

Evaluation of Fungal Flora and Mycotoxin in Some Important Nut Products in Erbil Local Markets

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Abstract: In this study a wide range of moulds representing several genera and species was recorded. Ten samples of each pure and salted (almond, cashewnut, hazelnut, Peanut and pistachio) were collected from different markets (Shekhalla, Tayrawa and Qaysari) in Erbil city, Iraq. High infection of fungal genera was found in pure samples, while low infection was found in salted nut products samples, application of nuts with salts was found to increase the resistance of nuts to invasion and colonization by the fungi during storage. The total counts of fungi were 138×10^3 cfu/g. Samples by dilution method and represented twelve genera and twenty species of fungi by all method. Detection of Mycotoxin on Dichloran Rose Bengal Chloramphenicol Agar (DRBC) for fungal isolation from nut samples showed, *A. flavus*, *A. fumigatus*, *A. niger* and *A. ochraceous*, positive results on the culture for mycotoxin production, change in colony diameter and color change in compare with control Czapek Dox Agar (CDA). Estimation of natural occurrence of Aflatoxin (AT) and Ochratoxin (OT) in nut product samples by using ELISA method, the results show low AF and OT content in most samples which is lower than the normal rang for human consumption. The high results of aflatoxin and ochratoxin show in peanut while the low results show in cashewnut.

Keywords: Almond, *Aspergillus*, cashew nut, ELISA, mycotoxins, peanut, *Penicillium*

INTRODUCTION

Various nuts are used as a raw material in many industries as well as for a direct consumption. They contain an important of protein and fat and their products have wide acceptances food throughout the world (Watt and Merrill, 1950). Due to the extremely high fat, protein and low water content of various nuts such as hazelnut, almonds, walnuts, pistachio and cashewnut, these products are quite refractory to spoilage by microorganisms. Mould can grow upon them if they are stored under conditions that permit sufficient moisture for their propagation (Phillips *et al.*, 1979; Frank, 1981). Contamination of different kinds of nut by fungi may occur at three different stages. It may first occur prior to harvest while the nuts are on the tree. At this stage, particularly when the nuts have ripened and the hulls have opened, they are often attacked by airborne and insect-borne spores of fungal species. The second stage of contamination may occur after harvesting, when nuts are de-hulled, washed and sorted. The washing water can be a source of contamination and if the nuts are allowed to remain wet, they will be highly susceptible to mould growth and mycotoxin contamination. The third stage of contamination can be in storage, especially when nuts are stored under adverse conditions of temperature (Abdel-Hafez and Saber, 1993; Burdaspal *et al.*, 1990). The economic loss resulting from fungal and mycotoxin contamination of nuts is difficult to estimate. However, judging from the widespread occurrence of fungal and mycotoxin

contamination and the large number of nuts affected, one can assume that such losses must be large. These losses constitute direct nut losses, human illness and reduced productivity and livestock losses from deaths and lower growth rates (El-Magraby and El-Maraghy, 1988). Additional economic losses include indirect costs of various systems for control of mycoflora and mycotoxins in nuts reduced value of rejected nuts costs of detoxification to recover acceptable products and, occasionally, from loss of export markets. Although the natural contamination by mycoflora and mycotoxins of various kinds of nuts such as almond, brazil nut, cashew nut, coconut, hazelnut, peanut, pistachio nut and walnut and different nut products were investigated in many parts of the world (Jimenez *et al.*, 1991). A major problem related to fungal attack in nuts is the production of toxic secondary metabolites (Scott, 1993). Fungi can grow on simple and complex food products and produce various metabolites. These microorganisms exist in the environment and distribute by wind, insects and raining (Brus *et al.*, 2005). Up to now, more than 100000 fungal species are considered as natural contaminants of agricultural and food products (Kacaniova, 2003). There are more and more indications that primary liver carcinoma and other serious diseases may be induced by consuming food or using raw materials for food processing contaminated with fungi or mycotoxins. Aflatoxins, ochratoxin and sterigmatocystin proved resistant to heat and have an ability to accumulate in the organism. The majority of the toxic species belongs to the genera *Aspergillus*,

Table 1: English and scientific names of the tested nuts products

No	English name	Scientific names
1	Almond (pure and salted)	<i>Prunus dulcis</i>
2	Cashews (pure and salted)	<i>Anacardium occidentale</i>
3	Hazelnut (pure and salted)	<i>Corylus colurna</i>
4	Peanut (pure and salted)	<i>Arachis hypogaea</i>
5	Pistachio (pure and salted)	<i>Pistacia vera</i>

Penicillium and *Fusarium*. Other species of molds are frequently isolated, including *Scopulariopsis* and *Sporendonema* (Galvano *et al.*, 2005; Zinedine *et al.*, 2006).

The aim of the present study was to determine mycoflora distribution of nuts such as pure and salted (almond, cashewnut, hazelnut, Peanut and pistachio) and to determine the level of aflatoxin and ochratoxin in nut products.

MATERIALS AND METHODS

Sampling: Sampling was done randomly on some human food products such as nut products. A total of ten samples, representing various types of Nut Products salted (roasted and salted), unsalted (pure), shelled and unshelled were collected randomly from different markets (Shekhalla, Tayrawa and Qaysari) of different localities in the Erbil city during the months of November and December 2012. The nuts include Almonds (*Prunus dulcis*), Cashewnuts (*Anacardium occidentale*), Hazelnuts (*Corylus colurna*), Peanut (*Arachis hypogaea*) and Pistachio (*Pistacia vera*). A total of different markets were investigated and each sample was purchased from three different sellers. These products were chosen on the basis of their availability in the market and popularity of usage. Nut Products samples were usually found outside, kept in metal or plastic containers or wooden boxes. For each samples, 3 replicates were taken and mixed to prepare one composite sample. Each sample was sealed in a double sterile polyethylene bag and stored at 3-5°C until mycoflora determination and mycotoxin analysis were performed. In the laboratory, samples were individually finely ground in a common household blender. The blender's cup was rinsed in 85% alcohol between samples. The powder kept tightly packed in a new study bags and stored at 4°C for further analysis. The common names and scientific name of each sample are presented in Table 1.

Mycological analysis (isolation techniques):

Isolation of fungi by direct plating method:

- **Isolation of sample-borne mycoflora:** The method used for isolation of fungi was previously described by Abdullah *et al.* (2002), 10 gm of each sample were surface sterilized by 6% sodium hypochlorite solution (NaOCl) in a sterile conical flask for 1-2 min and then washed by distill water 3

times for 1.5-2 min to removing the toxic activity of the chemical agent on the samples. The disinfected samples transferred with sterile forceps into Petri dish contain sterilized Czapek Dox Agar (CDA), at the rate of (5-10) pieces per plate, depending on the size of the particles, larger samples cut into small pieces. CDA supplemented with 0.5 mg chloramphenicol/mL to restrict bacterial growth. Three replicates were made and the plates were incubated at 25°C for 5-7 days. Fungi colonies were identified according to morphological and microscopic characteristics.

- **Isolation of sample surface mycoflora:** The samples transferred with sterile forceps into Petri dish contain sterilized CDA. Three replicates were made and the plates were incubated at 25°C for 5-7 days. Fungi colonies were identified according to morphological and microscopic characteristics (Pitt *et al.*, 1992).

Standard dilution plate: For fungal analysis, dilution method was used to determine total fungal counts in nut products samples. One grams of each composite sample (fine powder) were transferred into screw-capped medicinal bottle containing 9 mL of sterile distilled water and were mechanically homogenized at constant speed for 15 min. The sample-water suspension was allowed to stand for 10 min with intermittent shaking before being plated. Appropriate tenfold serial dilutions (1:10) were prepared and one mL portions of suitable dilutions of the resulting samples suspension (10^{-3}) were used to inoculate Petri dishes each containing 15 mL Potato Dextrose Agar (PDA). Plates were then incubated for 7 days at 28°C. Three replicates plates per medium were used for each sample and the developing fungi were counted and identified according to several key processes. After incubation, the results were expressed in Colony-Forming Units (CFU) /g of samples; all plates were examined visually, directly and with a microscope (Aziz *et al.*, 1998; Suleiman and Taiga, 2009).

Diagnosis:

Identification of the fungal genera: The fungal isolates were transferred to sterilized plates for purification and identification. The grown fungi were mounted on a slide, stained with lactophenol cotton blue to detect fungal structures (Basu, 1980), covered with a cover slip, examined under microscope and identified on the basis of their colony morphology and spore characteristics (Rajankar *et al.*, 2007; Ronhede *et al.*, 2005).

The texts (books) used for identification of fungi, depending on their taxonomic keys; (Moubasher, 1993; Larone, 2011; Pitt and Hocking, 1997; Guarro *et al.*, 1999; Howard, 2002; Watanabe, 2002; Ulhan *et al.*, 2006; Pornsuriya *et al.*, 2008).

DETERMINATION OF TOXIGENIC POTENTIAL OF FUNGI IN CULTURE MEDIA

Dichloran rose bengal chloramphenicol agar test: DRBC is a selective medium that supports good growth of fungi. The substances in the DRBC are dichloran (is added to the medium to reduce colony diameters of spreading fungi), rose bengal (suppresses the growth of bacteria and restricts the size height of colonies of the more rapidly growing moulds) and chloramphenicol (is included in this medium to inhibit the growth of bacteria present in environmental and food samples). The reduced pH of the medium from (7.2 to 5.6) helps inhibition of the spreading fungi (Jarvis, 1973; King *et al.*, 1979). The isolated fungi were inoculated in the solidified DRBC medium after incubation for 7 days at 25°C search for pigmentation and color change observed due to toxigenic compound in compare to CDA medium control. DRBC containing compounds to inhibit or reduce spreading growth of moulds such as *Mucor* sp., *Rhizopus* sp., (Samson *et al.*, 1992; Hocking *et al.*, 2006). Dichloran and rose Bengal effectively slow down the growth of fast-growing fungi, thus readily allowing detection of other yeast and mold propagules, which have lower growth rates (Tournas *et al.*, 2001).

Sample preparation for natural aflatoxin and ochratoxin determination by ELISA method: Samples were homogenized and kept in glass bottle and stored at 2-8°C until further analysis. For the quantitative analysis of mycotoxins (aflatoxin and ochratoxin), Enzyme Linked Immunosorbent Assay technique (ELISA) was used. Samples (10 g) were taken in 50 mL of 70% methanol for aflatoxins and ochratoxin analysis. Sample powder was blended individually with high speed blender for three minutes. After blending the material was filtered with Whatman filter paper number 1, the filtrate was used for further analysis. Commercially available immunoassay kit Veratox for quantitative analysis of aflatoxin and ochratoxin test-NEOGEN Crop, Lansing, MI was used. The assay kit was based on Competitive Direct Enzyme Linked Immunosorbent Assay (CD-ELISA). The antibodies captured the analyte and conjugated to the enzyme (horse reddish peroxidase). Tetra methylbenzidine/hydrogen peroxide was used as a substrate for color development. Finally stopping solution was added to stop the reaction. The color intensity was inversely proportional to the mycotoxin concentration and measured with the ELISA reader. All necessary reagents were present in the kit. Concentration of mycotoxins was calculated by Log/logit Software Awareness Technology Inc. (Anonymous, 2000; Stoloff *et al.*, 1991).

RESULTS AND DISCUSSION

Totally 10 samples were analyzed for enumeration of fungal isolates. Fungal genera were isolated by different method on two types of media: Czapek Dox Agar (CDA) and Potato Dextrose Agar (PDA). Common and scientific names of the nut products were tabulated in Table 1.

The results presented in Table 2 show fungi were isolated by using Agar Plate Method (APM) plated on CDA medium. Agar plate methods was employed for this study and two sets of samples were analyzed i.e., unsterilized and surface sterilized nut samples. A total of seven different fungal genera and sixteen species were isolated. All fungi were identified on the basis of their cultural and morphological characteristics. These were identified as *Aspergillus* spp. *Cladosporium* sp., *Mucor* sp., *Rhizopus stolonifer*, *Penicillium* spp. sterile mycellium and yeasts. It was observed that treated samples (with sodium hypochlorite) yielded less population of samples-borne fungi than the untreated

Table 2: Isolated fungi from nut products by using (APM) directly with and without sodium hypochlorite

Sample no.	Sample name	Czapex dox agar	
		Sterilized surface	Unsterilized surface
1	Almond (pure)	<i>Penicillium chrysogenum</i>	<i>Mucor</i> sp. <i>Penicillium chrysogenum</i> <i>Penicillium</i> sp.
2	Almond (salted)	<i>Rhizopus stolonifer</i> <i>Penicillium chrysogenum</i> yeast	<i>Rhizopus stolonifer</i> <i>Aspergillus flavus</i> <i>Penicillium chrysogenum</i> <i>Rhizopus stolonifer</i> Yeast
3	Cashews (pure)	<i>Penicillium citrinum</i>	<i>Penicillium citrinum</i> <i>Penicillium</i> sp.
4	Cashews (salted)	<i>Cladosporium</i> sp.	<i>Rhizopus oryzae</i> <i>Aspergillus</i> sp. <i>Mucor</i> sp.
5	Hazelnut (pure)	<i>Penicillium citrinum</i> <i>Penicillium italicum</i>	<i>Penicillium citrinum</i> <i>Aspergillus flavus</i> <i>Aspergillus niger</i> <i>Penicillium italicum</i> <i>Penicillium</i> sp.
6	Hazelnut (salted)	<i>Penicillium</i> sp. Yeast	<i>Aspergillus flavus</i> <i>Cladosporium</i> sp. <i>Penicillium</i> sp. Yeast
7	Peanut (pure)	<i>Aspergillus flavus</i> <i>Aspergillus niger</i>	<i>Aspergillus flavus</i> <i>Aspergillus niger</i> <i>Aspergillus ochraceous</i> <i>Penicillium fellutanum</i> <i>Rhizopus stolonifer</i>
8	Peanut (salted)	<i>Aspergillus fumigatus</i> <i>Penicillium fellutanum</i> Yeast	<i>Aspergillus flavus</i> <i>Aspergillus fumigatus</i> <i>Aspergillus niger</i> <i>Penicillium fellutanum</i> Yeast
9	Pistachio (pure)	<i>Penicillium crustosum</i>	<i>Aspergillus flavus</i> <i>Penicillium crustosum</i> <i>Penicillium</i> sp.
10	Pistachio (salted)	<i>Aspergillus niger</i> <i>Penicillium crustosum</i>	<i>Aspergillus niger</i> <i>Penicillium crustosum</i> <i>Rhizopus stolonifer</i> <i>Mycelia sterilia</i>

Table 3: The isolated fungi from nut product samples by standard dilution plate method

Fungi	Sample											TCFU/gm.s
	Al (p)	Al (s)	Ca (p)	Ca (s)	Ha (p)	Ha (s)	Pe (p)	Pe (s)	Pi (p)	Pi (s)		
<i>Aspergillus flavus</i>	1	0	0	0	1	0	1	0	1	0	4	
<i>A. fumigatus</i>	0	0	0	0	0	0	1	0	0	0	1	
<i>A. niger</i>	8	0	2	2	2	0	2	0	1	0	17	
<i>A. ochraceous</i>	0	0	0	0	0	0	0	0	15	4	19	
<i>Bispora antennata</i>	0	0	0	0	0	0	0	0	0	4	4	
<i>Cladosporium</i> sp.	0	0	1	0	1	0	0	0	0	0	2	
<i>Trichodochium disseminatum</i>	0	0	0	0	4	0	0	0	0	0	4	
<i>Gliocladium</i> sp.	0	0	1	0	0	0	0	0	0	0	1	
<i>Mucor</i> sp.	1	0	1	0	0	0	0	0	0	0	2	
<i>Rhizoctonia</i> sp.	0	0	0	1	0	0	0	0	0	0	1	
<i>Rhizopus stolonifer</i>	2	0	2	0	2	1	5	0	5	10	27	
<i>Penicillium chrysogenum</i>	1	6	0	0	0	0	0	0	0	0	7	
<i>Penicillium citrinum</i>	0	0	1	0	0	0	0	0	0	0	1	
<i>Penicillium crustosum</i>	0	0	0	0	0	0	0	0	1	0	1	
<i>Penicillium fellutanum</i>	0	0	0	0	0	0	1	0	0	0	1	
<i>Penicillium italicum</i>	0	0	0	0	1	3	0	0	0	0	4	
<i>Penicillium</i> sp.	1	5	1	0	0	1	0	0	0	0	8	
<i>Taeniocella exillis</i>	0	0	0	0	0	0	3	0	0	0	3	
<i>Trichoderma harzianum</i>	0	0	0	0	2	0	11	0	0	0	13	
Yeasts	2	1	0	0	3	1	0	8	1	2	18	
Total	16	12	9	3	16	6	24	8	24	20	138×10 ³	

Al: Almond; Ca: Cashewnut; Ha: Hazelnut; Pe: Peanut; Pi: Pistachio; (p): Pure; (s): Salted

samples, indicating partial elimination of some contaminating fungi. *Penicillium* spp. was isolated from all samples and out of 2 samples, *Aspergillus* spp. was isolated from all samples.

The results agree with many of the investigators working on seed pathology. Khomeiri *et al.* (2008), it was shown that collected peanut samples from Golestan, Mazandaran and Gilan provinces of Iran were contaminated by *Aspergillus flavus*. Sejiny *et al.* (1989) isolated fungi such as: *Aspergillus*, *Penicillium*, *Rhizopus*, *Mucor* and *Cladosporium*. Doster and Michailides (1994) showed the predominance of *Aspergillus* spp. and *Penicillium* spp. in stored nuts. Denizel *et al.* (2006), shows that the predominantly encountered species from the infected nuts in Turkey were: *Aspergillus niger*, *Cephalosporium* sp. and *Trichoderma viridae*. These species are known to have strains that cause toxic metabolites (Cole and Cox, 1981; Suleiman, 2010). Khosravi *et al.* (2007), showed a total of 60 nuts samples were analyzed and mycological analyses revealed that the most frequent isolated fungi from different nuts were *Aspergillus*, *Penicillium*, *Mucor*, *Fusarium*, *Paecilomyces* and yeast. Jecfa (2000) and Weidonborner (2001) showed that *Aspergillus*, *Penicillium* and *Fusarium* were the most important toxigenic fungi that decay food products. In addition, fungal contaminations due to *Aspergillus*, *Penicillium*, *Fusarium*, *Trichoderma* and *Cladosporium* in nuts especially almond and pistachio and other greasy edible seeds were reported in America, Brazil and western African countries, most of American pistachio products were contaminated by *Aspergillus* (Schatzki, 1995; Doster *et al.*, 2001). Research on Iranian pistachio in Kerman gardens showed that most of the pistachio products that have gapped or lost their shells during early period of growth and the immature splitted ones were contaminated by *A. flavus* and other *Aspergillus* sp., (Shahidi, 2004).

The results presented in Table 3 show the identity and the total Colony Forming Units (CFU) of fungi were found in all of the collected samples, they were serially diluted and plated on PDA medium. The total number of isolated fungi from the all 10 samples was (138×10³) cfu/g. samples. A total of twelve different fungal genera and twenty species were isolated. This study showed that contamination by fungal genera in pure nut products samples were more than salted samples. The minimum number of fungi was detected in Cashews (Salted) (3×10³) followed by Hazelnut (Salted) (6×10³) and Pistachio (Salted) (8×10³). In contrast, Peanut (pure) and Pistachio (pure) (24×10³) for each one, represented the highest infections of fungi. *Aspergillus*, *Rhizopus* and *Penicillium* genera were more frequently detected than other genera of fungi. *Aspergillus* spp. was found in the most examined samples. *Bispora antennata* found only in Pistachio (Salted), *Rhizoctonia* sp., in Cashews (Salted) *Gliocladium* sp., in Cashews (pure), *Trichodochium disseminatum* in Hazelnut (pure) and *Taeniocella exillis* in Peanut (pure).

Our results were in well agreement with those found by Adebajo and Diyaolu (2003), who isolated fourteen fungal species, mostly toxigenic and belonging to 5 genera were *Aspergillus*, *Penicillium*, *Rhizopus*, *Mucor* and *Syncephalastrum*, the most predominant isolates were: *A. niger*, *A. restrictus*, *A. flavus*, *A. fumigatus* and *Aspergillus* sp., the mean and range of total Fungal Counts (CFU/g) in samples were: 3,368. Rostami *et al.* (2009) who isolated fungal genera from nut products, found that the most common fungi isolated were extracted from 20 salted and 16 pure peanut samples: *Aspergillus flavus*, *Aspergillus niger*, *Penicillium*, *Rhizopus*, *Mucor*, *Alternaria* and *Nigrospora* were the most dominant genera, contamination by *Aspergillus flavus* in pure peanut

Table 4: Determination of aflatoxin and ochratoxin content in fungal culture by DRBC agar media

No	Fungi	Dichloran rosebengal chloramphenicol agar media
1	<i>Aspergillus flavus</i>	Positive
2	<i>A. fumigatus</i>	Positive
3	<i>A. niger</i>	Positive
4	<i>A. ochraceus</i>	Positive
5	<i>Penicillium spp</i>	Negative

Table 5: Total aflatoxins and ochratoxin content in nut product samples by ELISA method

Sample	Natural aflatoxin content (ppb)		Natural ochratoxin content (ppb)	
	OD	Results	OD	Results
Almond	0.628	1.7	0.427	6
Cashews	0.761	0.3	0.395	5.5
Hazelnut	0.616	5.3	0.625	1.5
Peanut	0.302	10.3	0.307	11.5
Pistachio	0.391	5.2	0.288	11.7

samples was more than salted samples, also roasting and processing with salt reduced the relative humidity of peanuts and the level of contamination, hence roasting, salting and provision of appropriate ambient conditions can be useful to peanut storage. Nawar (2008) evaluated the contamination risk of the improper storage of pistachio nuts in the major location of Saudi Arabia by studying the fungi associated with non and salted pistachio nuts, the infection with *Aspergillus flavus* and *A. niger*, high percentage infections were found in salted pistachio of Maddinah, while low infection was found in non salted pistachio of Jeddah. Abdel-Gawad and Zohri (1993) isolated fungal genera from 6 seed samples of each almond, cashewnut, chestnut, hazelnut, pistachio and walnut collected from different markets in Ar' Ar, Saudi Arabia, the prevalent fungi were *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *A. parasiticus*, *Eurotium* and *Penicillium chrysogenum*, *Rhizopus stolonifer* were isolated from all kinds of nut. El-Magraby and El-Maraghy (1987) found that *A. flavus*, *A. niger* and *A. fumigatus* were prevalent on peanut seeds in Egypt. Jimenez *et al.* (1991) isolated *A. flavus* and *A. niger* as the predominant fungi from sunflower, almond, peanut, hazelnut and pistachio nut. Abdel-Hafez and Saber (1993) isolated 12 species of *Aspergillus* from hazelnuts and walnuts with *A. flavus*, *A. niger* and *A. fumigatus* present in high frequencies. Jimenez *et al.* (1991) found *Rhizopus spp.* as the predominant fungi from almond, peanut, hazelnut and pistachio seeds in Spain.

Data represented in Table 4 show the result of mycotoxin detection on DRBC media for fungi isolated from nut samples, *A. flavus*, *A. fumigatus*, *A. niger* and *A. ochraceus*, show positive results on the culture for mycotoxin production and show change in colony diameter and color change in compare with control (CDA media), while *Penicillium spp.* show no any change in colony diameter and color.

Our results were in Contrary to this finding with Rajab (2011) show that the result of aflatoxin detection on DRBC media for fungi isolated from dried fruit samples, all the fungal culture were negative for aflatoxin production and show no any change in colony diameter or color change in compare with control (CDA media).

Table 5 shows results of Estimation of natural occurrence of Aflatoxin (AT) and Ochratoxin (OT) in pure nut sample products, by ELISA method, the concentrations of aflatoxin and ochratoxin were (1.7, 0.3, 5.3, 10.3 and 5.2), (6, 5.5, 1.5, 11.5 and 11.7) ppb. respectively. The results show low AF and OT content in most samples which is lower than the normal rang for mycotoxin (20 ppb) for human consumption, the high results of aflatoxin and ochratoxin show in peanut while the low results show in cashewnut. For the detection of mycotoxins, samples were analyzed quantitatively by CD ELISA technique (Competitive Direct Enzyme Linked Immunosorbent Assay). The quantity of mycotoxins in most of the samples was not detected within the detectable limits. However, in all samples, values of mycotoxins were below the permissible safe limits for human consumption and health. Mycotoxins can cause severe damage to liver, kidney and nervous system of man even in low dosages (Rodricks, 1976). *Fusarium* and *Aspergillus* species are common fungal contaminants of maize and also produce mycotoxins (Bakan *et al.*, 2002; Verga and Teren, 2005). The presence of *Aspergillus* in stored nuts results in deterioration, discolouration and bad odour, couple with toxins, posses potential hazard to consumer's health; a confirmation of that aflatoxin produced by *A. flavus* is found in cashewnuts and is hazardous to human health (Abdel-Gwada and Zohri, 1993).

REFERENCES

- Abdel-Gawad, K.M. and A.A. Zohri, 1993. Fungal flora and mycotoxins of six kinds of nut seeds for human consumption in Saudi Arabia. *Mycopathologia*, 124: 55-64.
- Abdel-Hafez, A.I. and S.M. Saber, 1993. Mycoflora and mycotoxin of hazelnut (*Corylus avellana* L.) and walnut (*Juglans regia* L.) seeds in Egypt. *Zentralbl Mikrobiol.*, 148: 137-48.
- Abdullah, S.K, I. Al-Saad and R.A. Essa, 2002. Mycobiota and natural occurrence of sterigmatocysin in herbal drugs in Iraq. *Basrah J. Sci. B.*, 20: 1-8.
- Adebajo, L.O. and S.A. Diyaolu, 2003. Mycology and spoilage of retail cashew nuts. *African J. Biotechnol.*, 2(10): 369-373.
- Anonymous, 2000. Veratox Software for Windows, Version 2, 0, II. Technology Inc., Palm City. FL.

- Aziz, N.H., Y.A. Youssef, M.Z. El-Fouly and L.A. Moussa, 1998. Contamination of some common medicinal plant samples and spices by fungi and their mycotoxins. *Bot Bull. Acad. Sinica*, 39(4): 279-285.
- Bakan, B., D. Richard, D. Molard and B. Cahagnier, 2002. Fungal growth and *Fusarium* mycotoxin content in isogenic traditional maize and genetically modified maize grown in France and Spain. *J. Agric. Food Chem.*, 50(4): 278-731.
- Basu, P.K., 1980. Production of Chlamydo spores of *Phytophthora megasperma* and their possible role in primary infection and survival in soil. *Canadian J. Plant Pathol.*, 2: 70-75.
- Brus, W., P. Horn and W. Joe, 2005. Colonization of wounded Peanut seeds by soil fungi in Africa and south eastern Asia. *Mycologia*, 97: 202.
- Burdaspal, P.A., A. Gorostidi and M.C. Tejedor, 1990. A survey of the occurrence of aflatoxins in edible nuts in Spain. Proceeding of the International Symposium and Workshop on Food Contamination 'Mycotoxins and Phycotoxins, Cairo, Egypt, November 4-15.
- Cole, R.J. and R.H. Cox, 1981. Handbook of Toxic Fungi Metabolites. Acad. Press, New York, pp: 780- 790.
- Denizel, T., B. Jarvis and E.J. Rolf, 2006. A field survey of pistachio (*Pistacia vera*) nut production and storage in Turkey with particular reference to aflatoxin contamination. *J. Sci. Food Agric.*, 27(11): 1021-1026.
- Doster, M.A. and T.J. Michailides, 1994. *Aspergillus* moulds and aflatoxins in pistachio tree in California. *Phytopathology*, 84(6): 583-590.
- Doster, M.A., T.J. Michailides, D.A. Goldhamer and D.P. Morgan, 2001. Insufficient spring irrigation increase abnormal splitting of pistachio nuts. *California Agric.*, 55: 28-31.
- E1-Magraby, O.M. and S.S. E1-Maraghy, 1987. Mycoflora and mycotoxins of peanut (*Arachis hypogaea* L.) seeds in Egypt, I: Sugar fungi and natural occurrence of mycotoxins. *Mycopathologia*, 98: 165-170.
- E1-Magraby, O.M. and S.S. E1-Maraghy, 1988. Mycoflora and mycotoxins of peanut (*Arachis hypogaea* L.) seeds in Egypt, III: Cellulose decomposing and mycotoxin producing fungi. *Mycopathologia*, 104: 19-24.
- Frank, H.K., 1981. Moulds and mycotoxins in nuts and nut products. Schimmelpilze and mycotoxin in Nuessen und daraus hergestellten producten. Mycotoxin in lebensmitteln, federal Republic of Germany, pp: 397-414.
- Galvano, F., A. Ritieni, G. Piva and A. Pietri, 2005. Mycotoxins in the Human Food Chain. In: Duarate, D. (Ed.), the Mycotoxin Blue Book. Nottingham University Press, England, pp: 187-225.
- Guarro, J., J. Gene and A.M. Stchigel, 1999. Clinical microbiology reviews, developments in fungal taxonomy. *Am. Soc. Microbiol. Rights Res.*, 12(3): 454-500.
- Hocking, A.D., J.I. Pitt, R.A. Samson and U. Thrane, 2006. *Advances in Food Mycology*. Springer, New York.
- Howard, D.H., 2002. *Pathogenic Fungi in Humans and Animals*. 2nd Edn., Marcel Dekker, INC., New York, pp: 790.
- Jarvis, B., 1973. Comparison of an improved rose-Bengal chlortetracycline agar with other media for the selective isolation and enumeration of moulds and yeasts in food. *J. Appl. Bac.*, 36: 723-727.
- Jecfa, 2000. Joint FAO/WHO Expert Committee on Food Additives. In: Ochratoxin, A., (Ed.), Safety Evaluation of Certain Mycotoxins in Food. Series 47, IPCS, WHO, Geneva.
- Jimenez, M., R. Mateo, A. Querol, T. Huerta and E. Hernandez, 1991. Mycotoxins and mycotoxigenic moulds in nuts and sunflower seeds for human consumption. *Mycopathologia*, 115: 122-128.
- Kacaniova, M., 2003. Feeding soybean colonization by microscopic fungi. *Trakya Univ.J. Sci.*, 4: 165-168.
- Khomeiri, M., Y. Maghsudlou, L. Kumar, F. Waliyar and S. Hasani, 2008. Determination of Aflatoxin contamination and *Aspergillus flavus* infection of groundnuts from Northern provinces of Iran. *J. Agric. Sci. Natur. Resour.*, 15: 77-85.
- Khosravi, A.R., H. Shokri and T. Ziglari, 2007. Evaluation of fungal flora in some important nut products (Pistachio, Peanut, Hazelnut and Almond) in Tehran, Iran. *Pakistan J. Nutr.*, 6(5): 460-462.
- King, D.A, A.D. Hocking and J. Pitt, 1979. Dichloran-rose bengal medium for enumeration and isolation of moulds from foods. *J. Appl. Environ. Microbiol.*, 37: 959-964.
- Larone, D.H., 2011. *Medically Important Fungi. A Guide to Identification*, ASM Press, Washington, DC, pp: 185, ISBN: 1555816606.
- Moubasher, A.H., 1993. *Soil Fungi Qatar and Other Arab Countries*. University of Qatar, Doha, pp: 566, ISBN: 9992121025.
- Nawar, L.S., 2008. Prevention and control of fungi contaminated stored pistachio nuts imported to Saudi Arabia. *Saudi J. Biol. Sci.*, 15(1): 105-112.
- Phillips, D.J., B. Mackey, W.R. Ellis and T.N. Hansan, 1979. Occurrence and interaction of *Aspergillus flavus* with other fungi on almonds. *Phytopathology*, 69: 829-831.
- Pitt, J.I. and A.D. Hocking, 1997. *Fungi and Food Spoilage*. 2nd Edn., Chapman and Hall, Gaithersburge, Maryland, pp: 593.
- Pitt, J.I., A.D. Hocking, R.A. Samson and A.D. King, 1992. Recommended Methods for the Mycological Examination of Foods, In: Modern Methods in Food Mycology. Elsevier Science Ltd., Amsterdam, pp: 388, ISBN: 0444889396.

- Pornsuriya, C., F.C. Lin, S. Kanokmedhakul and K. Soyong, 2008. New record of *Chaetomium* sp. isolated from soil under pineapple plantation in Thailand. *J. Agric. Technol.*, 4(2): 91-103.
- Rajab, N.N., 2011. Isolation of fungi from dried and concentrated fruits and study the effect of some plant extracts on the growth of aflatoxigenic fungi. M.Sc. Thesis, College of Science. Salahaddin University-Erbil.
- Rajankar, P.N., D.H. Tambekar and S.R. Wate, 2007. Study of phosphate solubilization efficiencies of fungi and bacteria isolated from saline belt of purna river basin. *Res. J. Agric. Biol. Sci.*, 3(6): 701-703.
- Rodricks, J.V., 1976. Mycotoxins and other Fungus Related Food Problems. *Advance in Chemistry, Series 149*. American Chemicals Society, Washington, pp: 239.
- Ronhede, S., B. Jenesen, S. Rosendahl, B.B. Kragelund, R.K. Juhler and J. Amand, 2005. Hydroxylation of the herbicide isoproturon by fungi isolated from agricultural soil. *Appl. Environ. Microbiol.*, 71(12): 7927-7932.
- Rostami, R., K. Naddafi, A. Aghamohamadi, H. Najafisaleh and M. Fazlzadeh Davil, 2009. Survey of peanut fungal contamination and its relationship with ambient conditions in the bazaar of zanja. *Iran J. Environ. Health Sci. Eng.*, 6(4): 295-300.
- Samson, R.A., A.D. Hocking, J.I. Pitt and A.D. King, 1992. *Modern Methods in Food Mycology*. Elsevier, Amsterdam.
- Schatzki, T.F., 1995. Distribution of aflatoxin in pistachios. *J. Agri. Food. Chem.*, 43: 1566-1569.
- Scott, P.M., 1993. Fumonisin. *Int. J. Food Microbiol.*, 18: 257-270.
- Sejiny, M.J., F.M. Thabet and M.K. Elshaieb, 1989. Microbial contamination of various nuts stored in commercial markets in Jeddah. *J. King Abdulaziz University Sci.*, 1(1): 61-71.
- Shahidi, B.H., 2004. Incidence of aflatoxin producing fungi in early split pistachio nuts of Iran. *J. Biological Sci.*, 4: 199.
- Stoloff, L., H.P. Egmond and D.L. Park, 1991. Rationales for the establishment of Limits and regulations for mycotoxins. *Food Addit Contam.*, 8: 213-216.
- Suleiman, M.N., 2010. Occurrence and distribution of fungi associated with biodeterioration of cashew in the eastern senatorial district, Kogi State, Nigeria. *Arch. Appl. Sci. Res.*, 2(5): 462-465.
- Suleiman, M.N. and A. Taiga, 2009. Efficacy of aqueous extracts of neem and sharf for the control of fungi associated with milled and unmilled stored rice grains. 5th Proceeding of the Humboldt kellogg Annual Agric. Conference, pp: 71-73.
- Tournas, V., M.E. Stack, B.M. Philip, A.K. Herbert and R. Bandler, 2001. *Center for Food Safety and Applied Nutrition. 8th Edn., Bacteriological Analytical Manual. Yeasts, Molds and Mycotoxins. Revision A. Chapter 18*.
- Ulhan, S., R. Demurel, A. Asan, C. Baycu and E. Kinaci, 2006. Colonial & morphological characteristics of some microfungus species isolated from agricultural soils in Eskişehir Province (Turkey). *Turk. J. Bot.*, 30: 95-104.
- Verga, B.T. and J. Teren, 2005. Mycotoxin producing fungi and mycotoxins in foods in Hungary. *J. Acta Aliment. Akademiai*, 34(3): 267-275.
- Watanabe, T., 2002. *Pictorial Atlas of Soil and Seed Fungi. Morphologies of Cultured Fungi and Key to Species. 2nd Edn., CRC Press LLC., Boca Raton*, pp: 484.
- Watt, B.K. and A.I. Merrill, 1950. *Composition of Food, Raw, Processed, Prepared, Agric. Handbook No.8, U.S.D.A., Washington, D.C.*, pp: 250-275.
- Weidonborner, M., 2001. The mycobiota and potential mycotoxins in peanuts. *Can. J. Microbiol.*, 47: 460-463.
- Zinedine, A., C. Brera, S. Elakhdari, C. Catano, F. Debegnach, S. Angelini, B. DeSantis, M. Faid, M. Benlemlih, V. Minardi and M. Miraglia, 2006. Natural occurrence of mycotoxins in cereals and spices commercialized in Morocco. *Food Chem.*, 17: 868-874.