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Full Length Research Paper

Fungal flora and deoxynivalenol (DON) level in wheat from Jeddah market, Saudi Arabia

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Deoxynivalenol (DON) is one of the least toxic trichothecenes, however, it is the most prevalent trichothecenes in human foods and its presence is an indicator of the possible incidence of other more toxic trichothecenes. This study aimed to explore the fungal flora along with the DON concentration in the collected wheat samples from Jeddah market to correlate between this flora and the detected DON. Whole grain wheat samples were collected from Jeddah market and this represents imported and locally produced wheat. The results indicated in this study showed high incidence of *Aspergilli*. The high-performance liquid chromatography (HPLC) chromatogram of the samples showed high DON resolution. DON was detected in a range of 15 to 800 µg/kg in the collected samples although no *Fusarium* species was detected in these samples. The 200 µg/kg DON level (the safe limit for baby foods and young children) was exceeded by 50% of some of the imported samples. The presence of some toxigenic fungi in these samples should set the alarm of possible contamination of these samples with other mycotoxins during storage. However, the level of DON in all wheat samples was within the permissible level of DON in unprocessed wheat which is 1750 µg/kg according to the European Commission.

Key words: Mycoflora, mycotoxins, deoxynivalenol (DON), wheat, Jeddah, Saudi Arabia.

INTRODUCTION

Mycotoxins are considered one of the most challenging human health hazards with different toxic compounds and different mode of actions. Among these mycotoxins are the highly toxic trichothecenes, which are produced by some *Fusarium* species in grains under certain conditions (Arseniuk et al., 1999; Bennett and Klich, 2003; Bottalico and Perrone, 2002). Beside *Fusarium* species, there are other fungi which are capable of producing trichothecens that exist all over the world and they include certain species of *Mycothecium*, *Trichoderma*, *Cephalosporium* and *Stachyobotrys* (Childress et al., 1990; Sudakin, 2003).

Deoxynivalenol (DON) is one of the most prevalent trichothecenes in human foods. It was first isolated in Japan and named “Rd-toxin” (Moorooka et al., 1972). The main source of DON contamination in food and feed-stuff are cereals (maize, oats, barley and wheat) which are infected by *Fusarium* fungi (Zöllner and Mayer-Helm, 2006). DON initiates a wide range of effects in farm animals and man, and these include reduced consumption of feed, skin irritation, diarrhea, multiple hemorrhage and immunosuppressive effects (Miller et al., 1991; Miller and Trenholm, 1997).

Even at a low level of contamination, the consumption of feeds by livestock has been associated with a variety of adverse health effects including feed refusal, reduced weight gain, diarrhea and emesis. At higher levels, it induces vomiting (named after this mode of action as vomitoxin) and causes growth depression (Schollenberger et al., 2002; Krska et al., 2007). All trichothecenes contain an epoxide at the C12, 13 positions, which is responsible for their toxicological activity. Cool-wet conditions (frequent rainfall and high humidity during the flowering period) are described as factors that stimulate *Fusarium* infection and DON synthesis (Cahill et al., 1999; Papadopoulou-Bouraoui et al., 2004).

Abbreviations: HPLC, High-performance liquid chromatography; **DON,** deoxynivalenol; **PDA,** potato dextrose agar.

Experimentally, horses are resistant to DON's effects on body weight gain (JECFA 56th, 2001). However, there is one report suggesting that DON exposure via contaminated bedding straw was the cause of sudden weight loss in stabled horses (Newman and Raymond, 2005). Shrimp, dogs and cats are sensitive to the emetic effects of DON (JECFA 56th, 2001).

Although DON is among the least toxic trichothecenes, it is the most frequently detected throughout the world and its occurrence is considered to be an indicator of the possible presence of other more toxic trichothecenes (Krska et al., 2007). Therefore, the objective of the current study was to explore the fungal flora along with the DON concentration in the wheat samples collected from Jeddah market, Saudi Arabia which represents the different sources of wheat production.

MATERIALS AND METHODS

Sampling sources

Whole grain wheat samples were collected from Jeddah market in the year 2010. The total sample number was 42 which represent imported wheat samples (30 samples) from 5 areas (Emirate, Oman, Syria, Australia and Egypt) and 12 samples from locally produced wheat from Najran and Al-Qassim Provinces. All samples were stored at low temperature in a dark place immediately after collection.

Mycological analysis

Samples were well mixed and out of 3000 g, a subsample of 100 g was first withdrawn, then a second subsample of 10 g was taken randomly for the analysis. The seeds were added to 100 ml distilled water and shaken for 15 min at 200 rpm. A series of dilutions 1:10 to $1:10^6$ in sterile distilled water were prepared. Aliquots (1 ml) of each dilution were dispensed into individual sterile Petri dishes, mixed with potato dextrose agar (PDA), and incubated at 25°C in the dark for 7 days. Developed fungal colonies were microscopically identified and the number of colonies was expressed as colony forming units per gram of the wheat sample (cfu/g). The fungal species were identified according to Raper and Fennell (1965), Von Arx (1974), Nelson et al. (1983), Mislivec et al. (1992) and Nelson (1992).

DON extraction and clean up procedures

The extraction and clean up of DON were carried out according to AOAC (2007). Wheat samples were grinded, 50 g were weighed and blended with 200 ml acetonitrile : H_2O (84:16) v/v. Extracted material was filtered and 20 ml filtrate was applied to column containing mixture of charcoal:alumina:celite (7:5:3) using vacuum at flow rate of 2 to 3 ml/min. The column was rinsed by 10 ml CH_3CN:H_2O (84:16), and then 3 ml ethyl acetate was used to elute the DON from the column. The elution was evaporated on steam bath under stream of nitrogen. The dry film was dissolved in 50 µl of methanol + 500 µl of water and cleaned up on C18 solid phase extraction column. The column was washed twice with 50 µl of methanol + 500 µl of water and the toxin were eluted with 6 ml methanol:water (80:20) v/v. The eluate was evaporated to dryness in a rotary evaporator (temperature below 50°C).

High-performance liquid chromatography (HPLC) conditions

HPLC was used for the detection and determination of DON where the dried residue was dissolved in 1 ml of 20% methanol. 100 µl was injected in HPLC (Agilent 1100) equipped with Diode Array Detector set at 220 nm and the column C18 (150×4.6 mm) was used to separate the DON by a mobile phase of methanol : water (30:70) with flow rate 1 ml/min (Visconti and Bottalico, 1983; Omurtag and Beyoğlu, 2007). The obtained data were integrated and calculated using Chemstation soft ware program.

RESULTS AND DISCUSSION

As a result of the trichothecenes high occurrence, frequency and especially the vomitoxin DON in wheat samples around the world, this study was aimed to explore the fungal flora along with the DON concentration in the wheat samples collected from Jeddah market to correlate between this flora and the detected DON that may be present in these samples. Table 1 shows that wheat samples fungal flora obviously show the environmental condition that existed in these samples during the storage period rather than its flora during growth in the field. This mean that the dominant genus found in these samples, the *Aspergilli* (44.8×10^3), is commonly found in the warm and subtropical environment and the absence of *Fusarium* also indicated the absence of common flora in cold weather where wheat is normally cultivated.

Although the *Aspergilli* showed the highest incidence, other genus, that is, *Alternaria*, *Penicillium*, *Acremonium*, *Eurotium* and *Emericella*, were also present in the wheat samples in lower count ranging from 3×10^3 to 5×10^3 (Table 1). It was also observed that some of the detected species are probable toxigenic species such as *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus parasiticus*, *Aspergillus japonicus* and *Alternaria alternate*. It implies that in the case of a favorite environmental condition during storage, these species may be a source of hazardous mycotoxins. *Fusarium* species is normally among the dominant fungal species in wheat during preharvest condition in the temperate zone and in the subtropical areas in winter season throughout Europe, Africa, Asia and the Americas (Hajieghrari, 2009; Kammoun et al., 2010; Brown et al., 2010). In Saudi Arabia, *Fusarium* species was found in wheat as well as in other crops in different regions in the Kingdom including Qassim, Najran, Riyadh, Hofuf, Hail and Tabouk (El-Meleigi et al., 1990; Al-Kherb et al., 1996). It should be noted that absence of *Fusarium* species is not an indication of the absence of *Fusarium* toxins. That is why we explored the presence of DON in the wheat samples used for this study although no *Fusarium* species were detected in these samples.

The results illustrated in Table 2 confirm the presence of DON although no *Fusarium* species was found where all samples showed different concentrations of DON with a range of 15 to 800 µg/kg. The lower DON mean concentrations were found in the samples from Egypt,

Table 1. Fungal species occurrence in locally and imported wheat samples collected from Jeddah market, Saudi Arabia.

Species	Najran	Qassim	Emirates	Egypt	Syria	Oman	Australia	Total	Total of Species
Aspergillus flavus	1×10^3	3×10^3	3×10^3	3×10^3	3×10^3	-	3×10^3	1.6×10^4	
A. niger	-	2×10^3	2×10^2	2×10^3	-	-	2×10^3	6.2×10^3	
A. parasiticus	2×10^3	-	2×10^3	3×10^2	3×10^2	3×10^3	3×10^2	7.9×10^3	
A. versicolor	2×10^2	2×10^3	1×10^3	-	-	-	-	3.2×10^3	44.8×10^3
A. tamari	-	2×10^2	2×10^3	-	-	2×10^3	-	4.2×10^3	
A. japonicas	-	1×10^3	1×10^3	-	-	-	-	2×10^3	
A. carneus	-	-	2×10^2	-	-	-	3×10^3	3.2×10^3	
A. fumigates	-	2×10^3	-	1×10^2	-	-	-	2.1×10^3	
Penicillium canesens	2×10^3	-	-	-	2×10^3	-	-	4×10^3	
P. chrysogenum	-	-	-	2×10^2	-	-	3×10^2	5×10^2	4.6×10^3
P. corylophilum	-	1×10^2	-	-	-	-	-	1×10^2	
Alternaria alternate	3×10^3	2×10^3	-	-	-	-	-	5×10^3	5×10^3
Acremonium sp.	-	-	2×10^3	1×10^3	-	-	-	3×10^3	3×10^3
Eurotium chievalieri	-	-	-	-	-	2×10^3	3×10^3	5×10^3	5×10^3
Emericella nidulans	1×10^2	-	3×10^3	3×10^2	-	-	-	3.4×10^3	3.4×10^3
Total	8.3×10^3	12.3×10^3	14.4×10^3	6.9×10^3	5.3×10^3	7×10^3	11.5×10^3	-	-

Table 2. Deoxynivalenol level (µg/kg) detected in wheat samples obtained from different sources in Jeddah market, Saudi Arabia.

S/N	Source	No of sample	No of positive sample	Range of deoxynivalenol level µg/kg	Mean ± SE µg/kg
1	Najran	6	6	70.71 - 506.23	219.02±89.1
2	Qassim	6	6	38.88 - 617.39	237.9±119.8
3	Emirates	6	6	38.94 - 466.36	197.75±80.7
4	Egypt	6	6	15.24 - 40.31	30.96 ±4.3
5	Syria	6	6	35.41 - 60.31	44.85 ±3.8
6	Oman	6	6	28.21 - 43.93	34.65 ±2.35
7	Australia	6	6	34.22 - 803.22	340.35 ±139.5

Oman and Syria (31, 35 and 45 µg/kg, respectively), while the highest mean concentrations were recorded in the Australian samples (340 µg/kg) followed by the locally produced wheat samples from Qassim and Najran, (238 and 219 µg/kg, respectively). The high percentage of the DON positive samples (100%) found in this study was reported in different studies (Schollenberger et al., 2002; Tutelyan, 2004; Pan et al., 2007; Bensassi et al., 2010). The DON level range of 15 to 803 µg/kg detected in this study was similar to that of Schollenberger et al. (2002) who recorded DON level in Germany (15 to 1379 µg/kg) and that of Jajić et al. (2008) who reported a range of 57 to 1840 µg/kg in Serbia as well as that reported by Muthomi et al. (2008) of DON detected in Kenya ranged from 105 to 303 µg/kg. However, the DON level detected in this study was lower than some other reports such as that of Pan et al. (2007) who recorded a DON range of 500 to 10000 µg/kg in Uruguay and that of Bensassi et al. (2010) who found a range of 7200 to 54000 µg/kg in Tunisia. In Russia, the range of DON was between 50 and 8600 µg/kg (Tutelyan, 2004) and also in Saudi Arabia, Al-Julaifi and Al-Falih (2001), DON concentration was found to range from 2 to 4000 µg/kg.

The safe limit of DON was set at 200 µg/kg for processed cereal-based foods and baby foods for infants and young children by the Commission of the European Communities (European Commission, 2005). If these wheat samples under study were intended for use in baby foods, 24.3% of the analyzed samples would be exceeding this safe limit. The percentage of wheat samples under study that exceeded the level of 200 µg/kg of DON for each sample source is illustrated in Figure 1. Although samples imported from Egypt, Oman and Syria contained different concentrations of DON, however, none of these samples exceeded the 200 µg/kg level. The highest percentage (50%) that exceeded the 200 µg/kg level was shown in the samples imported from Australia and Emirates followed by the local samples that

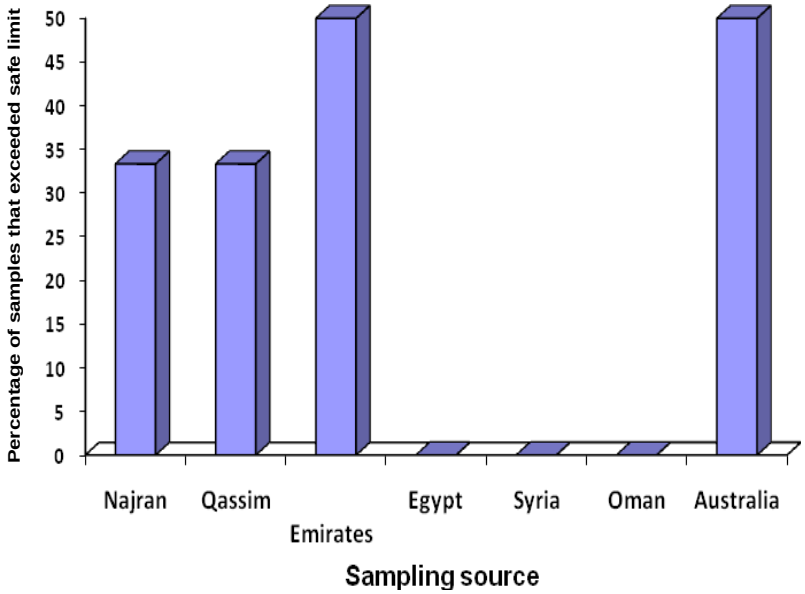

Figure 1. The percentage of wheat samples that contained DON that exceeded the safe limit (200 ppb) for baby foods according to the Commission of the European Communities (Commission Regulation No 856/2005).

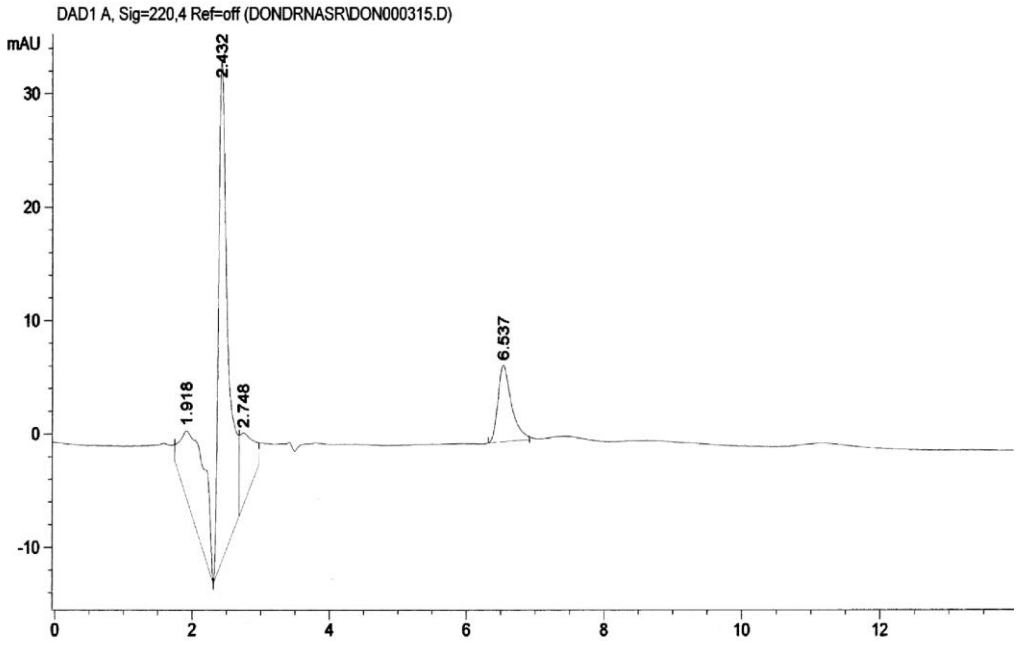

Figure 2. The HPLC chromatogram of the DON standard after 10 µl which was injected in C18 column (150 x 4.6 mm) and a mobile phase of methanol : water (30:70) with flow rate 1 ml/min was used for elution. DON was detected by Diode Array Detector set at 220 nm. DON retention time was at 6.5 min.

recorded 33%. Figure 2 shows the HPLC chromatogram of the DON standards with a retention time of 6.5 min. The HPLC chromatogram of one of the samples showed a peak at the same DON retention time (Figure 3). No contaminant overlapping in the sample chromatogram was observed and the DON peak clearly indicated the high selectivity of the extraction and the HPLC method.

The uses of HPLC in the detection of DON facilitate the

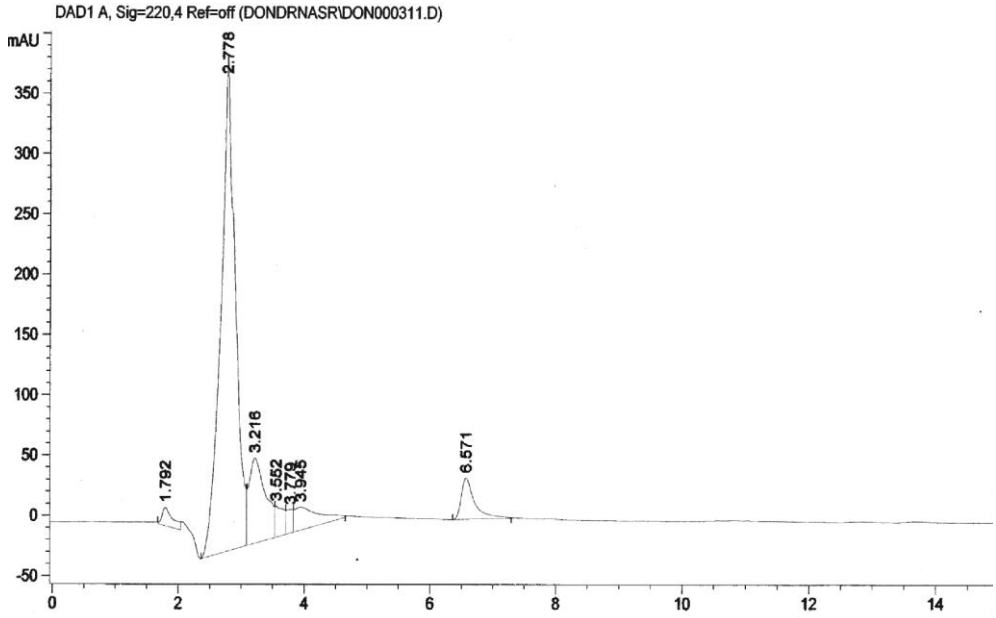

Figure 3. The HPLC chromatogram of wheat sample with DON concentration of 506.23 µg/kg after 100 µl of the extracted sample was injected in C18 column (150 x 4.6 mm) and a mobile phase of methanol : water (30:70) with flow rate 1 ml/min was used for elution. DON was detected by Diode Array Detector set at 220 nm. DON retention time was at 6.5 min.

detection of low level of mycotoxins in general and DON in particular. Razzazi-Fazeli et al. (1999) reported that accurate, rapid and efficient assay are necessary to determine the contamination of food and feed so that exposure to mycotoxins can be prevented and the use of HPLC in such determination especially with DON is of great importance. Moazami and Jinap (2009) determined DON in wheat based noodles using HPLC and they found a concentration of DON which is as low as 1.24 µg/kg. Bensassi et al. (2010) determined DON in 65 wheat samples in Tunisia by HPLC where they detected DON in 83% of the tested samples in concentrations ranging from 7.2 to 54 mg/kg.

In conclusion, because the least toxic trichothecenes, DON, is the most widely spread trichothecenes throughout the world and its presence would display the possible incidence of other more toxic trichothecenes, this study was keen to explore the level of DON in wheat from Jeddah market of Saudi Arabia. Also, the presence of some toxigenic fungi in these samples should set the alarm of possible contamination of these samples with other mycotoxins during storage. The result of this study shows that the level of DON in all wheat samples were within the permissible level of DON in unprocessed wheat which is 1750 µg/kg according to the European Commission (2006).

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