ISOLATION AND CHARACTERIZATION OF FUNGI AND MYCOTOXINS (DEOXYNIVALENOL AND ZARALENONE) IN FISH FEED FROM BAGHDAD CITY.

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ABSTRACT

This research provides a brief review of approaches for the early detection of fungi and its metabolites in feedof fish from some Baghdad farms. During a mycological analysis of complete feed mixes(15 samples), a total of five genera of moulds were identified. *Penicillium* spp. was present in considerably more samples than any other genus 36.4%, followed by the genera *Fusarium* spp.24.5%. Other fungi from the genera *Aspergillus* spp. 20%, *Mucor* spp. 11.1% and *Alternaria* spp. 8% were represented in a smaller amount.The mycotoxinsdeoxynivalenol and zearalenone were detected. Deoxynivalenol was detected in 10 samples in the concentration range 0.25–2.5 mg/kg. Zaralenone were detected in 8 samples in the concentration range 0.2–5.0 mg/kg.Thesefindings indicate that there may be a risk for animal exposure to mycotoxins through the consumption of moldy infected feeds.

Key words:*Fusarium*mycotoxins, Deoxynivalenol, Zaralenone, Fungal.

INTRODUCTION

The contamination of agricultural commodities with fungi able to produce toxic metabolites is a worldwide concern. Discoloration, quality deterioration, reduction in commercial value and mycotoxin production has been linked to moldy contaminated foods and feeds (Pardo *et al.,* 2005). Mould contamination not only generates great economic losses, but also represents a threat to human and animal health, particularly through the synthesis of mycotoxins (Mishra and Sopori, 2012).

Mycotoxins are a structurally diverse group of mostly small molecular weight compounds, produced by the secondary metabolism of fungi that contaminate the whole food chain, from the harvested products to the plate of consumers. Mycotoxins occur sporadically both seasonally and geographically (Pestka, 2011). The main mycotoxin classes of concern produced by fungi in the genera *Aspergillus*, *Penicillium* and *Fusarium* include the aflatoxins, ochratoxin A, trichothecenes and fumonisins(Danicke *et al.,* 2011). Environmental factors

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such as nutrients, light, temperature, pH and water activity (aw), either as single factors or in combination, are known to control mycotoxin production in many filamentous fungi (Georgianna and Payne, 2009). These environmental factors typically exert their influence on mycotoxin biosynthesis atthe level of gene transcription(Mostrom and Raisbeck, 2012). Limited information exists regarding the effects of low levels of multiple mycotoxins in fishes. It has been suggested that combination of mycotoxins at low concentrations may have negative effects on animals, even though the concentrations of individual mycotoxins are well below concentrations reported to cause negative effects. Therefore, rapid and specific detection of mycotoxogenicmoulds is important for ensuring both microbiological quality and safety of both feed and food (Sultana and Hanif, 2011).Deoxynivalenol (DON) is one of the most common *Fusarium* based mycotoxins found in human foodstuffs. It is also called vomitoxin, because of its impact on farm animals consuming contaminated feed. DON is much more frequently found in barley, corn, sunflower, wheat, and compound animal feeds. The fungal pathogens producing DON cause ear rot in corn and head blight in wheat, two of the most common sources of DON in the feed supply. Rain during the flowering period in small grains clearly increases the risk of DON contamination (Mosse, 2012). Zearalenone (ZON) is a secondary metabolite of *Fusarium*species. Unlike other mycotoxins, zearalenone is virtually non-toxic to mammals following acute ingestion(Shekhany, 2008). Nonetheless, it is extremely potent in other ways, since it resembles a key hormone produced by human ovaries, 17β- estradiol, and as a result, can disrupt the human endocrine system (Dombroski, 2012). As little as 0.0001 ppm of zearalenone has been shown to create a detectable, hormone-related uterogenic response in females (Bennett and Klich, 2003). In other words, zearalenone exposure has been shown to impact reproductive processes at a dose that is 100 million times less than the lethal dose in a mice LD_{50} study (Mosse, 2012).Therefore, the aims of this research were to: Isolation of fungi and detection of mycotoxins (Deoxynivalenol and Zearalenone) in fish feeds.

MATERIAL AND METHODS

Sample collection

The research materials consisted of 15 representative fishfeed samples which were collected directly at fish farms from different parts of Baghdad city during a 2 month period.The samples (each about 1 kg) were stored at -4˚C andanalyzed the day after collection.

Material

Standards of zearalenone(ZON) and deoxynivalenol (DON) were purchasedfrom Sigma. All othersolvents and reagents were analytical grade.

Isolation and identification of fungal strains

Isolation and identification of fungal strains were done on solid media using the potato dextrose agar (PDA). Plates were incubated at 25˚C for 7 days. Each isolated mould colony was observed microscopically for morphological characterization and identification to genera/species level. This was done by their macro- and micro morphology features using appropriate identification keys (Samson *et al.*, 2002).

Extraction and Cleanup of Mycotoxins

The procedure described by Sreenivasa*et al.*,(2009) was used for extraction and clean up ofmycotoxins.

Mycotoxin analysis

Analysis of twomycotoxins in all samples was performed by High Performance Liquid Chromatography (HPLC). The optimized instrumental conditions are summarized in Table 1.A standard chromatogram of the Fusariummycotoxins of major interest is shown in Figure 1.The mycotoxin concentration in the samples was calculated by comparing the area of chromatographic peak of the samples with that of the standard calibration curve by densitometry analysis.

Table 1.*Instrumental conditions of HPLC.*

Instrumental conditions		
LC Column	Shimadzu Shim 150 mm x 2 mm x5µm	
Flow-rate	0.1mL/min	
Detection wavelength	230 nm	
Injection volume	10µL	
Column temperature	40 °C	
Mobile phase	10mM ammonium acetate – methanol	
Time (minutes)	10mM ammonium acetate %	methanol %
0	90	10
5	50	50
20	0	100

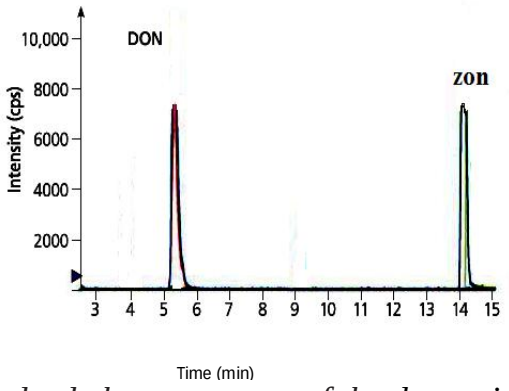

Figure 1. *Standard chromatogram of the deoxynivalenol (DON) andzearalenone(ZON)mycotoxins.*

Statistical analysis

Differences in the mean levels of mycotoxin contamination across the two groups of positive samples were calculated by a Student's *t*-test.

RESULTS AND DISSCUSION

The results obtained from the mycoflora analysis and incidence of mycotoxins contamination in thesamples originating from the region where the sampleswere collected is presented in Table 2 and Figure2.

Mycoflora analysis

The most frequently isolated fungus was *Penicillium* spp., a 36.4%. Other frequently isolated moulds included *Fusarium*spp. 24.5%,*Aspergillus*spp. 20%, *Mucor* spp.11.1% and *Alternaria*spp. 8% were represented in smaller amount Figure 2.

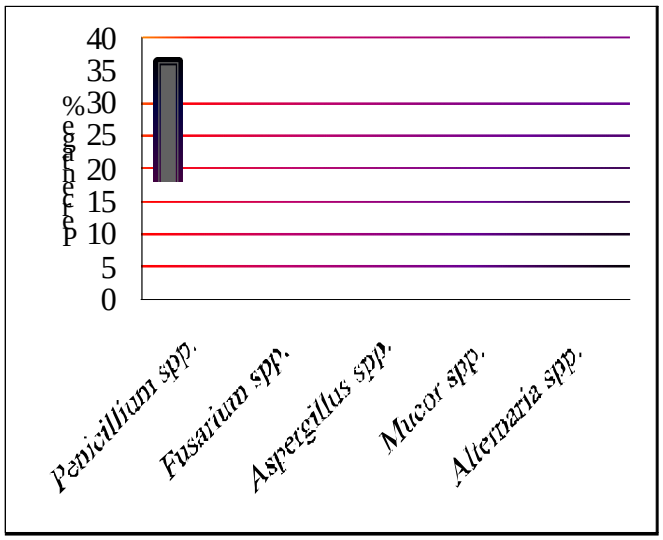

Figure 2. *Percentage of moulds contamination fish feed.*

Occurrence of mycotoxins in samples

The results obtained from the analysis of mycotoxins in the fish feed samples are presented in Table 2. The predominant mycotoxin for all analyzed samples was DON. The incidence of DON and ZON in all the samples was 51.7% and 48.3%, respectively figure 3. No significant differences were found between the median DON and ZON contents for all feed items ($P<0.05$).

Table 2. *HPLC data for occurrence of mycotoxins in fish feeds***.**

Mycotoxins	Positive samples (Incidence %)	Rang (mg/kg)	mean ± SD* (mg/kg)
DON	10 (51.7%)	0.25-2.5	0.78 ± 0.85[a]
ZON	8 (48.3%)	0.2-5.0	0.85 ±1.416[a]

* SD Standard deviation

Means with same letters within the same column are no significantly differences.

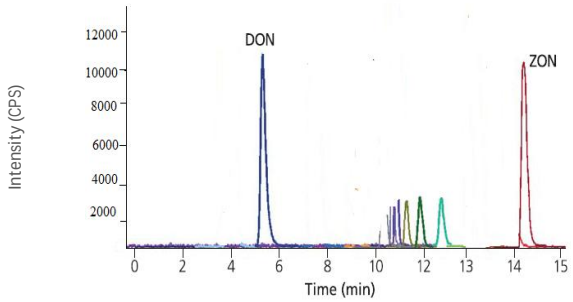

Figure 3.*Samples chromatogram of the deoxynivalenol (DON) andzearalenone (ZON) mycotoxins.*

Isolated species in our case are mostly storage contaminators, implicating that the high number of contaminated feed is most probably the result of manipulative mistakes (storage duration, temperature and humidity levels, etc.), during storage of feedstuffs or feed. Fungal colonization, growth and synthesis of toxins, results from the complex interaction of several factors (water availability, temperature and incubation time) and therefore, an understanding of each factor involved is essential for understanding the overall process and predicting fungal spoilage in agricultural and food products (Pardo*et al.*, 2005). Improper storage accompanied by too high a temperature and elevated moisture content in the grain favour further mycotoxin production and lead to reduction in grain quality (Ramirez *et al.*, 2004). It is well known that cereal infection with moulds and toxin production depends strongly on environmental conditions (damp climate, cool temperatures). However, these data must be interpreted with caution, as they were calculated from a limited number of

samples. The results of the mycoflora analysis carried out in this study are similar to previous results found by other authors (Stankovic*et al.*, 2007;Milicevic, 2008). This shows that the incidence of these various species was important, as the produce was stored for prolonged periods of time.The main advantages of the HPLC technique include its general applicability to a broad range of compounds, high sensitivity and outstanding selectivity. Several methods already have been reported for the simultaneous determination of mycotoxins, which offer significant advantages over conventional techniques (Scarlett *et al.*, 2012).

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عزل وتصنيف الفطريات والسموم الفطرية (الديوكسينيفالينول و الزيرالينون) في علائق الاسماك لمدينة بغداد.

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المستخلص

استعرض هذا البحث مختصراً عن تحديد الفطريات وسمومها في خمسة عشر نموذجاً من اعلاف الاسماك من بعض المزارع السمكية لمدينة بغداد خلال مدة شهرين. حيث تم عزل خمسة أجناس من الفطريات وبنسب مختلفة، حيث وجد ان جنس *Penicillium* spp. كان الغالب في النماذج وبنسبة ٣٦.٤% يتبعه جنس*Fusarium* spp. وبنسبة ٢٤.٥%.أما جنس*Aspergillus* spp.فكان بنسبة ٢٠% ثم جنس*Mucor* spp. بنسبة ١١.١% وحل جنس*Alternaria* spp. بالمرتبة الاخيرة وبنسبة ٨%. كما تم تحديد السم الفطري Deoxynivalenol في عشرة نماذج وبتركيز تراوح بين 0.25 ـ ٢.٥ ملغم/ كغم عليقة ، فيما حدد Zaralenone في ثمانية نماذج وبتركيز تراوح بين ٠.٢ ـ ٥ ملغم/ كغم عليقة. ان ما تم تحديده من فطريات وسمومها ربما يمثل خطراً على الحيوانات وخصوصاً الثروة السمكية.

الكلمات المفتاحية: سموم الفيوزاريم، الديوكسينيفالينول، الزيرالينون، الفطريات.