed samples was higher of total fungal colonies which was about54.6 % compared with chicken feed samples which record 45.4%. Seven fungal species belonging to four fungal genera were isolated and identified from these samples. These genera are Asperigillus (*A. Flavus, A. parasiticus, A. niger* and *A. ochraceous*), *Penicillum, Fusarium* and *Alternaria* spp. Tested of mycotoxin production resulted that, six fungal isolates associated fish feeds were found to be produce one or more mycotoxin i. e. aflatoxin(s), ochratoxin A (OTA) and fumonisin B_1 (FB_1),out of them five fungal isolates of *A. flavus*, one of *A. parasiticus* were found to produce aflatoxin(s), two isolates of *A. ochraceous*, as well as two isolates of *Penicillum* sp.,were found to produce ochratoxin A (OTA) and one isolate of *Fusarium* sp. produce Fumonisin FB_1. While, chicken feed isolates presented that, four fungal isolates of *A. flavus*, one isolate of *A. parasiticus* were found to produce aflatoxin(s) and one isolate of *Penicillum* sp. was found to produce ochratoxin A (OTA).

Key words: Chicken, Fish feeds, Fungi, Mycotoxins, HPLC

Introduction

Information about fungi associated with food and feeds is important in assessing risk of mycotoxin contamination. They are usually in form of dry complex feeds composed of plant (cereal seeds, bran, rapeseed or soybean meal or cake, legume seeds) and animal components (meat-bone and fish meal, poultry off fall meat, powdered milk, animal fats) supplemented with vitamins and minerals (Zmyslowska and Lewandowska, 2000 and Almeida, *et al*., 2011).This type of raw materials has been associated with contaminants produced by moulds during the initial stages of the crop production. During manufacturing, feeds can be contaminated with mould spores, especially when the cereal grains are ground and the feeds are pelleted. Mould contamination not only causes deterioration of food and feeds, but also can adversely affect the health of humans and animals, once they may produce toxic metabolites (Joseph, 1971 and Cole and Cox, 1981).The quality of the products used in feeds for farmed fish has become a limiting factor for activity because these feeds are ideal substrates for the growth of fungi, which, under favorable conditions, may favor the synthesis of mycotoxins. Production of these toxic metabolites can occur during the growth of the crop, during post-harvest storage, or during the storage of the compounded feed (CAST Council for Agricultural Science and Technology 2003).

Mycoflora in the feed was determined. Nine fungi genera were isolated. The most frequently isolated fungi genera in both privately milled and commercial feed was *Aspergillus* spp. which was about 40% of mould isolate. *Penicillium* spp. is 20% in private feed and 13% in commercial feed. A total of 874 fungi were isolated consisting of 458 fungi species in privately milled feed and 416 fungi species found in commercial feed. Mycotoxigenic fungi genera, *Aspergillus*, *Penicillium, Fusarium*, *Alternaria, Cladosporum, Mucor, Rhizopus, Torula* and Yeast were isolated. *Aspergillus flavus* is the commonest isolated fungi species (Ariyo, *et al*., 2013).The fungi contamination in 87 samples finished fish feed samples for sea bass (52 extruded feed and 35 pellet), were *Aspergillus flavus, Aspergillus niger, Aspergillus glaucus, Fusarium, Penicillium* and *Cladosporium*. Fish meal, wheat, sunflower, soybean, and nutritionally complete feeds can also be contaminated with aflatoxins and other

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mycotoxins. The contamination of agriculture commodities with toxigenic fungi under favorable conditions may lead to mycotoxin build-up reaching to injurious levels for farm animals and human health (Almeida, *et al*., 2011). *Aspergillus flavus, Aspergillus parasiticus* and *Aspergillus nomius* are the widespread fungi isolated from a wide range of animal and human foods, which produce highly hepato-carcinogenic aflatoxins (Anjum, *et al*., 2012).

In Egypt and under local markets, there is relatively little information related to the natural occurrence of fungi and mycotoxins in feeds. The investigation aimed to study feedstuff used for chicken and fish nutrition in Egypt, analyzed for fungal flora and natural incidence of selected mycotoxins.

Material and Methods

Collected samples:

Randomly collected of commercial chicken and fish feed samples were collected from three different Governorates i.e. Cairo, Qalubyia and Sharkiya, Egypt.

Mycological analysis:

Isolation and counting of fungal flora:

The dilution-plate method was applied. Randomized samples of chicken and fish feeds were suspended as follow:-

added 10g of each sample with 90ml sterilized water then shacked for 3-5 min and filtered to give 1:100 of dilution and routinely diluted to 1:1000,1:10000.,1:100000 and 1:1000000. Either dilution i.e. 10^4,10^5,$10^6$was used for experiment of fungal isolate**.** Added 1ml of each dilution in sterilized Petri dishes 9cm then mixed with 10ml of sterilized PDA medium and incubated at 26±2 °C for 7-10 days. After incubation period, total fungal count (TFC) and frequency percent of fungi in fish and chicken feeds were recorded.

Fungi were isolated and cultured according to the method described by Pitt and Hocking (1997). About 1 g of the ground grain was diluted in 9 ml of sterile distilled water which was followed by five other serial dilutions. One mil liter of the extract was placed at random in each of the Petri-dishes containing potato dextrose agar (PDA). These plates were then incubated at 25± 2°C for five days. The isolation frequency (Fq) of fungi was recorded as recorded by Bensassi *et al*., (2011) as follow:

$$Fq(\%) = \frac{\text{Number of isolates of a genus}}{\text{Total number of fungi or genera}} \times 100$$

Purification of fungal isolates:

All devolving fungi were purified on plates of PDA by using hyphal tip or single spore techniques (Maliha *et al*., 2010).

Identification of fungal isolates:

All fungal isolates were identified in Plant Pathology Dept., National Research Centre, (NRC), Cairo, Egypt. These fungi were observed under a microscope and identification with the help of available literature by Raper, and Fennell, (1965) and Ainsworth, *et al*., (1972), Biligrami *et al*., (1991) and Barnett and Hunter, (1999).Different frequencies of fungi were noted. Species of *Aspergillus, Penicillium* and *Fusarium* were identified according to Klich(2002) and Nelson *et al*. (1983).

Mycotoxin production:

The different fungal isolates were propagated as pure culture in yeast extract sucrose (YES) which containing 2% yeast extract and 15% sucrose / liter distilled water to produce mycotoxin according to Munimbazi and Bullerman (1998). Each flask was inoculated with 0·1 ml of a spore suspension containing approximately 10^6 spores ml^{-1}. Cultures were incubated at 26±2 °C for 14 days for *Aspergillus* and *Penicillium* and 21 days for *Fusarium* isolates.

Determination of aflatoxins

Determination of aflatoxins was carried out by using HPLC according to (AOAC, 2007). The HPLC instrument used was waters (474) system, equipped with quaternary pump. The fluorescence detector system was set at 360 nm excitation and 440 nm emission wavelengths. The chromatography column was phenomenex c_{18} (250x 4.6 mm), 5 µm. The mobile phase system (H_2O: MeOH: CH_3CN, 30:60:10 v/v/v) was isocratic-ally at flow rate of 1 ml /min. The data were collected and integrated using Totalchrom Navigator Chromatography Manager Software. According Han, *et al*., (2004), AOAC, (2007) and Embaby, *et al*., (2007 and 2012).

Fusarium mycotoxins assay:

The samples were analyzed using the Fumonisin test (FB_1) by using HPLC, (Ammar, and El-Naggar, 2014).

Penicillium mycotoxins assay:

Penicillium was tested by using HPLC. Official method of the Association of Official Analytical Chemists (AOAC, 2007) for the analysis of mycotoxins was carried out.

Ochratoxin analyses in vitro:

Fungi were grown in 50 ml of liquid medium in 125-ml Erlenmeyer flasks. Yeast extract-sucrose broth (YES; 2% yeast extract, 15% sucrose) was used (Bayman, *et al*., 2002). Cultures were incubated for 10 days at 30°C in the dark. Then 2 ml of culture fluid was removed from each flask, filtered through a 0.2-µm syringe filter, and extracted with 2 ml of chloroform. The organic phase was collected, evaporated, and re-suspended in 500 µl of methanol. Then 20 µl was injected into a high-performance liquid chromatograph (HPLC). The HPLC was run on a VYDAC 218TP54 C_{18} column (4.6 by 250 mm; VYDAC/The Separations Group, Inc., Hesperia, Calif.), with methanol-H_2O-H_3PO_4 (87%), 70:30:0.1, as the mobile phase and a flow rate of 1 ml/min. Excitation was at 333 nm, with detection at 418 nm. Peak areas were calculated from a standard curve based on concentrations from 0.005 to 15 µg/ml of an ochratoxin A standard (Sigma Chemical Co., St. Louis, Mo).

Results

Percentage of total fungal count associated chicken and fish feed collected from different governorate in Egypt

Total fungal count associated collected samples of chicken and fish feeds which collected from three different governorates i.e. Cairo, Qalubiya and Sharkiya were recorded in Table (1). Isolation from chicken and fish feed samples yielded 885 fungal colonies. Fish feed samples was higher total fungal colonies compared with chicken feed samples. Fish feed samples resulted 483 of total fungal colonies equal 54.6 % while, Fish feed samples yielded 402 of total fungal colonies equal 45.4%.On the other hand, Sharkiya governorate was the highest total fungal counts which gave 388 isolates equal 43.8% of fungal frequency followed by Qalubiya with 270 isolates equal 30.5%. Cairo governorate recorded the lowest fungal counts which record 227 isolates equal 25.7%.

Table 1: Total fungal count associated of chicken and fish feed samples

Governorate	Type of feeds				Total	
	Chicken		Fish		T.C.	%
	T.C.	%	T.C.	%		
Sharqiya	150	17	238	26.9	388	43.8
Qalubiya	134	15.1	136	15.4	270	30.5
Cairo	118	13.3	109	12.3	227	25.7
Total	402	45.4	483	54.6	885	100

T.C. = Total fungal colony

Fungal frequency contaminated chicken feeds Collected from three different localities in Egypt

Fungal frequency contaminated chicken feed samples which collected from three different localities i.e. Cairo, Qalubiya and Sharkiya governorates in Egypt were recorded in Table (2). Data in this table show that four hundred and two fungal isolates were isolated then identified to seven fungal species belonging to four fungal genera. These genera are Asperigillus (*A. Flavus, A. parasiticus, A. niger* and *A. ochraceous*), *Penicillum, Fusarium* and *Alternaria* spp. Also data in the same table presented that *Aspergillus* spp. was the most fungal frequency compared with others. *A. flavus* was the most fungal frequency record225 isolates equal 56% followed by genus *Penicillium* with 76 isolates equal 19% and *Fusarium* with 21 isolates equal 5.2%. *Alternaria* genus was less fungal frequency contaminated chicken feed samples which gave 9 isolates equal 2.2%.

Table 2: Fungal frequency contaminated chicken feeds

Fungi	Sharqiya		Qalubiya		Cairo		Total	
	T.F	%	T.F	%	T.F	%	T.F	%
A. flavus	60	14.9	87	21.6	78	19.4	225	56
A. parasiticus	23	5.7	5	1.24	23	5.7	51	12.7
A. niger	8	2	2	0.5	1	0.3	11	2.7
A. ochraceous	2	0.5	5	1.24	2	0.5	9	2.2
Penicillium sp.	34	8.5	28	7	14	3.5	76	19
Fusarium sp.	14	3.5	7	1.74	NF	0	21	5.2
Alternaria sp.	9	2.2	NF	NF	NF	NF	9	2.2
Total	150	37.3	134	33.3	118	29.4	402	100

T.F = Total frequency NF = Not found

Fungal frequency associated fish feed samples collected from three different localities in Egypt

Fungal frequency associated fish feeds which collected from three different localities i.e. Cairo, Qalubiya and Sharkiya governorates in Egypt were recorded in Table (3). Data in this table presented that, Seven fungal species belonging to four fungal genera were identified. These are *Asperigillus* (*A. flavus, A. parasiticus, A. niger* and *A. ochraceous*) *Penicillium, Fusarium* and *Alternaria. Aspergillus* genus was the most fungal frequency compared with others. *A. flavus* was the most fungal frequency which record166 isolates equal 34.4%, *Penicillium* with 118 isolates equal 24.4% and *Fusarium* gave 26 colonies equal 5.4%. *Alternaria* was less fungal counts which gave 17 isolates with 3.5%.

Table 3: Fungal frequency contaminated fish feeds

Fungi	Sharqiya		Qalubiya		Cairo		Total	
	T.F	%	T.F	%	T.F	%	T.F	%
A. flavus	86	17.8	20	4.14	60	12.4	166	34.4
A. parasiticus	45	9.3	47	9.73	18	3.7	110	22.8
A. niger	14	2.9	2	0.41	3	0.6	19	3.9
A. ochraceous	27	5.6	NF	NF	NF	NF	27	5.6
Penicillium sp.	41	8.5	54	11.2	23	4.8	118	24.4
Fusarium sp.	8	1.7	13	2.7	5	1	26	5.4
Alternaria sp.	17	3.5	NF	NF	NF	NF	17	3.5
Total	238	49.3	136	28.2	109	22.5	483	100

T.F = Total frequency NF = Not found

Mycotoxins production from some mycoflora contaminated chicken feed samples.

Identification and determination of aflatoxins could easily be deduced from the constant retention time compared with the standard spiked in the HPLC chromatogram (Fig. 1). Data in table (4) presented that Qalubiya sample was higher of aflatoxin concentrate (ng/ml) than others. Five fungal isolates were found to be produce one or more aflatoxins. Four fungal isolates which were identified as *A. flavus.* Both isolate No. 1, 2 from Qalubiya samples were found to be produce 67.5 and 0.128 ng / ml of aflatoxin AFB_1 respectively (Fig. 2). *A. flavus* isolate No 3 from Sharkiya sample was found to be produce 0 0.054 ng/ml of aflatoxin AFB_1 and isolate No 4 from Cairo sample was found to be produce 0 0.143 ng / ml of aflatoxin AFB_1 (Fig. 3). *A. parasiticus* No. 5 from Qalubiya sample was found to produce AFB_1, AFB_2, AFG_1 and AFG_2 which produce 39.6, 0.527, 0.484 , 0.26 ng / ml (Fig. 4). On the other hand identification and determination of ochratoxin A (OTA) could easily be deduced from the constant retention time compared with the standard spiked in the HPLC chromatogram (Fig. 5). One isolate of *Penicillium* sp. from Cairo chicken feed sample was found to produce ochratoxin A (OTA) at 9.63 ng / ml (Fig. 6).

Table 4: Mycotoxins contaminated chicken feed samples

Sample	Location	Type of fungi	Type of mycotoxin	Mycotoxin conc. (ng/ml)				
				AFB_1	AFB_2	AFG_1	AFG_2	Total
1	Qalubiya	*A. flavus*	Aflatoxins Afs	67.5	ND	ND	ND	67.5
2	Qalubiya			0.128	ND	ND	ND	0.128
3	Sharkiya			0 054	ND	ND	ND	0 .054
4	Cairo			0.143	ND	ND	ND	0.143
5	Qalubiya	*A. parasiticus*		39.6	0.527	0.484	0.26	40.87
6	Cairo	*Penicillium*sp.	OchratoxinA (OTA)	9.63				9.63

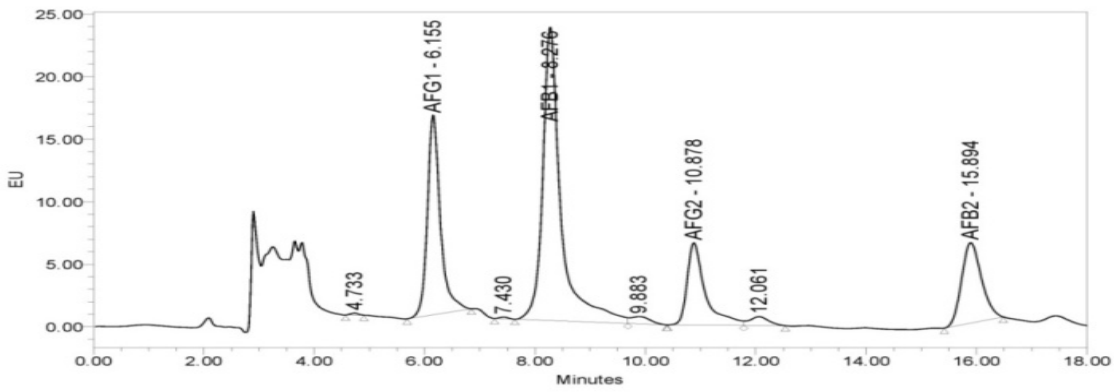

Fig. 1: Standard spiked in the HPLC chromatogram of aflatoxins AF G_1, B_1, G_2& B_2 .

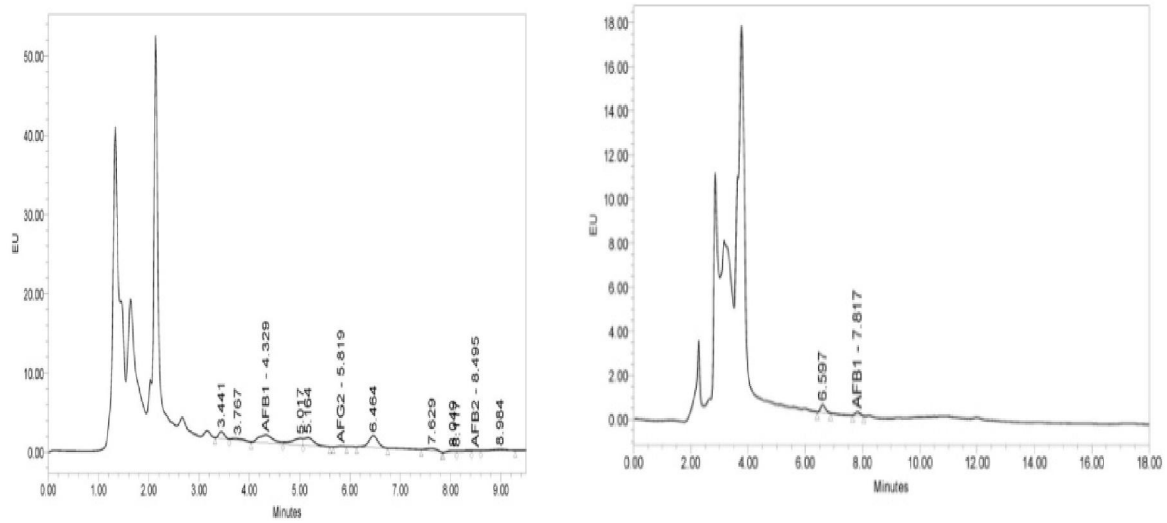

Fig. 2: HPLC chromatogram of aflatoxins produced by *Aspergillus flavus* (isolate No. 1(Left) & isolate No.2 (Right) isolated from chicken feed Qalubiya samples .

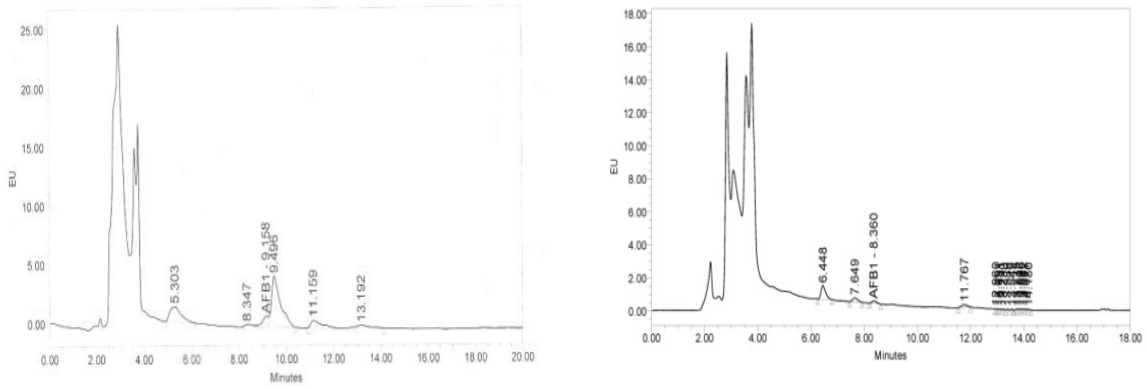

Fig. 3: HPLC chromatogram of aflatoxins produced by *Aspergillus flavus*(No. 3) isolated from chicken feed Sharkiya sample(Left) *and Aspergillus flavus* (No. 4) isolated from chicken feed Cairo sample (Right).

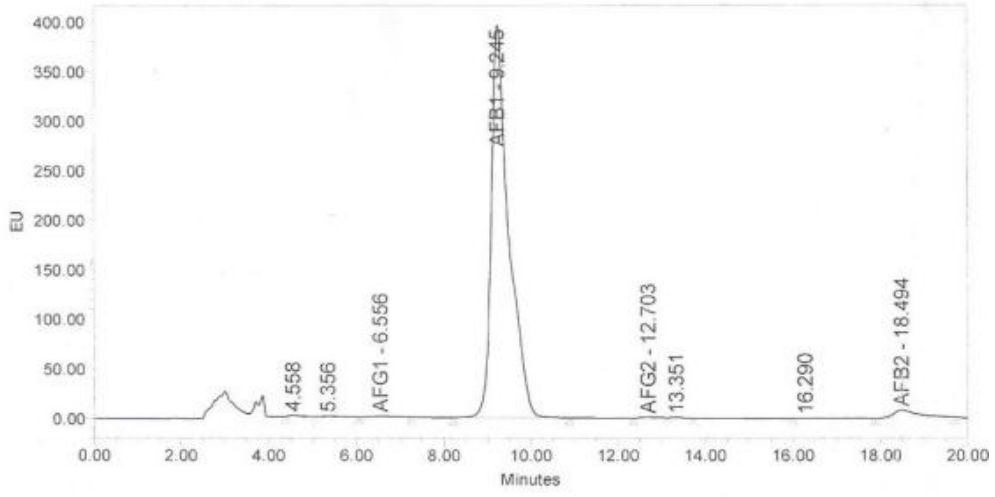

Fig. 4: HPLC chromatogram of aflatoxins produced by *Aspergillus parasiticus* (No. 5) isolated from chicken feed Qalubiya sample.

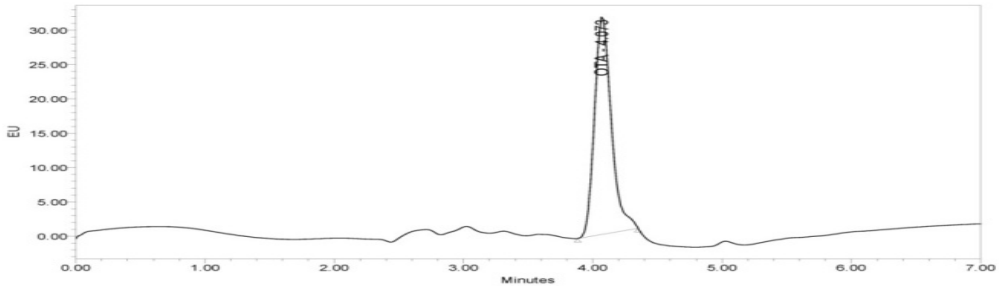

Fig. 5: Standard spiked in the HPLC chromatogram of ochratoxin A (OTA).

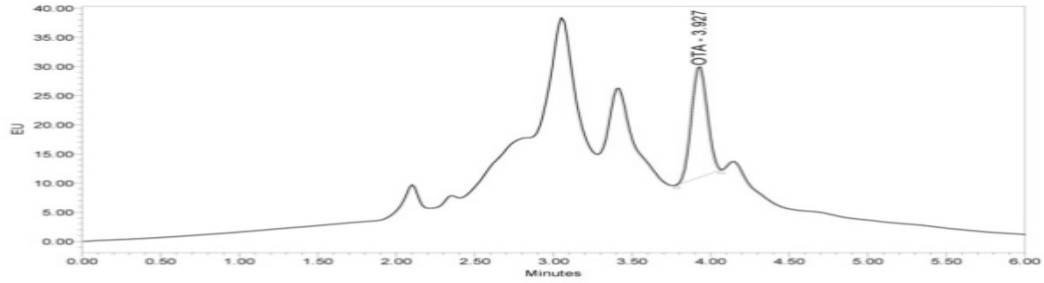

Fig. 6: HPLC chromatogram of ochratoxin A (OTA) produced by *Peicillium* sp. (No. 6) isolated from chicken feed Cairo sample.

Mycotoxins production from some mycoflora contaminated fish feed samples

Identification and determination of aflatoxions could easily be deduced from the constant retention time compared with the standard spiked in the HPLC chromatogram (Fig.7). Determination of aflatoxins produced by *Aspergillus flavus* and *A. parasiticus* after inoculated artificially and incubated for two weeks at 28 ± 2ºC were tabulated in Table (5).

Data in this table presented that, tested of mycotoxin production resulted that six fungal isolates were found to be produced one or more aflatoxin(s), out of them five fungal isolates which were identified as *A. flavus* and one as *A. parasiticus*. *A. flavus* isolate No. 1 from Sharkiya sample was found to be produce 1.5 ng / ml of aflatoxin AFB_1 and *A. flavus* isolate No. 2 from Cairo sample produce 1.4 ng / ml of aflatoxin AFB_1 (Fig. 8). *A. flavus* isolate No. 3 from Cairo sample was found to produce 0.05 ng/ml of aflatoxin AFB_1, *A. flavus* isolate No. 4 from Qalubiya sample was found to be produce 40.0 ng/ml of aflatoxin AFB_1 (Fig. 9), isolate No. 5 from Qalubiya sample was found to be produce 0.018 ng / ml of aflatoxin AFB_1 and *A. parasiticus* No. 6 from Qalubiya fish feed samples was found to produce AFB_1, AFB_2, AFG_1 and AFG_2 which produced 1.91, 0.018,0.038 and 0.027 ng / ml respectively equal 2.155 ng/ml of total aflatoxins (Fig. 10). *Fusarium* sp. isolate No. 7 from Sharkiya fish feed sample was found to produces Fumonisin FB_1 with 0.412 ng / ml (Fig. 11). Higher aflatoxin concentration was determined with Qalubiya fish feed sample, while Sharkiya sample was higher with Fumonisin FB_1 and ochratoxin A (OTA).

On the other hand, Identification and determination of ochratoxin could easily be deduced from the constant retention time compared with the standard spiked in the HPLC chromatogram (Fig. 12), two isolates of *Penicillium* sp. No. 8 & 9 from Qalubiya and Cairo fish feed samples were found to produce ochratoxin A (OTA) at 0.025 ng / ml and 0.03 ng / ml respectively (Fig. 13). While *A. ochraceous* No. 10 & 11 isolates of Sharkiya sample were found to be produce 125.92 ng / ml and 4.44 ng / ml of ochratoxin A (OTA) respectively (Fig. 14).

Table 5: Mycotoxins contaminated fish feed samples

Sample		Type of		Mycotoxin conc. (ng/ml)				
No	Location	fungi	mycotoxin	AFG_1	AFB_1	AFG_2	AFB_2	Total
1	Sharkiya	*A. flavus*	Aflatoxin Afs	ND	1.5	ND	ND	1.5
2	Cairo			ND	1.4	ND	ND	1.4
3				ND	.05	ND	ND	0.05
4	Qalubiya			ND	40.0	ND	ND	40.0
5	Qalubiya			ND	0 .018	ND	ND	0.018
6	Qalubiya	*A. parasiticus*		0.027	1.91	0.038	0.018	2.155
7	Sharkiya	*Fusarium* sp.	Fumonisin FB_1	0.412				0.412
8	Qalubiya	*Penicillium* sp.	Ochratoxin OTA	0.03				0.03
9	Cairo			0.025				0.025
10	Sharkiya	*A. ochraceous*		125.92				125.92
11				4.44				4.44

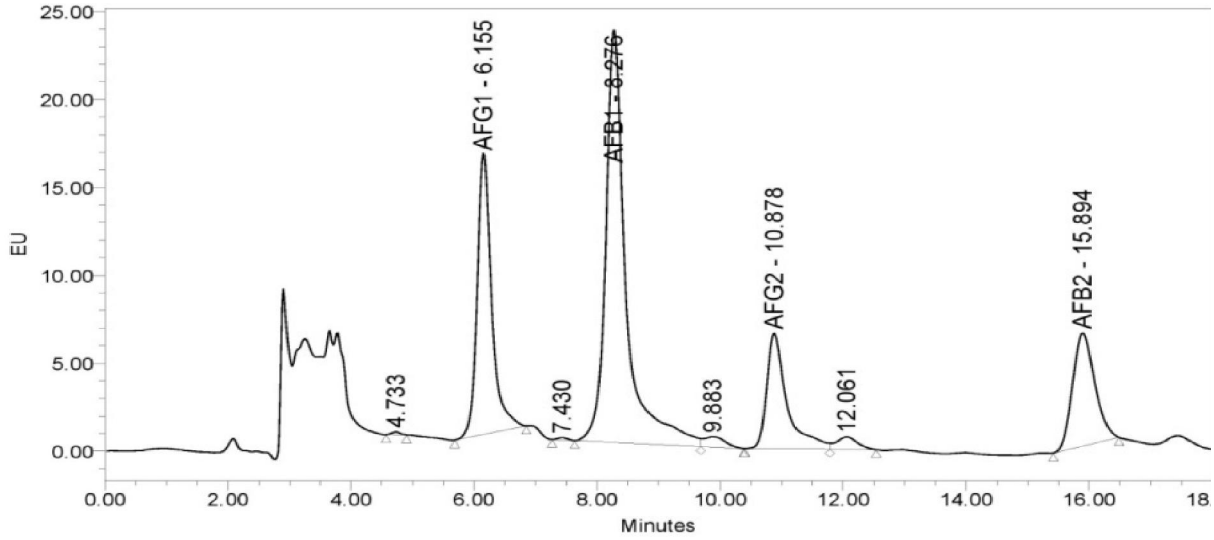

Fig. 7: Standard spiked in the HPLC chromatogram of aflatoxins AF G_1, B_1, G_2& B_2.

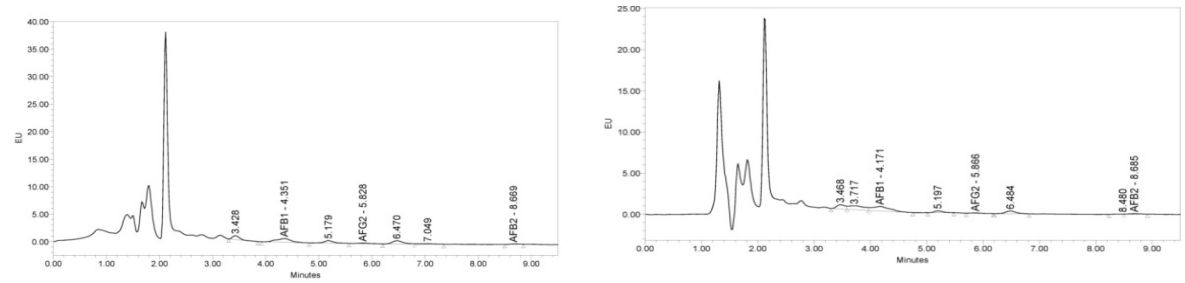

Fig. 8: HPLC chromatogram of aflatoxins produced by *Aspergillus flavus* (No. 1) isolated from fish feed Sharkiya sample (Left) and *Aspergillus flavus* (No.2) isolated from fish feed Cairo sample (Right).

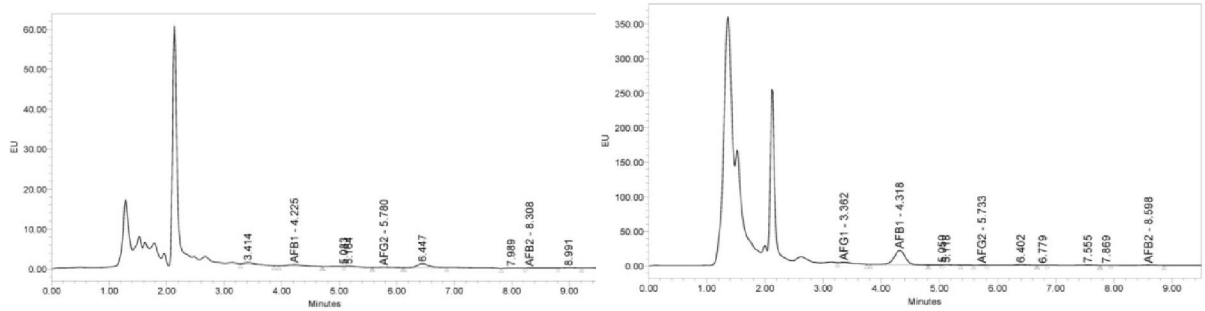

Fig. 9: HPLC chromatogram of aflatoxins produced by *Aspergillus flavus* (No.3) isolated from fish feed Cairo sample (Left) and *Aspergillus flavus* (No.4) isolated from fish feed Qalubiya sample (Right).

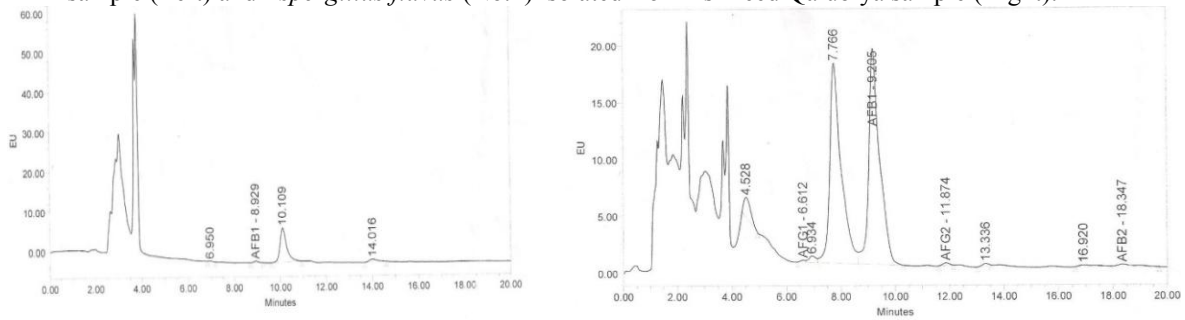

Fig. 10: HPLC chromatogram of aflatoxins produced by *Aspergillus flavus* (No.5) isolated from fish feed Qalubiya sample (Left) and *Aspergillus parasiticus* (No.6) isolated from fish feed Qalubiya sample(Right).

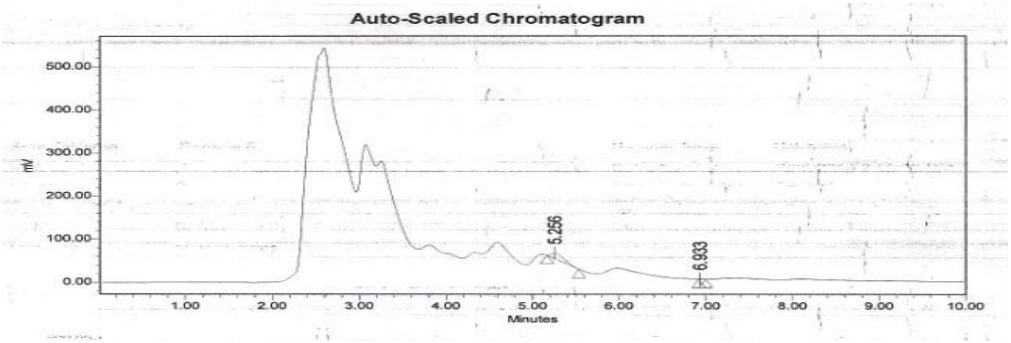

Fig. 11: HPLC chromatogram of fumonisin produced by *Fusarium* (No.7) isolated from fish feed Sharkiya sample

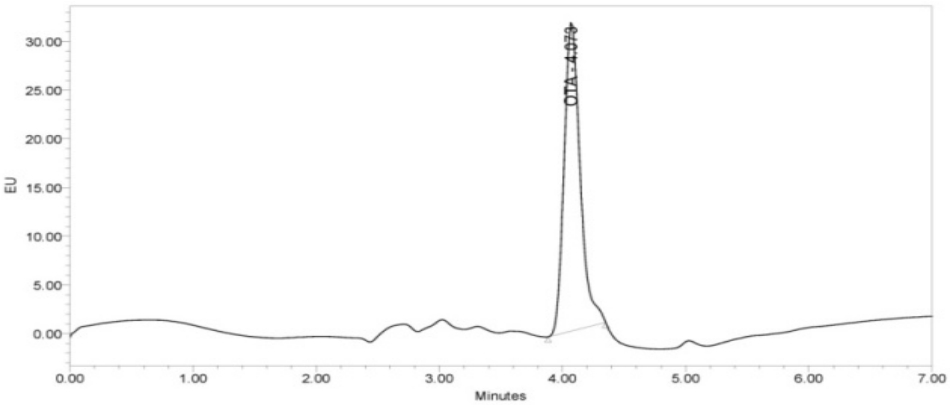

Fig. 12: Standard spiked in the HPLC chromatogram of ochratoxin A (OTA).

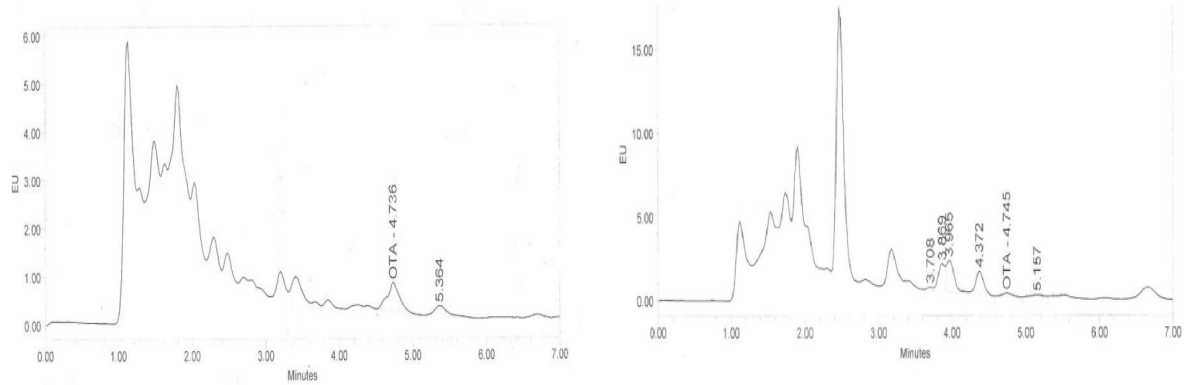

Fig. 13: HPLC chromatogram of ochratoxin A (OTA) produced by *Penicillium* sp.(No. 8) isolated from fish feed Qalubiya sample (Left) and *Penicillium* sp. (No. 9) isolated from fish feed Cairo sample (Right).

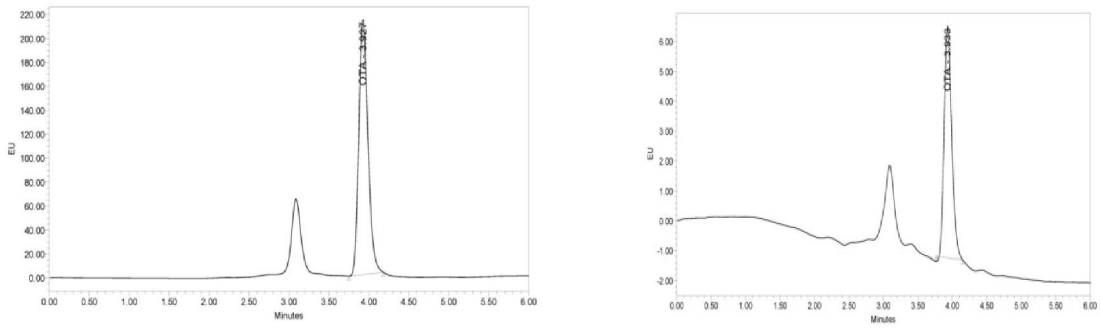

Fig. 14: HPLC chromatogram of ochratoxin A (OTA) produced by *Aspergillus ochraceous* No. 10 (Left) & 11(Right) isolated from fish feed Sharkiya sample .

Discussion

Information about fungi associated with food and feeds is important in assessing risk of mycotoxin contamination. Mycotoxin-contaminated feed has adverse effects on chicken, fish and animal health and productivity. Analyzed fungal flora of chicken and fish feed samples which collected from three different localities i.e. Cairo, Qalubiya and Sharkiya governorates in Egypt yielded 885 fungal isolates. Fish feed samples was higher of total fungal colonies which was about 54.6 % compared with chicken feed samples which record 45.4%. Four fungal genera belonging to seven fungal species were isolated and identified from these samples. These genera are *Asperigillus* (*A. Flavus, A. parasiticus, A. niger*and *A. ochraceous*), *Penicillum, Fusarium* and *Alternaria* spp.

Similar results were obtained by many investigators Krnjaja, *et al*., (2008), Anjum, *et al*., (2012) and Ariyo, *et al*., (2013) stated that, the most prevalent fungal genera isolated from poultry feeds were *Fusarium* (56.09%) and *Aspergillus* (54.35%), followed by *Rhizopus* (40%), *Penicillium* (30.87%), *Mucor* (30.04%) and the least frequency species were from genus *Alternaria* (3.48%). Also, Almeida, *et al*., (2011) and Anjum, *et al*., (2012); reported that, the fungi contamination in 87 samples finished fish feed samples for sea bass (52 extruded feed and 35 pellet), were *Aspergillus flavus, Aspergillus niger, Aspergillus glaucus, Fusarium, Penicillium* and *Cladosporium*.

Tested of mycotoxin production of chicken feed isolates resulted that, four fungal isolates of *A. flavus*, one isolate of *A. parasiticus* were found to produced aflatoxin (s) and one isolate of *Penicillum* sp. was found to produce ochratoxin A (OTA). While, six fungal isolates from fish feeds were found to be produced one or more mycotoxin i. e. aflatoxin(s), ochratoxin A (OTA) and fumonisin B_1 (FB_1), out of them five fungal isolates of *A. flavus*, one of *A. parasiticus* were found to produce aflatoxin(s), two isolate of *A. ochraceous*, two isolates of *Penicillum* sp., were found to produce ochratoxin A (OTA) and one isolate of *Fusarium* sp. produce Fumonisin FB_1.

Different researchers demonstrated the presence of aflatoxin B_1 (AFB_1) in shrimp and fish feed (Abdel-hamid, *et al*. 1998). Aflatoxin was the first of the mycotoxins to be investigated in aquaculture. As in other animal species, aflatoxin exerts carcinogenic effects in fish (Spring and Fegan 2005). There are several studies demonstrating the toxic effects of ochratoxin A (OTA) toxin in different fish species (Manning etal.2003). Several *Aspergillus* and *Penicillium* species have been described as producers of ochratoxin A (OTA). Ochratoxin contamination of foods and feeds poses a serious health hazard to animals and humans (Varga, *et al*., 2009 & 2010). Overall incidence of AFB_1 in feed ingredients was 60 percent. The average contamination and maximum levels of AFB_1 were 37.62 and 56 µg/kg, respectively, whereas, the incidence in poultry feed samples was 44.39 percent with average contamination and maximum levels of 23.75 and 78 µg/kg, respectively. However, maximum level of AFB_1 was higher (78 µg /kg) in poultry feed samples as compared to feed ingredients (56 µg /kg) (Anjum, *et al*., 2012). Fumonisin is of concern to the aquaculture industry because it commonly contaminates corn and its by-products. There are several publications on the toxic effects of fumonisin B_1 (FB_1) in different fish species (Yildirim *et al*. 2000 and Nguyenetal.2003). The fungal genera *Aspergillus, Fusarium, and Penicillium* were isolated and identified from feed intended for fish farms. Fumonisin B_1 (FB_1), aflatoxin B_1 (AFB_1), and ochratoxin A (OTA) were determined (Barbosa, *et al*., 2013). Also, Greco, *et al*., (2014) found that, Fumonisins were detected in all the samples of feedstuff (median 1,750 ppb). Forty-four out of 49 samples (90%) were contaminated with deoxynivalenol (DON) (median 222 ppb) and ochratoxin OTA (median 5 ppb). Also, 44 out of 49 samples were contaminated with aflatoxins (median 2.685 ppb), 42 samples (86%) with zearalenone (ZEN) (median 50 ppb), and 38 samples (78%) with trichothecene (T-2) (median 50 ppb). Ninety percent of the samples had at least one type of nutritional deficiency. This study indicates the need for continuous assessment of the mycological status of animal feed production, in order to feed animals for optimal performance ensuring food safety.

Conclusion

Moulds are capable of reducing the nutritional value of feedstuff as well as elaborating several mycotoxins. The mycotoxin produced by the fungi poses a serious economic impact worldwide. The economic impact result from lowered productivity reduced feed conversion efficiency which causes reduced weight gain and less meat production, increased disease incidence because of immune-suppression, damage to organs in the body. The presence of microscopic fungi affects the quality of feeds, their organoleptic attributes, and nutritional quality**.** In addition to their negative impact on nutritional and organoleptic properties, moulds can synthesize different mycotoxins. Mycotoxin-contaminated feed has adverse effects on chicken and fish health and productivity. Consumption of a mycotoxin-contaminated diet may induce acute and long-term chronic toxic effects**.** HPLC is the most frequently used technique for the measurement of main mycotoxins occurring than other techniques.

Recommendation

Good quality of the products used and proper hygiene of the technological processes decrease the risk of microbiological contamination of chicken and fish feeds. Storage conditions, especially temperature and humidity represent another important factor affecting microbiological quality of feeds.

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