Mycotoxin Research, 2010, vol 26, 133-140

Mycotoxigenic fungi in peanuts from different geographic regions of Egypt

Yousef Sultan and Naresh Magan

Applied Mycology Group, Cranfield Health, Cranfield University, Bedford MK43 0AL, U.K.

Corresponding author: Prof. N. Magan, Applied Mycology Group, Cranfield Health, Cranfield University, Bedford MK43 0AL, U.K. Tel: 01234 758308; Fax: 01234 758380; E.mail: n.magan@cranfield.ac.uk

Key words: Peanuts, mycobiota, mycotoxigenic fungi, *Aspergillus* section *Flavi*, *Aspergillus* section *Nigri*, *Aspergillus* section *Circumdati*

Abstract
To understand the importance of mycotoxigenic fungi in Egyptian peanuts, samples from five regions (Alexandria, El-Beheira, El-Sharqiya, El-Daqahelaya in northern Egypt and Asyut, southern Egypt) in two seasons (2007, 2008) were collected. *Aspergillus* was consistently the most frequent genus in seeds and in-shell peanuts and was the dominant mycotoxigenic component of the mycobiota. There was no direct correlation between the moisture content of the samples and the fungal populations on peanut seeds tested from different regions. The most common species were from *Aspergillus* section *Flavi* (4.7-78.3%), *Aspergillus* section *Nigri* (9.4-52.6%) and *Aspergillus* section *Circumdati* (5.1-30.9%). In the in-shell peanut samples the lowest populations were recorded in El-Beheira and Asyut (3.7-4.0 log_{10} CFU g^{-1}) and the highest in Alexandria and Elsharqiya (4.1-6.0 log_{10} CFU g^{-1}). *Aspergillus* section *Flavi* and section *Nigri* were the most dominant and *Aspergillus* section *Circumdati* were only found in samples in 2008. Both qualitative (coconut cream agar) and quantitative analyses (HPLC) were used to analyse the potential mycotoxin production by strains isolated from peanuts. Of a total of 88 *Aspergillus* section *Flavi* strains examined, 95% were *A. flavus* based on production of aflatoxin B_1 on YES medium and confirmation using molecular analyses. Of 64 *Aspergillus* section *Circumdati* strains only 28% produced ochratoxin A (OTA), and were identified as *A. westerdijkiae*. No *Aspergillus* section *Nigri* strains produced OTA, and they were identified as *A. niger* (uniseriate). The presence of these toxigenic fungi indicates that there is a potential risk of mycotoxin contamination in Egyptian peanuts and suggests that problems can arise from contamination with both aflatoxins, and perhaps also OTA.

Introduction
The mycotoxins produced by *Aspergillus* spp. of greatest significance in peanuts (*Arachis hypogaea* L.) and peanut products include aflatoxins and

ochratoxin A (OTA) (Pittet 1998). Peanuts are important substrates for the growth and subsequent aflatoxin production by different members of *Aspergillus* section *Flavi*: *A. flavus* Link, *A. parasiticus* Speare, *A. nomius*, *A. pseudotamarii* and *A. bombycis*. Recently, additional new aflatoxin producing species have been isolated from peanuts in Argentina (*A.arachidicola* sp. nov. and *A.minisclerotigenes* sp. nov (Pildain et al. 2008). The commonest species can infect peanuts and maize both pre- and post-harvest (Cotty, 1997; Barros et al. 2003). Usually, aflatoxin contamination pre-harvest occurs under conditions of heat and drought stress during the latter stages of the growing season (Hill and Lacey 1983; Blankenship et al. 1984; Dorner and Cole 2002). Because peanuts are primarily used for food, strict regulatory limits for the amount of aflatoxin in peanut products have been established. For raw peanuts entering the EU, the level must be <4 μg kg^{-1} total aflatoxins and have not more than 2 μg kg^{-1} B1 (European Commission 2006).

Egypt is a major peanut exporting country and the European markets account for 68% of its peanut exports. Its production has increased steadily, as a result of increased growing areas as well as significant increases in yields during the last two decades (1990 to 2005) from 2.1 to 3.1 t ha^{-1} (Diaz Rios and Jafee 2008). Peanuts are grown mostly in the north of the country. There are a large number of peanut producers, with many small holders resulting in an overall peanut cultivation area covering 61,000 ha (FAO 2003). Although some studies, especially in South America, have isolated ochratoxigenic fungi from peanuts, there are no legislative limits for this mycotoxin in peanuts or derived products although these are in place for cereals (Van Egmond 2004).

In May, 1999 the European Commission suspended the import of peanuts from Egypt due to the presence of aflatoxins in concentrations in excess of the maximum levels specified in the EU regulations. Since then the Egyptian Government has been actively trying to reduce the aflatoxin content in peanuts. Since 2003, the EU only required random sampling in 20% of peanut consignments imported from Egypt for aflatoxin B1 and total aflatoxins. Subsequently, Egypt has increased its market share in 2005 by >60 M US$ (Diaz Rios and Jafee 2008).

There have been previous studies on the mycobiota contamination of Egyptian peanuts (Mobasher et al. 1979; Moubasher et al. 1980; El-Hissy et al. 1981; El-Maghraby and El-Maraghy 1987; El-Magraby and El-Maraghy 1988; Youssef et al. 2008). However, none of these studies compared the toxigenic potential of the strains in peanut samples from different regions of Egypt. Furthermore, where mycotoxin analyses were carried out this was qualitative only predominantly using TLC, with very limited quantification of aflatoxins, or other mycotoxigenic fungi present. For example, recent studies in Argentina have found that *Aspergillus carbonarius* was commonly isolated from peanuts under South American cultivation conditions and that many of the strains isolated could produce ochratoxin A (OTA) (Magnoli et al. 2007). No information is available on the isolation of *Aspergillus* section *Circumdati* species, or *Aspergillus* section *Nigri* and whether these may produce OTA.

The objectives of this study were to (a) identify the dominant mycotoxigenic fungi in Egyptian peanuts (in shell, and seeds) in two seasons (2007, 2008) in five different regions of Egypt and (b) to examine and quantify the contributions of the mycotoxgenic fungi to the total populations, and (c) to examine the relative ratios of species from *Aspergillus* section *Flavi*, *Aspergillus* section *Nigri* and *Aspergillus* section *Circumdati* which were able to produce aflatoxins and OTA.

Material and methods

Collection of peanut samples

Triplicate raw peanut in shell samples (each 500 g) were collected after harvest in two seasons (2007, 2008) from five provinces in Egypt (Alexandria, El-Beheira, El-Daqahliya, El-Sharqiya, all from northern Egypt; and Asyut from southern Egypt). Half of each sample was shelled in a sterile flow bench to obtain the peanut seeds for mycobiota analyses. Samples were kept at 4°C, until fungal enumeration.

Determination of water activity and moisture content of peanuts

The water activity (a_w) of the peanut in shell and peanut seed samples was determined using the AquaLab 3TE (Decagon Devices, Inc., Pullman, Washington, USA). A 10 grams sub-sample of each replicate was also oven dried at 105°C for 24 h, and then cooled in desiccators and re-weighed to a constant weight. The moisture content (MC) was calculated as the percentage of the wet weight (Deshpande et al. 1993).

Mycotoxigenic fungi isolation from samples

Direct plating: A total of 45 peanut seeds (5 x 3 x 3 replicates) were direct plated onto Petri plates of Malt Extract Agar (MEA, Oxoid, Basingstoke, Hampshire, U.K.), Dichloran 18% glycerol agar (DG18, Oxoid) and MEA + 10% NaCl (0.95 a_w, MS; Oxoid) from each region. After incubation at 25°C for 7 days, the frequency of fungi was recorded. The isolates of *Aspergillus* section *Flavi, Aspergillus* section *Nigri and Aspergillus* section *Circumdati* were recovered from the peanut seeds and maintained on slants of MEA at 4°C, until examination for their toxigenic potential.

Serial dilutions: The colonization was assessed as CFU g^{-1} of dry weight peanut in-shell samples. Triplicate 10g sub-samples from each region were weighed in stomacher bags containing 90 ml of distilled water and left for 10 min to soak. A total of four samples were homogenised for 15 min in the Colworth Stomacher 400. Serial dilutions (10^{-2}, 10^{-3}, 10^{-4}) were done and 200 µl from each dilution spread plated on MEA, DG18 and MS agar in triplicate. The plates were incubated at 25°C for 7-10 days and examined periodically. A colony counter was used for total counts and microscopic examinations were made for fungal genera classification. Individual colonies were sub-cultured for detailed examination. Data obtained on DG18 and MS showed no statistically significant differences so data for only one of these media is presented.

For identification of species, comparisons were made with type strains from culture collections and reference to Pitt and Hocking (2009). Type strains in our laboratories included *Aspergillus flavus* (SRKC-G1907; USDA,USA); *A. parasiticus* (SSWT 2999, USA), *Aspergillus carbonarius* (IMI 388653), *Aspergillus niger*. var *niger* (biseriate; IMI 388550); *A. niger* var. *niger* (uniseriate, IMI 387209), *Aspergillus westerdijkiae* (IBT 21991), *A. steynii* (IBT 22339), and *A. ochraceus* (IBT 11952).

Evaluation of toxigenic potential and identification

Two methods were used to assess this. We used a medium-based qualitative system and HPLC analyses for quantification of aflatoxins and ochratoxin A (OTA) production and molecular identification of *Aspergillus* section *Flavi* strains.

Species from the *Aspergillus* section *Flavi*, *Aspergillus* section *Nigiri* and *Aspergillus* section *Circumdati* isolated from peanut seeds were tested, respectively, for their ability to produce aflatoxins (B_1, B_2, G_1, G_2; AFs) or OTA in coconut cream agar 50% (Dyer and McCammon 1994). The reverse side of the colony was observed daily for 6 days at 25°C under ultraviolet light at 365 nm in a dark cabinet to verify the presence of a blue fluorescent ring, an indication of the presence of AFs or OTA, thus characterizing it as a toxigenic strain.

Quantitative analyses of mycotoxins

AFs and OTA analyses was done using HPLC: Three replicates of the peanut samples from each region were analysed for AFs in the two seasons by extraction using aflaprep columns (Neogene Europe, wide bore). For the strains isolated from peanuts three to five plugs were extracted from each colony after 10 days incubation at 25°C on Yeast Extract Sucrose Agar (YES, Fisher, Loughborough, Leicestershire, U.K.) , transferred to a 2ml Eppendorf tube and weighed (Filtenborg and Frisvad 1980). For aflatoxin extraction, 800 µl chloroform was added to each Eppendorf and shaken well for 1 hour. The chloroform extract was transferred to a new vial and dried gently under air for derivatization using the AOAC method (2000) and then analysed quantitatively using HPLC. A 200 µl stock solution of Aflatoxin mix standard in methanol (Supelco, Bellefonte, PA USA) ,containing 200 ng B1, 60 ng B2, 200 ng G1 and 60 ng G1, was dried under nitrogen gas and derivatized as for samples. Four concentrations were prepared for HPLC injection. For OTA extraction, 750 µl methanol was added, the sample shaken for 30 minutes and centrifuged for 10 minutes at 15000xg. After filtration of the supernatant, the samples were analysed by HPLC. OTA 50 µg ml^{-1} benzene:acetic acid (99:1, Supelco, Bellefonte, PA., USA) was used. 50 ul of the stock was evaporated under nitrogen and dissolved with methanol to inject 10 ul from four different concentration (125, 250, 375, 500 ng ml^{-1}). Detection limit of AFB_1 using HPLC was 0.012 and 0.8 ng g^{-1} peanut seeds and YES media respectively whereas detection limit of OTA in YES media was 0.4 ng g^{-1} YES media.

HPLC conditions**:** The HPLC system used for AFs and OTA analyses was an Agilent 1200 series system (Agilent, Berks., UK) with a fluorescence detector (FLD G1321A), an auto sampler ALS G1329A, FC/ALS therm G1330B, Degasser G1379B, Bin Bump G1312A and a C18 (Phenomonex, Luna 5 micron, 150 x 4.6 mm) column joined to a pre- column (security guard, 4x3mm cartridge, Phenomenex Luna). The mobile phase was methanol:water:acetonitrile (30:60:10, v/v/v) using an isocratic flow rate of 1ml min^{-1} at 360 nm excitation and 440 nm emission wavelengths and a 25 min run time for AFs analyses. For OTA analyses, acetonitrile (57%): water (41%): acetic acid (2%) were isocratically used at the same flow rate at 333 nm excitation, 460 nm emission wavelengths. The run time for samples was 15 min with OTA being detected at 5.75 min.

Molecular discrimination of *A. flavus* and *A. parasiticus*

DNA extraction and PCR amplification: Genomic DNA of the strains was obtained using the DNAeasy Plant Mini kit (Qiagen, Hilden, Germany), according to the

manufacturer's instructions. Differentiation between *A. flavus* and *A. parasiticus* strains isolated from Egyptian peanut samples was carried out according to González-Salgado et al. (2008). A pair of primers (Invetrogen), FLA1 (5´-GTAGGGTTCCTAGCGAGCC-3´) and FLA2 (5´-GGAAAAAGATTGATTTGCGTTC-3´), specific to *A. flavus* was used for that differentiation. PCR reactions were performed in 0.2 ml PCR Eppendorf (Starlab Ltd, Milton Keynes, UK) using an Alpha Unit ™ Block Assembly for PTC DNA Engine ™ Systems (MJ Research Inc., Waltham, USA). The PCR amplification protocol was as follows: 1 cycle of 5 min at 95°C, 26 cycles of 30s at 95°C (denaturation), 30 s at 58°C (annealing), 45 s at 72°C (extension) and, finally, 1 cycle of 5 min at 72°C. Amplification reactions were carried out in volumes of 25 ml containing 1µl of template DNA, 0.25 µl of each primer (100 µM), 2.5 µl of 10xPCR buffer, 2 µl of $MgCl_2$ (25 mM), 0.625 µl of dNTPs (40mM) and 0.2 µl of Taq DNA polymerase ($5U\mu l^{-1}$) supplied by Qiagen (Hilden, Germany). PCR products were detected on 2% agarose ethidiumbromide gels in TAE 1xbuffer (Tris–acetate 40mM and EDTA 1.0 mM). The 100-bp DNA ladder (Qiagen, Hilden, Germany) was used as the molecular size marker. Genomic DNA samples used in this work were tested using the transcription of the housekeeping gene (β-tubulin), Tub1-F (5´-GTCCGGTGCTGGTAACAACT-3´) and Tub1-R (5´-GGAGGTGGAGTTTCCAATGA-3´), obtained from IDT (Integrated DNA Technology Inc. Iowa, USA) as a control. The PCR amplification protocol was as previously detailed except for the changing of the annealing temperature to 65°C and the number of denaturation cycles to 35.

The references strains used in this work were *A. flavus* SRKC-G1907, *A. flavus* A 2092, *A. parasiticus* SSWT BN009-E, and *A. parasiticus* SSWT 2999. We are grateful to Dr. D. Bhatnagar and Dr. P. Cotty for supply of strains).

Statistical analysis

Data on populations of mycotoxigenic fungi and total counts (CFU g^{-1} of dry weight peanut in-shell) on DG18 and MS medium were logarithmically transformed before statistical analysis. This was required because of the wide range of variability (from single-digit numbers to numbers in hundreds/thousands) (Clewer and Scarisbrick 2001). Statistical significance were determined using Statistica version 9 (StatSoft Inc., Tulsa, OK, USA). Means of log CFU units were determined by analysis of variance (ANOVA, tow and three ways) ($p<0.05$). Fisher's LSD Method ($\alpha=0.05$) was applied to compare significant differences between regions and seasons.

Results and Discussion

Water activity and moisture content of peanut samples

Water activity levels (a_w) and moisture contents (MC%) were measured in an attempt to find any correlation with the fungal density of peanut samples from different regions of Egypt in 2007 and 2008 (Table 1). Values of either a_w or MC% of in-shell peanuts and seeds from each region were similar. Most of the samples had <0.59 a_w and 9.3% moisture content respectively. However, samples from Alexandria and El-Sharqiya in 2008 had the highest values (0.89-0.91 a_w, 23.4-26.1% moisture content). Only in these two areas in 2008 were conditions conducive to colonisation by *A. flavus* and AFs contamination which can occur at >0.78-0.8 a_w (Lacey et al. 1991; Sanchis and Magan, 2004). The background aflatoxin B1 concentrations found in the peanuts in 2007 and 2008 were however very low with the range being 0.8-0.41 and 0.09 to 0.23 µg kg^{-1} respectively.

Previous studies from markets as opposed to directly from farmers, also suggest that *Aspergillus spp.* are predominant in Egypt (Moubasher et al. 1979; El-Maghraby and El-Maraghy, 1987, 1988; Youssef et al. 2008).

Frequency of isolation and populations of mycotoxigenic fungi in peanuts from different geographic regions of Egypt in two seasons

Table 2 shows the populations (log_{10} CFU g^{-1} of in-shell peanut sample) of mycotoxigenic fungi and the total fungal populations from the five Egyptian regions in 2007 and 2008. Overall, the total fungal populations of samples collected in 2008, in particular from Alexandria and El-Sharqiya, was significantly higher when compared with 2007. High populations of *Cladosporium* and *Penicillium* species were present in samples from Alexandria and El-Sharqiya in 2008 (4-5 log units g^{-1}). However, *Wallemia sebi*, yeasts, *Acremonium* species were present in samples from the other regions in both seasons. *Aspergillus* section *Flavi* and *Aspergillus* section *Nigri* groups were the most prevalent mycotoxigenic fungi in both seasons. Samples from Alexandria and El-Sharqiya collected in 2008 had the highest total populations among the regions examined, and subsequently the highest populations of mycotoxigenic fungi. The *Aspergillus* section *Circumdati* group were only found in samples from the 2008 season (0.7 - 4.8 log_{10} units g^{-1}).

Aspergillus section *Circumdati* was observed from all regions in 2008, but none in 2007. However, the other two mycotoxigenic groups were isolated in 2.9-5.6 and 2.6-4.7 log units g^{-1} respectively for the two seasons. A similar frequency was also found by Gonçalez et al. (2008) for *A. flavus* in full pod maturity (33.6%). In Brazil, Nakai et al. (2008) reported that in kernels, in addition to *A. flavus* (21.2%), *A. niger* was isolated but at a very low frequency (0.6%). However, they found that among representatives of the genus *Aspergillus*, only *A. flavus* (10.3%) were isolated from shells. Moubasher et al. (1979) found *A. flavus* to constitute 16.1, 8.4 and 27.2% of the total count in the shells, in covered and uncovered peanut seeds respectively. They also found that *A. niger* was recorded from 95% of peanut samples tested, constituting 20.7% of the total fungi and 35% of the total *Aspergillus*. Previously, El-Maghraby and El-Maraghy (1987) found *A. flavus* and *A. niger* comprised only 8.1 and 2.2% of the total fungi in Egyptian peanuts. With regard to *Aspergillus* section *Circumdati* group, Tripathi and Kumar (2007) found them to be present in only 12.7% of the total fungi in India. However, none of the studies in Egypt examined potential for mycotoxin production by these genera.

Table 3 shows the statistical analyses of the single and interaction between single factors of mycotoxigenic groups in relation to region and season. This shows that these were statistically significant effects of region and season on *Aspergillus* section *Flavi,* while for *Aspergillus* section *Nigri* season had no effect.

Figure 1 shows the frequency of isolation of the mycotoxigenic fungal groups from peanut seeds in the different regions in 2007 and 2008. The frequency of these fungal groups ranged from 0 to 78.3 and from 4.7 to 52.6% collected in the two years. Generally, the percentage of *Aspergillus* section *Flavi*, *Aspergillus* section *Nigri* and *Aspergillus* section *Circumdati* groups in 2007 was higher than that isolated in 2008. In 2007, *Aspergillus* section *Flavi* group represented 78, 44, 27, and 25% of the total count in samples from El-Daqahelaya, Alexandria, El-Beheira, El-Sharqiya regions respectively. While, there was none found in samples from the southern region (Asyut). However, they were contaminated with *Aspergillus* section *Circumdati* (33%) in addition to *Eurotium* and *Alternaria* as the

dominant other genera (33 and 25% respectively). The second most important group was *Aspergillus* section *Nigri* which can produce OTA. Peanut seeds from El-Beheira contained the highest percentage frequency of *Aspergillus* section *Nigri* (45%) with the other regions having less in 2008. The frequency of isolation of *Eurotium* and *Acremonium* species from all regions was high when compared to that of the *Aspergillus* section *Flavi* group (4.7-10.2%), *Aspergillus* section *Circumdati* group (5.1-21.2%) and *Aspergillus* section *Nigri* groups (9.4-52.6%). Xerophilic *Eurotium* species represented 26%, 47% and 70% of the total fungal count in samples from El-Beheira, El-Daqahliya and Asyut region, respectively. However, *Acremonium* was dominant in Alexandria and El-Sharqiya samples.

El-Maghraby and El-Maraghy (1988) and Youssef et al. (2008) reported that *A. flavus* represented 4 and 4.9% respectively of the total fungi on untreated seeds. In the present study *Aspergillus* section *Circumdati* group represented 5.1-21.2% of mycobiota populations in samples from all regions except from El-Beheira. In contrast, Yousef et al. (2008) did not find any *Aspergillus* section *Circumdati* group in peanut samples. In 2008, in the Alexandria and El-Sharqiya samples a higher frequency of *Penicillium* and *Acremonium* species were found in contrast to 2007 where *Eurotium* species were dominant. This may be linked to the higher a_w of these samples. This also suggests that samples from these regions were stored poorly after harvest (Pitt and Hocking 1997).

Toxigenic potential of isolated strains of mycotoxigenic genera

Screening of fungi isolated from peanut seeds for aflatoxin production by the fluorescence method on coconut cream agar medium revealed that among 56 and 32 *Aspergillus* section *Flavi* isolates in 2007 and 2008 almost all except for 6 and 8 strains respectively, produced a blue fluorescence (Table 4). The same medium was also used to examine *Aspergillus* section *Nigri* and *Aspergillus* section *Circumdati* isolates for ochratoxin A (OTA) production. No blue fluorescence was observed in any of the former group isolates tested (28 and 50 isolates in 2007 and 2008). Of the *Aspergillus* section *Circumdati* isolates in 2007 and 2008 (12 and 52 respectively) 42 and 25 %, were ochratoxigenic.

A total of 88 *Aspergillus* section *Flavi* isolates were tested by HPLC for quantification of the ability to produce aflatoxins (AFAs; B1, B2, G1, G2). A further 64 *Aspergillus* section *Circumdati and* 78 *Aspergillus* section *Nigri* were screened for production of OTA on a conducive YES medium. Table 4 also shows that there were differences between the qualitative and quantitative methods. For example, of 88 *Aspergillus* section *Flavi* isolates only 5 gave false negative results. For *Aspergillus* section *Circumdati* of 64 isolates, only 4 gave a false positive in the two seasons when compared with HPLC results. The mean aflatoxigenic *Aspergillus* section *Flavi* isolates for both seasons was 90% based on HPLC analysis. The concentrations of aflatoxin B1 produced ranged from 10 to 100,000 ng g^{-1}. However, 2 (2.3%) isolates were able to produce AFB_1 >100,000 ng g^{-1}. The mean percentage ochratoxigenic *Aspergillus* section *Circumdati* isolates was 28% and the concentration range of OTA was from 1-3000 ng g^{-1}. None of the 78 *Aspergillus* section *Nigri* isolates produced OTA. This suggests that most of the isolates from these groups were, respectively, *A. flavus*, *A. westerdijkiae* and *A. niger* (uniseriate).

Molecular analyses of *Aspergillus* section *Flavi* strains isolated from Egyptian peanuts were further tested using the primer pair FLA1 and FLA2. A

single fragment of about 500 bp was only amplified when genomic DNA from *A. flavus* strains was used, but not from *A. parasiticus* strains where no product was observed. More than 95% of the tested *Aspergillus* section *Flavi* were *A. flavus* and this is shown in Figure 2 as an example of the results. Isolate in lane 16 was *A. parasiticus* and non-aflatoxigenic as well. These result confirmed those obtained from analysis of isolates on YES medium for AFAs. This showed that all the aflatoxigenic isolates produced AFB_1 and B_2 and none produced AFG_1 and G_2.

There was approximately 90% compatibility of the results between coconut agar method and HPLC. Similar results were obtained by Giorni et al. (2006) who found that 73% of *Aspergillus* section *Flavi* strains isolated from maize showed fluorescence when inoculated on Coconut extract agar and 70% of strains were positive when tested by HPLC. For both seasons, the mean *Aspergillus* section *Flavi* isolates producing aflatoxins was 89.3%, and for ochratoxin A, 33.5% of *Aspergillus* section *Circumdati* isolates. However, no *Aspergillus* section *Nigri* group produced OTA. Nakai et al. (2008) found in Brazil that 93.8% of *Aspergillus* section *Flavi* isolated from Brazilian peanuts produced aflatoxins. Also, Sánchez-Harvás et al. (2008) reported 64.1% of isolates from cocoa beans produced aflatoxins. The results for OTA production by *Aspergillus* section *Nigri* and *Aspergillus* section *Circumdati* group in this study differ from those obtained by Sánchez-Harvás et al. (2008) who found that 49.2% of black *Aspergilli* strains were able to produce OTA in cocoa beans and suggested that *Aspergillus* section *Circumdati* is probably a relatively unimportant source of OTA in cocoa production. In contrast, Recently, *Aspergillus* section *Circumdati* species, especially *A. westerdijkiae*, were found to be commonly present on coffee beans (Noomin et al. 2008). Magnoli et al. (2007) reported that 32% of *Aspergillus* section *Nigri* isolated from Argentinean peanut seeds were OTA producers in culture. Additionally, Bayman et al. (2002) demonstrated that none of the isolates of *Aspergillus* section *Circumdati* or *Aspergillus melleus* from tree nuts and fig orchards produced OTA, although all isolates of *Aspergillus alliaceus* did produce the toxin. This is unusual as *Aspergillus* section *Nigri* isolates normally contaminate grapes, grape products and wine with OTA (Mateo et al. 2007).

In conclusion the present study demonstrated the susceptibility of Egyptian peanut to colonization with *Aspergillus* section *Flavi,* especially *A. flavus* isolates*, Aspergillus* section *Nigri* and *Aspergillus* section *Circumdati* groups as dominant fungi from different regions of Egypt. The a_w of the samples, season, and regions were important factors affecting the fungal populations. It may be important to not overlook the presence of ochratoxigenic fungi (28%) in peanuts in addition to aflatoxigenic species (90%) and to perhaps formulate guidelines for OTA as well. *Aspergillus* section *Nigri* group isolates from Egypt were unable to produce OTA in peanuts. This contrasts with results from South America. Poor post-harvest storage of peanuts in Egypt could increase the risk from contamination with aflatoxins above the EU legislative limits.

REFERENCES

AOAC (2000) Association of Official Analytical Chemist, Official methods of analysis. Natural toxins, 17th edn., Chapter 49, Washington, D.C.

Barros G, Torres, A, Palacio G, Chulze S (2003) *Aspergillus* species from section *Flavi* isolated from soil at planting and harvest time in peanut-growing regions of Argentina. J Sci Fd Agric 83:1303-1307.

Bayman P, Baker JL, Doster MA, Michailides TJ, Mahoney NE (2002) Ochratoxin production by the *Aspergillus ochraceus* group and *Aspergillus alliaceus*. Appl Environ Microbiol 68: 2326-2329.

Blankenship PD, Cole RJ, Sanders TH, Hill RA (1984) Effect of geocarposphere temperature on pre-harvest colonization of drought-stressed peanuts by *Aspergillus flavus* and subsequent aflatoxin contamination. Mycopathologia 85:69-74.

Clewer AG, Scarisbrick DH (2001) Practical statistics and experimental design for plant and crop science. John Wiley & Sons, Ltd, England, p. 332.

Cotty PJ (1997) Aflatoxin-producing potential of communities of *Aspergillus* section *Flavi* from cotton producing areas in the United States. Mycol Res 101:698-704.

Deshpande SO, Bal S, Ojha TP (1993) Physical properties of soybean. J Agric Eng Res 56:89-98.

Diaz Rios LB, Jaffee S (2008) Barrier, Catalyst, or Distraction? Standards, Competitiveness, and Africa's Groundnut Exports to Europe. Agriculture & Rural Development Discussion Paper 39, World Bank, Washington, DC 20433. http://www.worldbank.org/rural

Dorner JW, Cole RJ (2002) Effect of application of nontoxigenic strain of *Aspergillus flavus* and *A. parasiticus* on subsequent aflatoxin contamination of peanut in storage. J Stored Prod Res 38:329-339.

Dyer SK, McCammon S (1994) Detection of aflatoxigenic isolates of *Aspergillus flavus* and related species on coconut cream agar. J Appl Bacteriol 76:75-78.

El-Hissy FT, Abdel-Hafez SII, Hassan SKM (1981) Effect of level of relative humidity on fungi and germinability of Egyptian peanut seeds. Zent Bacteriol 136:49-55.

El-Maghraby OMO, El-Maraghy SSM (1987) Mycroflora and mycotoxins of peanut (*Archis hypogaea* L.) seeds in Egypt. 1- sugar fungi and natural occurrence of mycotoxins. Mycopathologia 98:165-170.

El-Magraby OM, El-Maraghy SS (1988) Mycoflora and mycotoxins of peanut (*Arachis hypogaea* L.) seeds in Egypt. III. Cellulose-decomposing and mycotoxin-producing fungi. Mycopathogia 104:19-24.

European Commission (2006) Final report of a mission carried out in China, in order to assess the facilities and measures in place for the control of aflatoxin contamination in peanuts intended for export into the EU. Report DG (SANCO)/8126/2006.

Filtenborg O, Frisvad JC (1980) A simple screening method for toxigenic moulds in pure cultures. Lebensmittle Wissenschaft und Technologie 13:128-130.

FAO (2003) Trade, market access and food safety in the near east region. In: High-Level Technical Workshop "Regional Programmes for Food Security in the Near East: Towards Sustainable Food Security and Poverty Alleviation". Jeddah, 8-9 October 2003. Food and Agriculture Organisation http://www.fao.org/docrep/MEETING/007/AD388E/ad388e00.htm

Giorni P, Magan N, Pietri A, Bertuzzi T, Battilani P (2007) Studies on *Aspergillus* section *Flavi* isolated from maize in northern Italy. Int J Fd Microbiol 113:330-338.

Gonçalez E, Nogueira JHC, Fonseca H, Felicio JD, Pino FA, Corrêa B (2008) Mycobiota and mycotoxins in Brazilian peanut kernels from sowing to harvest. Int J Fd Microbiol 123:184-190.

González-Salgado, A; González-Jaén, T, Vázquez, C and Patiňo, B (2008) Highly sensitive PCR-based detection method specific for Aspergillus flavus in wheat flour. Food Add Contam 25: 758-764.

Hill RA, Lacey J (1983) Factors determining the microflora of stored barley grain. Ann Appl Biol 102:467-483.

Lacey J, Ramakrishna N, Hamer A, Magan N, Marfleet I (1991) Grain fungi. In: Arora D.K., Mukerji, K.G. and Marth E.H.(eds.) Handbook of applied mycology: Foods and feeds, Marcel Dekker, New York, pp. 12–77.

Magnoli C, Astoreca A, Ponsone ML, Fernández-Juri MJ, Barberis C, Dalcero AM (2007) Ochratoxin A and *Aspergillus* section *Nigri* in peanut seeds at different months of storage in Córdoba, Argentina. Int J Fd Microbiol 119:213-218.

Mateo R, Medina Á, Mateo EM, Mateo F, Jiménez M (2007) An overview of ochratoxin A in beer and wine. Int J Fd Microbiol 119:79-83.

Moubasher AM, El-Hissy FT, Abdel-Hafez SI, Hassan SKM (1979) The mycoflora of peanuts in Egypt. Mycopathologia 68:39-46.

Moubasher H, Abdel-Hafez SI, El-Hissy FT, Hassan SK (1980) Effect of temperature and moisture content on Egyptian peanut seed-borne fungi. Mycopathologia 70:49-54.

Nakai VK, Rocha L, Gonçalez E, Fonseca H, Ortega EM, Corrêa B (2008) Distribution of fungi and aflatoxins in a stored peanut variety. Food Chem 106:285-290.

Noonim, P, Mahakarnchanakul W, Nielsen KF, Frisvad JC, Samson RA (2008) Isolation, identification and toxigenic potential of ochratoxin A-producing *Aspergillus* species from coffee beans grown in two regions of Thailand. Int J Food Microbiol 128:197-202.

Pildain, MB, Frisvad, JC, Vaamonde, G, Cabral D, Varga J, Samson RA (2008) Two novel aflatoxin-producing *Aspergillus* species from Argentinian peanuts. Int J Syst Evol Microbiol 58:725-735.

Pitt JI, Hocking AD (2009) Fungi and Food Spoilage (3rd ed), Spinger Verlag, Germany,540 pp.

Pittet A (1998) Natural occurrence of mycotoxins in foods and feeds: an updated review. Rev Mèd Véter 149:479-492.

Sánchez-Hervás M, Gil JV, Bisbal F, Ramón D, Martínez-Culebras PV (2008) Mycobiota and mycotoxin producing fungi from cocoa beans. Int J Fd Microbiol 125:336-340.

Sanchis V, Magan N (2004) Environmental conditions affecting mycotoxins. In: N. Magan and M. Olsen, Editors, Mycotoxins in Food: detection and control, pp. 174–189.

Tripathi NN, Kumar N (2007) *Putranjiva roxburghii* oil- A potential herbal preservative for peanut during storage. J Stored Prod Res 43:435-442.

Van Egmond HP (2004) Worldwide regulations for mycotoxins in food and feed in 2003. FAO FOOD and Nutrition paper, 81. http://www.fao.org/docrep/007/y5499e/y5499e00.HTM.

Youssef MS, El-Maghraby OMO, Ibrahim YM (2008) Mycobiota and Mycotoxins of Egyptian peanut (*Arachis hypogeae* L.) seeds. Int J Bot 4:349-360.

Table 1. Water activity levels (a_w) and moisture content (MC%) of peanut in-shell and seeds collected from different Egyptian regions

		2007		2008	
Regions	**Type of sample**	a_w	**MC%**	a_w	**MC%**
Alexandria	Peanut in-shell	0.58	8.5	0.91	24.1
	Peanut seeds	0.56	7.0	0.91	26.1
El-Behiera	Peanut in-shell	0.50	8.3	0.54	7.9
	Peanut seeds	0.54	7.6	0.52	6.9
El-Sharqiya	Peanut in-shell	0.48	9.3	0.89	23.4
	Peanut seeds	0.51	8.4	0.92	24.4
El-Daqahliya	Peanut in-shell	0.54	8.8	0.56	8.9
	Peanut seeds	0.56	7.6	0.59	7.5
Asyut	Peanut in-shell	0.56	8.3	0.54	7.3
	Peanut seeds	0.53	6.1	0.50	5.8

Table 2. The toxigenic mycobiota populations which were isolated from in-shell peanuts in two seasons based on serial dilution.

Means followed by different letters are significantly different (P=0.05).

Season	2007				2008			
Regions	*Aspergillus* section *Flavi*	*A.* section *Nigri*	*A. section Circumdati*	Total fungal counts	*Aspergillus* section *Flavi*	*A.* section *Nigri*	*A.*section *Circumdati*	Total fungal counts
Alexandria	$4.24^{a}\pm0.22$	$3.96^{a}\pm0.15$	0.0	$4.45^{a}\pm0.17$	$5.57^{a}\pm0.09$	$4.71^{a}\pm0.11$	$4.84^{a}\pm0.02$	$5.97^{a}\pm0.06$
El-Behiera	$3.00^{b}\pm0.21$	$3.84^{ab}\pm0.06$	0.0	$3.95^{b}\pm0.07$	$2.91^{b}\pm0.10$	$3.51^{b}\pm0.06$	$0.72^{b}\pm0.37$	$3.68^{b}\pm0.06$
El-Sharqiya	$3.68^{c}\pm0.30$	$3.54^{ab}\pm0.22$	0.0	$4.10^{ab}\pm0.12$	$5.56^{a}\pm0.01$	$4.79^{a}\pm0.17$	$4.27^{c}\pm0.14$	$5.93^{a}\pm0.03$
El-Daqahilya	$3.78^{c}\pm0.17$	$3.47^{bc}\pm0.16$	0.0	$4.06^{ab}\pm0.12$	$3.25^{b}\pm0.07$	$3.38^{b}\pm0.04$	$2.26^{d}\pm0.32$	$4.37^{c}\pm0.17$
Asyut	$3.21^{b}\pm0.18$	$3.74^{ac}\pm0.16$	0.0	$3.90^{b}\pm0.10$	$3.11^{b}\pm0.05$	$2.55^{c}\pm0.19$	$2.51^{d}\pm0.05$	$3.86^{b}\pm0.31$

Table 3. Statistical analyses of significance of (a) *Aspergillus* section *Flavi* ((log CFU g^{-1} in-shell peanuts) (b) *Aspergillus* section *Nigri* and (c) *Aspergillus* section *Circumdati* in relation to regions, season and their interaction.

(a)

Effect	SS	DF	MS	F	P
Intercept	440.3368	1	440.3368	5525.136	0.000000
Regions	18.5865	4	4.6466	58.304	0.000000
Season	1.8346	1	1.8346	23.019	0.000110
Regions x season	6.5233	4	1.6308	20.463	0.000001
Error	1.5939	20	0.0797		

(b)

Effect	SS	DF	MS	F	P
Intercept	419.3993	1	419.3993	6745.462	0.000000
Regions	5.6958	4	1.4239	22.902	0.000000
Season	0.0281	1	0.0281	0.451	0.509343
Regions x season	5.1241	4	1.2810	20.604	0.000001
Error	1.2435	20	0.0622		

(c)

Effect	SS	DF	MS	F	P
Intercept	63.92400	1	63.92400	833.1498	0.000000
Regions	16.41431	4	4.10358	53.4837	0.000000
Season	63.92400	1	63.92400	833.1498	0.000000
Regions x season	16.41431	4	4.10358	53.4837	0.000000
Error	1.53451	20	0.07673		

SS: sum of squares, DF: degree of freedom, MS: mean square, P: probability at P=0.05.

Figure legends

Figure 1. Frequency of isolation of dominant groups from peanut seeds from different regions in (a) 2007 and (b) 2008 based on direct plating on MS medium for 7 d at 25°C.

Figure 2. PCR-based detection of *A. flavus* using FLA1/FLA2 primers. Lanes 1–8: Tested strains. Lane 9: non-template control. Lane 10: *A. flavus* 2092. Lane 11: *A. flavus* SRKC-G1907. Lane 12: *A. parasiticus* SSWT BN009-E. Lane 13: *A. parasiticus* SSWT 2999. Lanes 14-22: Tested strains. M: DNA molecular size marker.

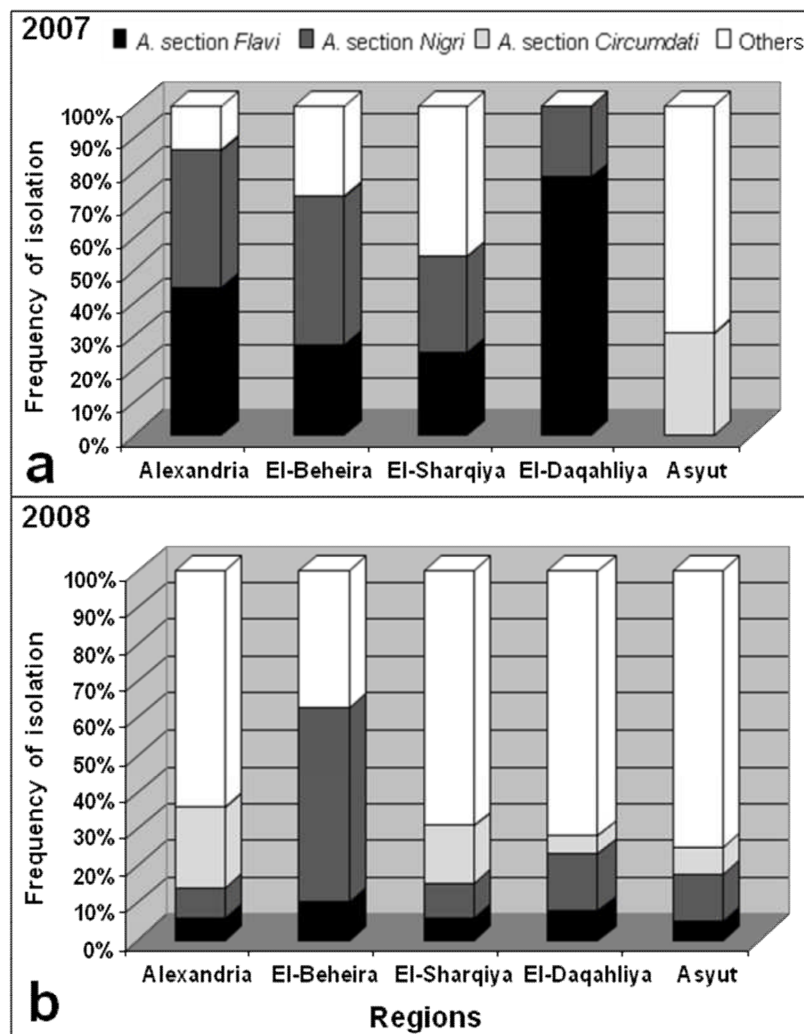
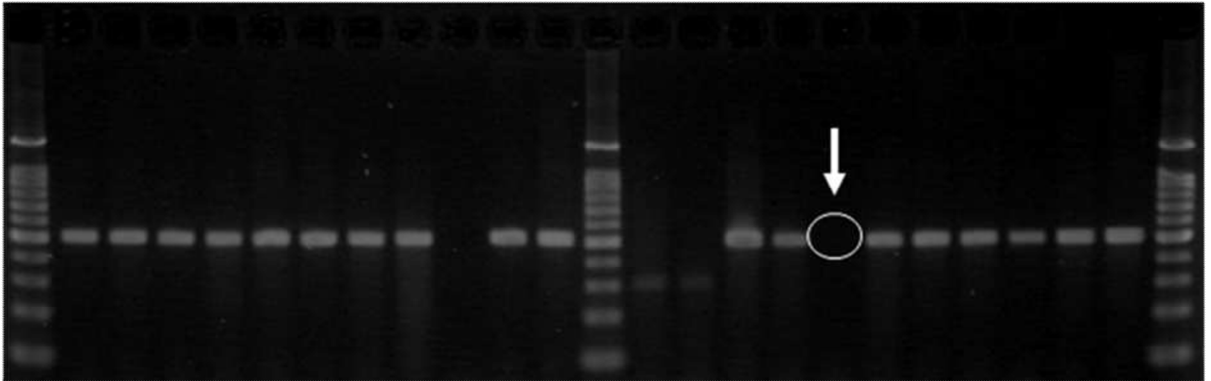


Figure 1. Sultan and Magan


M 1 2 3 4 5 6 7 8 9 10 11 M 12 13 14 15 16 17 18 19 20 21 22 M