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THE INFLUENCE OF OZONE GASEOUS ON FUNGAL SPOILAGE AND AFLATOXIN DEGRADATION IN PEANUTS

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ABSTRACT

Peanut or groundnut (*Arachis hypogea* L.), a member of the legume family, is an important food and oil crop. It is currently grown on approximately 42 million acres worldwide and considers the third major oilseed of the world after soybean and cotton. The aims of the current study were to identify the toxigenic fungi associated with peanut and to study the effect of ozone gaseous (O_3) on fungal spoilage and aflatoxin concentration in peanuts. Peanut samples were collected from three Egyptian governorates , i.e., Sharkia, Cairo and Ismailia during the season of 2007. Peanut samples were exposed to O_3 at doses of 20 ppm for 5 min, 40 ppm for 10 min and 50 ppm for 5 min. Total fungal counts were estimated in ozonated and non-ozonated peanuts shells and seeds using tow different media and aflatoxin concentration was determined in the ozone treated and non-treated peanuts. The results indicated that all the shell and seed samples were infected with fungi and the samples from Sharkia were the most infected recording the highest total fungal counts followed by the samples collected from Cairo governorate. *Aspergillus flavus* was isolated from all seed samples but did not isolated from peanut shells. Exposure to O_3 gaseous was effective to reduce total fungal counts in a dose dependent manner and succeeded to eliminate *A. flavus* in seed samples. All seed samples were contaminated with aflatoxin. Exposure to O_3 at 40 ppm for 10 min succeeded to degrade aflatoxin in peanut seeds.

Keywords: Peanut, fungi, mycotoxins, antifungal, ozone, Egypt.

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INTRODUCTION

Peanut or groundnut (*Arachis hypogea* L.), a member of the legume family, is an important food and oil crop. It is currently grown on approximately 42 million acres worldwide. It is the third major oilseed of the world after soybean and cotton (FAO, 1999). India, China, and the United States have been the leading producers for over the last 25 years and grow about 70% of the world's crop. In Egypt, peanut is one of the most important leguminous crops as well as in many parts of the world. It is used for human consumption, oil production, food industries and animal feeding. The total production of peanut in Egypt was 26255 metric tons harvested from 29338 feddan, with an average yield of 895 kg/ feddan (CAPMAS, 2006). Egypt is a major peanut exporting country and the European markets accounts for 68 percent of its peanut exports. In 1999, the European Commission suspended the import of peanuts from Egypt due to the presence of aflatoxin in concentrations in excess of maximum levels specified in EU regulations. The Egyptian government is anxious to restore Egyptian peanuts full access to the European market.

Although aflatoxin contamination of peanuts occurs during post-harvest curing and storage, the most significant contamination usually occurs prior to harvest during periods of late season drought stress as peanuts are maturing. The losses caused by fungal infection are mainly due to the rejection of food with visible fungal growth and /or to its probable content of mycotoxins. Several types of aflatoxins exist, but the four main types are Aflatoxin B_1, B_2, G_1 and G_2, with Aflatoxin B_1 being the most toxic (Olaru *et al.,* 2008). *Aspergiulls flavus* and *A. parasiticus* can produce the B toxins; *A. parasiticus* (more prevalent in peanuts than in other crops) also produces the G toxins (Diener *et al.*, 1987; Klitch and Pitt, 1988). Optimum growth conditions for *A. flavus* during post harvest are between 25°C and 30°C and humidity levels of 0.99 aw, with production of aflatoxin occurring optimally at 25°C and 0.99 aw (Giorni *et al.,* 2009). To minimize aflatoxins contamination and fungal growth in peanuts, several strategies included physical, chemical and biological means have been reported (CAST, 2003).

The most recent approaches is the use of ozone gas (O_3) technology for successful detoxification and elimination of mycotoxins from agricultural commodities. In 1997, the FDA approved O_3 for use in the U.S. food processing and fresh produced industries. O_3 gas has been used with success to inactivate contaminant microflora on meat, poultry, eggs, fish, fruits, vegetables and dry foods. It extends the shelf life of such products while preserving its sensory attributes with minimal destruction of nutrients. The aims of the current study were to identify the toxigenic fungi associated with peanut collected from different Egyptian governorates, determination of aflatoxins in peanut samples and to evaluate the effect of O_3 treatment to reduce fungal growth and aflatoxins production in peanut samples.

MATERIALS AND METHODS

Peanut Samples

Peanut samples (*Arachis hypogea* L.) were collected from different Egyptian Governorates (Sharkia, Cairo and Ismailia) during the season of 2007. Sixty samples from each location (10 kg each) of peanut shells and seeds from each governorate were stored in polyethylene bag in the frigidaire for different studies.

Chemicals, Media and Reagents

Aflatoxin B_1 standards, methanol, acetonitrile, toluene, acetic acid and sodium chloride were purchased from Sigma, Chemical Co. (St. Louis, MO, U.S.A.). The immunoaffinity column AflaTes® HPLC were obtained from VICAM (Watertown, MA, USA.) All solvents were of HPLC grade. The water was double distilled with millipore water purification system (Bedford, M A, USA).

Ozone (O_3) Production and Treatment

O_3 gas was produced from air using ozone generator unit model ozo 6VTTL (OZO MAX LTD, shefford, quebec, Canada). Peanut seeds or shell samples were exposed to O_3 at three concentrations for different time as follow: 20 ppm for 5 min, 40 ppm for 10 min and 50 ppm for 5 min (McKenzie *et al*., 1997)

Fungal Isolation and Identification

Fungi associated with control or O_3-treated seeds or shells were isolated according to the international groundnut *Aspergillus flavus* Nursery guide (Igafan, 1980). Each five seeds or 5 pods shells were placed in 20 ml sterile container and sterilized distilled water was added and left for 2 minutes to allow the samples to sink. Water was drained off and 2.5% equal solution of sodium hypochlorite was added and left for 3 minutes. Excess solution was drained off and immediately pods were rinsed in 3 changes sterilized distilled water. Water was drained off and the samples were dried between two layers of sterilized filter papers. Each sample was separated into shell and seed with sterilized scalpel. The shells and seeds were plated on each of Rose Bengal streptomycin agar medium (Allen, 1961) and *Aspergillus flavus* agar specific medium at rate of five seeds or shells/dish and all plates were incubated at 28 °C ± 2 for 5-8 days.

The fungal colonies were examined microscopically by observing the colonial morphology color of colony, texture, shape and surface appearance and cultural characteristic- a sexual and sexual reproductive structures like sporangia, conidial head, arthrospores, the vegetative mycelia and septate or non-septate. All fungal isolates were identified to the generic or species level according to Gilman (1957); Nilson *et al.* (1983) and Barnett and Hunter (1986).

The percentage of natural seeds and shells infection, the total fugal counts and the frequency occurrence of different fungi associated with shells were determined.

Production of Aflatoxins

Cultures were grown on standard Petri dishes (90 mm diameter) containing approximately 15 ml of solid medium prepared according to the method described by Gonzalez *et al.* (1987). For each combination, three plates of medium were inoculated with 10 ml of the spore suspension of each fungal strain (*Aspergillus flavus* and *A. parasiticus*) dispensed from a micropipette. Inoculated plates were incubated in an upright position at 25, 30 and 36 ^{O}C for 2 weeks.

Extraction of Aflatoxins

Aflatoxins were extracted according to the method described by VICAM (1999). In brief, 50g of sample were mixed with 10 g salt sodium chloride and place in blender jar. A 200 ml methanol: water (80:20) were added. The sample was blend at high speed for 1 min. The pour was extracted into fluted filter paper and the filtrate was collected in a clean vessel. Ten ml of the filtered extract were placed into a clean vessel, diluted with 40 ml of purified water and mixed well. The diluted extract was filtered through glass microfiber filter into a glass syringe barrel using markings on barrel to measure 4 ml.

Purification

Four ml filtered diluted extract (4 ml = 0.2 g sample equivalent) were completely passed through AflaTest ®-P affinity column at a rate of about 1-2 drops/second until air comes through column. Five ml of purified water were passed through the column at a rate of about 2 drops/second. The affinity column was eluted by passing 1.0 ml HPLC grade methanol through column at a rate of 1-2 drops/second and all of the sample elute (1ml) was collected in a glass vial. Methanol was evaporated to dryness under stream of nitrogen and aflatoxin was determined by HPLC.

Determination of AFB_1 by HPLC

Derivatization

The derivatives of samples and standard were done as follow: one hundred µl of trifluor acetic acid (TFA) were added and mixed well for 30 s and the mixture stand for 15 min. Nine hundreds µl of water: acetonitrile (9:1 v/v) were added and mixed well by vortex for 30 s and the mixture was used for HPLC analysis.

HPLC conditions

The mobile phase consists of acetonitrile/water/ methanol (1:6:3). The separation was performed at ambient temperature at a flow rate of 1.0 ml/min. The injection volume was 20 µl for both standard solutions and sample extracts. The fluorescence detector was operated at an excitation wavelength of 365 nm and an emission wavelength of 450 nm. AFB_1 concentration in samples was determined from the standard curve using peak area for quantitation.

RESULTS AND DISCUSSION

The results of the current study revealed that all peanut seeds and shell samples collected from different governorates were found to be infected with fungi, the seeds samples collected from Sharkia recorded the highest total fungal count (TFC) followed by the samples collected from Cairo then Ismailia when the Rose Bengal medium was used (Table 1). The most prevalent fungus was *A. niger* in all peanut seed samples. However, number of *A. flavus* isolates was the highest in all seed samples collected from Cairo and Ismalia compared to those collected from Sharkia. On the other hand, data presented in Table 2 revealed that all shell samples were infected with fungi and the most prominent fungus was *A. niger* . However; *A. flavus* was not found in all shell samples.

Data presented in Fig.1 indicated that treatment with O_3 succeeded to reduce TFC in all seed and shell samples for different governorates in a dose dependent manner. The reduction percentages due to O_3 treatment in the different doses tested i.e. 20 ppm for 5 min, 40 ppm for 10 min and 50 ppm for 5 min recorded 31, 44.8 and 65.5% in Sharkia samples; 39, 60.8 and 82.6% in Cairo samples and 13.4, 40.9 and 68.2% in Ismailia samples respectively. However, the reduction percentage in shell samples due to O_3 treatments at the three tested doses (Fig 1) recorded 21, 42 and 63% in the samples collected from Sharkia; 16.6, 33.3 and 66.7% in the samples collected from Cairo and 40.9, 50 and 68.2% in the samples collected from Ismalia.

When *Aspergillus flavus* agar specific medium was used, all peanut seed samples were found to be infected with fungi (Table 3) and the seed samples collected from Ismalia was the most infected followed by those collected from Sharkia then Cairo. *A. flavus* was the most prevalent in the seed samples collected from Sharkia however, *A. niger* was the most prevalent in seed samples collected from Cairo and Ismailia . It is interest to mention that *A. flavus* was completely absent in the shell samples collected from the three governorates when the same medium was used (Table 4). Similar to the current results, El-Magraby *et al.* (1988) isolated 43 species of fungi, belonging to 16 genera from peanuts samples collected from Egypt. Moreover, Youssef *et al.* (2008) found *A. flavus, A. niger, A. ficuum, Penicilliums* spp. and *Fusarium* spp. in Egyptian peanut kernels.

Treatment with O_3 at the three tested doses succeeded to induce a reduction in TFC in all seed samples and the percentage of reduction recorded 40, 60 and 72%

Table 1. TFC associated with control and O_3-treated* peanut seed samples collected from different governorates isolated using Rose Bengal medium

	Sharkia				Cairo				Ismailia			
Organism	**Control**	**Ozone 1**	**Ozone 2**	**Ozone 3**	**Control**	**Ozone 1**	**Ozone 2**	**Ozone 3**	**Control**	**Ozone 1**	**Ozone 2**	**Ozone 3**
A. Flavus	2	1	1	1	3	2	1	1	3	2	1	1
A. niger	13	7	5	4	9	6	4	2	9	7	5	3
A. sydow	5	4	4	2	4	2	1	0	3	3	2	1
A. terreus	1	1	0	0	0	0	0	0	0	0	0	0
A. ochraceaous	1	1	1	0	1	1	0	0	0	0	0	0
Fusarium	1	1	1	0	1	0	0	0	1	2	0	0
Penicillium	1	1	1	1	1	1	1	0	0	0	0	0
Rhizopus	3	2	1	1	2	2	1	1	3	3	3	1
Mucor	2	2	2	1	2	2	1	0	3	2	2	1
Macrophemena	0	0	0	0	0	0	0	0	0	0	0	0
Total	29	20	16	10	23	16	9	4	22	19	13	7

*Ozone 1: 20 ppm for 5 min, Ozone 2: 40 ppm for 10 min, Ozone 3: 50 ppm for 5 min.

Table 2. TFC associated with control and O_3-treated* peanut shell samples collected from different governorates isolated using Rose Bengal medium

	Sharkia				Cairo				Ismailia			
Organism	**Control**	**Ozone 1**	**Ozone 2**	**Ozone 3**	**Control**	**Ozone 1**	**Ozone 2**	**Ozone 3**	**Control**	**Ozone 1**	**Ozone 2**	**Ozone 3**
A. Flavus	0	0	0	0	0	0	0	0	0	0	0	0
A. niger	9	7	5	3	9	7	5	4	8	5	5	3
A. sydow	1	1	0	0	1	1	1	0	2	1	1	0
A. terreus	0	0	0	0	0	0	0	0	0	0	0	0
A. ochraceaous	0	0	0	0	0	0	0	0	0	0	0	0
Fusarium	0	0	0	0	0	0	0	0	1	0	0	0
Penicillium	2	0	0	0	1	0	0	0	3	1	0	0
Rhizopus	3	3	3	2	3	3	2	1	3	2	2	2
Mucor	3	3	2	1	3	3	3	1	4	3	2	2
Macrophemena	1	1	1	1	1	1	1	0	1	1	1	0
Total	19	15	11	7	18	15	12	6	22	13	11	7

*Ozone 1: 20 ppm for 5 min, Ozone 2: 40 ppm for 10 min, Ozone 3: 50 ppm for 5 min.

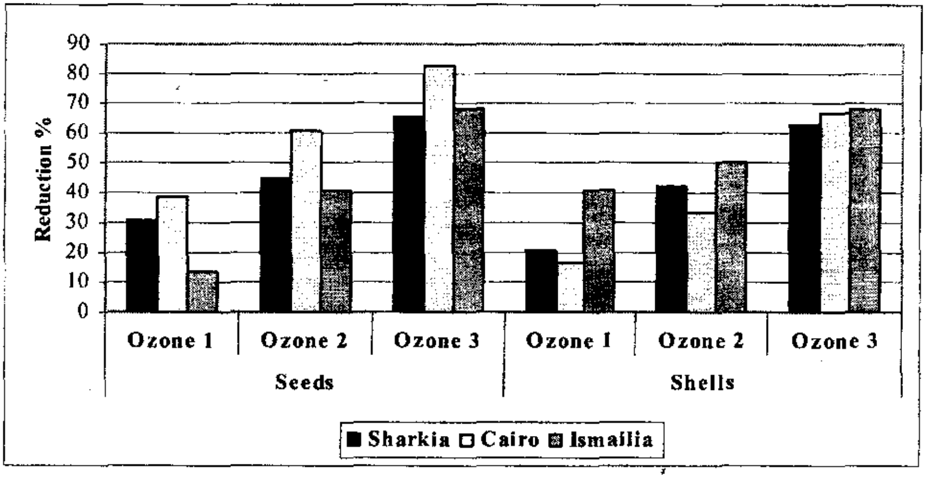

Fig. 1. Effect of different doses of O_3 on reduction percentages of TFC in peanuts seeds and shells collected from different governorates using RB medium (Ozone 1: 20 ppm for 5 min, Ozone 2: 40 ppm for 10 min and Ozone 3: 50 ppm for 5 min)

Table 3. TFC associated with control and O_3-treated* peanut seed samples collected from different governorates isolated using *Aspergillus flavus* agar specific medium

Organism	Sharkia				Cairo				Ismailia			
	Control	Ozone 1	Ozone 2	Ozone 3	Control	Ozone 1	Ozone 2	Ozone 3	Control	Ozone 1	Ozone 2	Ozone 3
A. Flavus	8	3	2	2	6	3	2	1	6	5	3	2
A. niger	6	4	2	1	7	5	2	1	9	5	3	2
A. sydow	3	2	2	1	2	1	1	0	5	4	3	1
A. terreus	0	0	0	0	0	0	0	0	0	0	0	0
A. ochraceaous	0	0	0	0	0	0	0	0	0	0	0	0
Fusarium	2	1	1	0	1	0	0	0	0	0	0	0
Penicillium	1	1	0	0	1	1	1	0	2	1	1	0
Rhizopus	2	2	2	2	1	1	1	0	3	2	1	1
Mucor	2	1	1	1	1	1	1	0	3	2	2	1
Macrophemena	1	1	0	0	0	0	0	0	0	0	0	0
Total	25	15	10	7	19	12	8	2	28	19	13	7

*Ozone 1: 20 ppm for 5 min, Ozone 2: 40 ppm for 10 min and Ozone 3: 50 ppm for 5 min.

Table 4. TFC associated with control and O_3-treated* peanut shell samples collected from different governorates isolated using *Aspergillus flavus* agar specific medium

Organism	Sharkia				Cairo				Ismailia			
	Control	Ozone 1	Ozone 2	Ozone 3	Control	Ozone 1	Ozone 2	Ozone 3	Control	Ozone 1	Ozone 2	Ozone 3
A. Flavus	0	0	0	0	0	0	0	0	0	0	0	0
A. niger	9	6	4	3	10	7	5	3	9	7	5	3
A. sydow	1	1	0	0	3	2	1	0	3	3	1	1
A. terreus	0	0	0	0	0	0	0	0	0	0	0	0
A. ochraceaous	0	0	0	0	0	0	0	0	0	0	0	0
Fusarium	1	0	0	0	1	0	0	0	2	0	0	0
Penicillium	2	0	0	0	0	0	0	0	2	1	0	0
Rhizopus	3	2	2	2	2	2	2	1	4	3	2	2
Mucor	3	2	2	1	2	2	1	1	3	2	2	2
Macrophemena	1	1	1	1	2	1	0	0	0	0	0	0
Total	20	12	9	7	20	14	9	5	23	16	10	8

*Ozone 1: 20 ppm for 5 min, Ozone 2: 40 ppm for 10 min and Ozone 3: 50 ppm for 5 min.

in Sharikia samples, 36.8, 57.9 and 89.5% in Cairo samples and 32, 53.6 and 75% in Ismailia samples (Fig. 2). Although all the shell samples were infected with fungi, *A. niger* was the most prevalent in all governorates and the samples collected from Ismailia recorded the highest TFC compared to those collected from Sharkia or Cairo. The reduction percentage due to O_3 treatment for shell samples recorded 40, 55 and 65% in Sharkia samples; 30, 55 and 75% in Cairo samples and 30.4, 56.5 and 65.2% in Ismailia samples for the three tested doses of O_3 respectively (Fig. 2). When *A. flavus* agar specific medium was used, *A. flavus* was the prominent and its total isolates recorded 8, 3, 2, 2 for Sharkia seed samples, 6, 3, 2, 1 for Cairo seed samples, and 6, 5, 3, 2 for Ismailia seed samples in the control and O_3-treated samples at the three tested doses respectively (Fig. 3). Moreover, the reduction percentage in *A. flavus* due to O_3 treatment recorded 62.5, 75, 75 for Sharkia samples, 50, 66.7. 83.3 for Cairo samples and 17.7, 50, 66.7 for Ismailia samples for the three tested doses of O_3 respectively (Fig. 4). Similar to the current results, El-Magraby *et al.* (1988) isolated 43 species of fungi, belonging to 16 genera from peanuts samples collected from Egypt. Moreover, Youssef *et al.* (2008) found *A. flavus, A. niger, A. ficuum, Penicilliums* spp. and *Fusarium* spp. in Egyptian peanut kernels.

Kumar *et al.* (2008) reported that soil samples in major peanut growing areas of Gujarat in India showed predominance *A. flavus* with a positive correlation between *A. flavus* soil population and aflatoxin contamination in peanut kernels. On the other hand, Gonzalez *et al.* (2008) found *A. flavus*, *Rhizopus* spp. and *Fusarium* spp. as the prevalent fungi in peanut hulls from Sao Paulo state in Brazil. In the same regards, Udagawa (1976) isolated *A. flavus*, *A. niger*, *Penicillium citrinum*, *P. cyclopium*, *P. funiculosum*, *P paraherquei*, *Fusarium* and *Rhizopus* from groundnut samples in Papua New Guinea and *A. flavus*, *A. terreus*, *A. niger* and *Mucor*. Furthermore, Richard and Abas, (2008); and Kumar *et al.* (2008) reported that *A. ochraceus*, *A. versicolor*, *P. citrinum* and *F. verticillioides* are other toxigenic strains of other mycotoxigenic fungi are associated with peanuts.

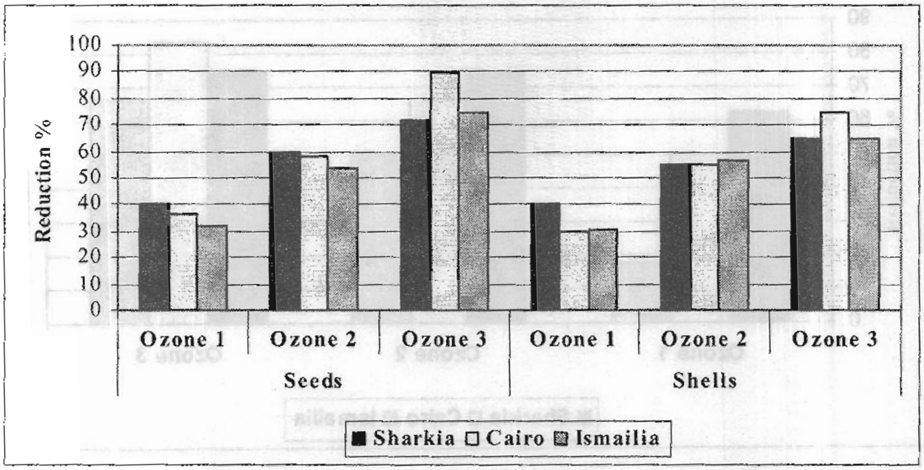

Fig. 2. Effect of different doses of O_3 on reduction percentages of TFC in peanuts seeds and shells collected from different governorates using *A. flavus* agar specific medium (Ozone 1: 20 ppm for 5 min, Ozone 2: 40 ppm for 10 min and Ozone 3: 50 ppm for 5 min).

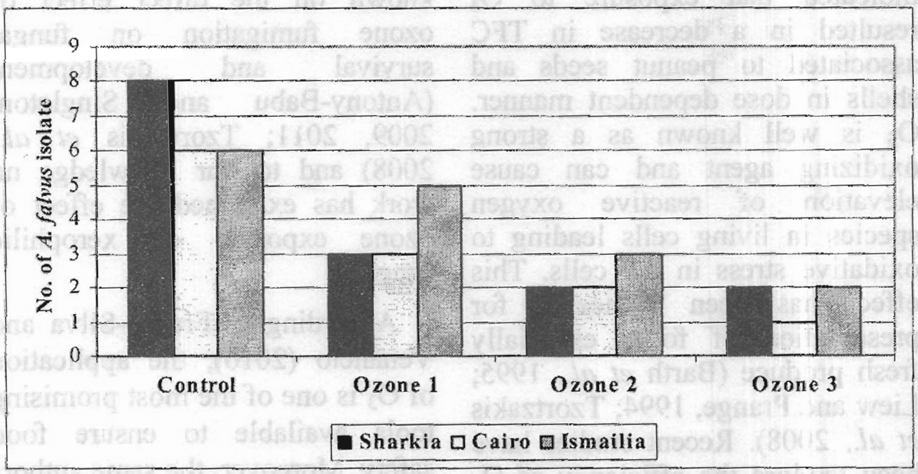

Fig. 3. Number of *A. flavus* isolated from control and O_3-treated peanuts seed samples collected from different governorates (Ozone 1: 20 ppm for 5 min, Ozone 2: 40 ppm for 10 min and Ozone 3: 50 ppm for 5 min).

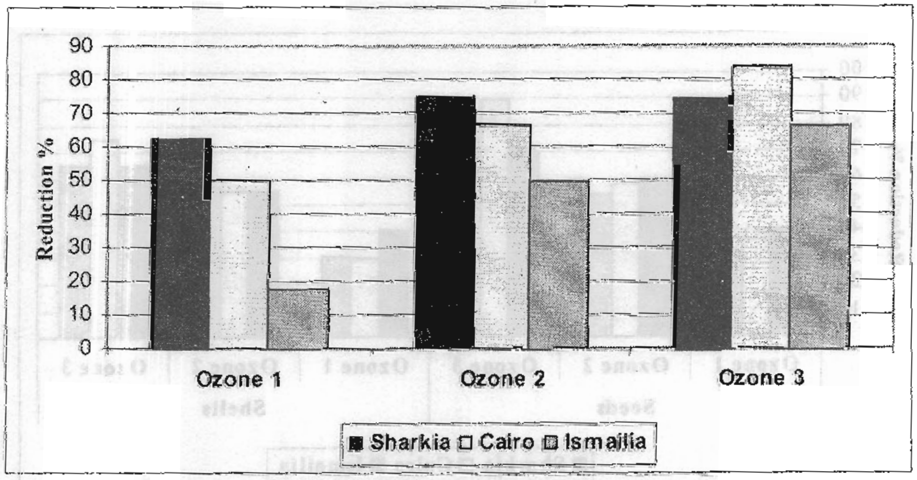

Fig. 4. Reduction percentage of *A. flavus* in peanuts seed samples after O_3 treatments at the three tested doses (Ozone 1: 20 ppm for 5 min, Ozone 2: 40 ppm for 10 min, and Ozone 3: 50 ppm for 5 min).

The present results clearly indicated that exposure to O_3 resulted in a decrease in TFC associated to peanut seeds and shells in dose dependent manner. O_3 is well known as a strong oxidizing agent and can cause elevation of reactive oxygen species in living cells leading to oxidative stress in the cells. This effect has been harnessed for preservation of food, especially fresh produce (Barth *et al.,* 1995; Liew and Prange, 1994; Tzortzakis *et al.,* 2008). Recent studies have been explore the efficiency of O_3 treatment in preservation of low moisture foods (Al-Ahmadi *et al.,* 2009; Najafi and Khodaparast, 2009). Nevertheless, very little is known on the direct effect of ozone fumigation on fungal survival and development (Antony-Babu and Singleton, 2009, 2011; Tzortzakis *et al.,* 2008) and to our knowledge no work has examined the effect of ozone exposure on xerophilic fungi.

According to Freitas-Silva and Venâncio (2010), the application of O_3 is one of the most promising tools available to ensure food safety. Moreover, the same authors reported that the application of O_3 in low doses can directly protect Brazil nuts from contamination, by reducing the growth of pathogenic microorganisms and decay and, consequently, ensuring product quality. The effect of O_3 on fungal growth may be explained as suggested by Adams and Moss, (2008) who reported that during food storage, spores are the major source of inoculum. Hence reduction or inhibition of spore production is very advantageous in food storage facilities. Such inhibition/reduction of spore production has been previously observed in fungi cultured under O_3 rich environment (Antony-Babu and Singleton, 2009 and Tzortzakis *et al.,* 2008). Interestingly, in the current work, the ability of O_3 to reduce spore production was dependent on the concentration of sucrose present in the growth media.

The results of the current study indicated that the control peanut seed samples were contaminated with aflatoxin B_1 and B_2 in concentration reached 10.07 and 0.46 ng/g. However, peanuts seeds inoculated with *A. flavus* were contained AFB_1 and AFB_2 in concentrations reached 38.43 and 1 ng/g. In this concern, Abbas *et al.* (2005) studied the relationships between aflatoxin productions among isolates of *Aspergillus* section *Flavis* from the Mississippi Delta and observed that about 50% of the isolates from peanut produced aflatoxins. Environmental conditions required to induce pre-harvest aflatoxin contamination of groundnuts was studied by Cole *et al.* (1989). These authors showed that groundnuts do not become contaminated with aflatoxins in the absence of severe and prolonged drought stress in spite of invasion levels of up to 80% by *A. flavus* and *A. parasiticus*The role of environmental stress in predisposition of groundnuts to aflatoxin contamination was demonstrated by several workers (Sanders *et al.,* 1985; Thai *et al.,* 1990; Lisker *et al.,* 1993 and Kumar *et al.,* 2008). Although, roots did not suffer drought stress, the risk of aflatoxin contamination increased (Sanders *et al.,* 1993). Consequently, the rainy season encourages *A. flavus* infection and aflatoxin contamination and combination of critical pre- and post-harvest factors at soil, plant and storage levels reduced aflatoxin risk substantially (Ghewande, 1997).

According to Freitas-Silva and Venâncio, (2010), the application of O_3 is one of the most promising tools available to ensure food safety. Moreover, the same authors reported that the application of O_3 in low doses can directly protect Brazil nuts from contamination, by reducing the growth of pathogenic microorganisms and decay and, consequently, ensuring product quality. The effect of O_3 on fungal growth may be explained as suggested by Adams and Moss, (2008) who reported that during food storage, spores are the major source of inoculum.

Hence reduction or inhibition of spore production is very advantageous in food storage facilities. Such inhibition/reduction of spore production has been previously observed in fungi cultured under ozone rich environment (Antony-Babu and Singleton, 2009; Tzortzakis *et al.*, 2008). Interestingly, in this work, the ability of ozone to reduce spore production was dependent on the concentration of sucrose present in the growth media.

The effect of O_3 treatment on aflatoxins concentration reported in the current study revealed that O_3 succeeded to degrade aflatoxin content at a dose of 40 ppm and exposure time 10 min and the recorded concentrations reached 2.08 and 0.52 ng/g for AFB_1 and AFB_2 respectively (Fig 5). These levels are blew the maximum level in the Egyptian regulations which set a maximum level of 5 μg/kg AFB_1 and 10 μg/kg total aflatoxins in human food. It is well knowen that ozonation is an oxidation method has been developed for the detoxification of aflatoxins in foods (Samarajeewa *et al.,* 1990). O_3 is a powerful disinfectant and oxidising agent (McKenzie *et al.,* 1997). It reacts across the 8, 9 double bond of the furan ring of aflatoxin through electrophilic attack, causing the formation of primary ozonides followed by rearrangement into monozonide derivatives such as aldehydes, ketones and organic acids (Proctor *et al.,* 2004). Several studies have been undertaken to evaluate the effects of O_3 in reducing aflatoxin levels in contaminated agricultural products. Maeba *et al.* (1988) have confirmed the destruction and detoxification of aflatoxins B_1 and G_1 with O_3.

Conclusion

It could be concluded from the current study that peanuts seed and shell collected from different Egyptian governorates was infected with different fungi. *A. falvus* was

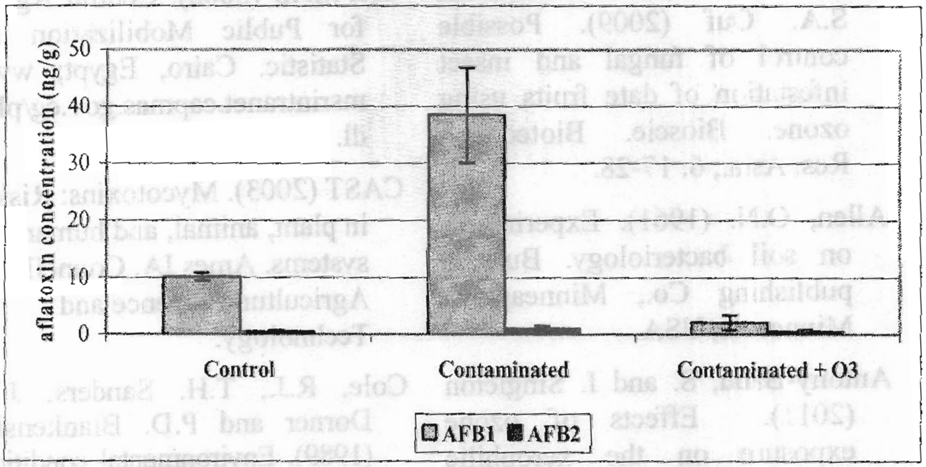

Fig. 5. Aflatoxin concentration in naturally-contaminated (control), artificial-contaminated and O_3-treated peanut seed samples exposed to O3 at 40 ppm for 10 min .

prominent in all peanut seeds but not peanut shell and was capable to produce aflatoxins. O_3 treatments succeeded to induce a significant reduction in TFC and degrade aflatoxins in peanut samples. Consequently, O_3 should be applied for peanuts to reduce fungal spoilage and aflatoxin contamination in the peanut prepared for export or for local consumption to reach the maximum level set in the Egyptian standards regulation as well as many EU countries.

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تأثير غاز الأوزون علي تلف الفطريات وتكسير الأفلاتوكسين في السوداني

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يعتبر الفول السوداني أحد أعضاء العائلة البقولية وهو من أهم الأغذية والمحاصيل الزيتية ويزرع علي مساحة تصل ٤٢ مليون أكر علي مستوي العالم كما انه يعتبر ثالث المحاصيل الزيتية الرئيسية بعد فول الصويا والقطن. استهدفت الدراسة الحالية تعريف الفطريات السامة الموجودة في الفول السوداني وكذلك دراسة تأثير غاز الأوزون (O_3) علي الفطريات والتلوث بالافلاتوكسين في عينات السوداني. تم تجميع عينات الفول السوداني من ثلاث محافظات هي(الشرقية والقاهرة والإسماعيلية) أثناء موسم عام ٢٠٠٧. تم تقدير العدد الكلي للفطريات في عينات قشور وبذور السوداني المعاملة وغير المعاملة بالاوزون علي جرعات ٢٠ جزء في المليون لمدة ٥ دقائق و ٤٠ جزء في المليون لمدة ١٠ دقائق و ٥٠ جزء في المليون لمدة ٥ دقائق باستخدام نوعين من البيئات. كما تم قياس تركيز الافلاتوكسين في بذور السوداني المعامل وغير المعامل بالأوزون. أثبتت النتائج أن كل عينات بذور وقشور السوداني ملوثه بالفطريات وكانت عينات محافظة الشرقية أكثرهم تلوثا يليها العينات المجمعة من محافظة القاهرة. كما وجد أن جميع عينات البذور المجمعة من المحافظات الثلاثة ملوثه بفطر الاسبراجلس بينما لم يتم عزل هذا الفطر من القشور. أدى التعريض للأوزون إلى نقص في العدد الكلي للفطريات وكان هذا التأثير مرتبط بالجرعة المستخدمة كما أدى إلى التخلص من فطر الاسبراجلس فلافس المنتج للافلاتوكسين. كما أثبتت النتائج أن جميع عينات البذور ملوثه بالافلاتوكسين ب١ و ب٢ وأن المعاملة بالأوزون أدت إلى حدوث نقص في تركيز الافلاتوكسين بدرجه تناسبت مع الجرعة المستخدمة. كما أن استخدام الأوزون بتركيز ٤٠ جزء في المليون لمدة ١٠ دقائق ادي الي التخلص الكامل من الأفلاتوكسين في بذور السوداني. نستخلص من هذه الدراسة أن استخدام الأوزون بتركيز ٤٠ جزء في المليون لمدة ١٠ دقائق يمكن تطبيقه بنجاح في التخلص من الفطريات والأفلاتوكسين في السوداني المعد للاستهلاك المحلي أو التصدير.