



## Short Communication: Scanning of Processed Food Contaminating Fungi and Determine the Potential Aflatoxigenic type

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**Abstract:** This study was conducted in the plant protection dept., College of Agriculture, University of Basrah to investigate the food contaminated fungi in several food products involved potato chips, pasta, and popcorn to specify the potential aflatoxigenic species. Eight samples of food products were randomly collected from local market included two samples of pasta, 5 samples potato chips, and one sample of popcorn. The primary isolation was performed on potato dextrose agar (PDA) in 9 cm Petri dishes; the isolated fungi were purified then diagnosed morphologically. The isolation results revealed a presence of several species within three main fungal genera, which included, *Penecillium* sp., *Alternaria alternata*, *Aspergillus flavus*, *A. niger*, *A. alliaceus*, *A. candidus*, *A. fumigatus* and *A. sclerotiorum* in prevalence percentages 43.75, 35.00, 18.75, 27.50, 5.64, 3.75, 3.75, 3.75, 3.75 % respectively and frequency percentage 7.15, 2.60, 9.07, 10.69, 0.46, 1.28, 0.46, 1.00% respectively. *A. flavus* was obtained to examine its ability to produce Aflatoxin using ammonia vapor test. The results revealed that nine isolates of *A. flavus* showed a possible ability to produce Aflatoxin B1.

**Keywords:** Aflatoxins, Contamination, Fungi.

## Introduction

The fungi are well known as pathogenic microorganisms that contaminating most of the economic crop causing direct and/or indirect damages. A twenty-five percent of the world's food crops and their products are contaminated with mycotoxin (Smith *et al*, 1994).

Mycotoxins are secondary metabolites produced by several genera of fungi like *Aspergillus* spp., *Pencilium* spp. and *Fusarium* spp., which are spread all over the world as natural contaminants in numerous commodities of plant origin, especially in cereals grains, nuts, oil seeds, fresh and dried fruits, vegetables, cocoa, coffee beans, beer,

and species. Also, they can be found in animal-derived food (if animals consumed a contaminated feed), namely meat, eggs and milk (Bryden, 2012; Marin *et al.*, 2013). More than 4.5 million people in developing countries are exposed to the Aflatoxins, which had a great interest as a hazardous food contaminant especially in developing countries (Bhat & Krishnamachari, 1977; Makun *et al.*, 2010). The consumption of foods contaminated with mycotoxins can affect human and animal health and causes several diseases such as urinary tract infection, immuno-deficiency, and genetic abnormalities lead to congenital malformations (Bhat & Vasanthi 2003, Carvajal, 2015).

Climate represents the key factor for fungal occurrence and mycotoxin production. Mycotoxin is climate-dependent compounds, but several factors can affect their presence, such as bioavailability of micronutrients, insect damage making it complex and multifactor phenomenon (Milani, 2013)

Indeed, the toxicity of mycotoxin cannot be confirmed precisely in natural contaminated food and feeds due to complicated infection with several toxigenic fungi that produce a mixture of mycotoxins that leads to generate a synergistic effect, which can cause activation or inhibition effect.

Aflatoxins are the most important group of the mycotoxins that produced by *Aspergillus* spp., especially in the tropical areas, which are causes liver cancer and immune deficiency in humans and animals (Da Rocha *et al.*, 2014). Aflatoxin B1 is the most important aflatoxins produced by *Aspergilli*, which is metabolized to M1 and P1 by hydrogenation, which is less toxic than the main toxin (Rustom *et al.*, 1993).

The most important physical and chemical properties of aflatoxins are its yellowish-white-colored, odorless and bitter, crystalline, dissolves in organic solutions and dissolve in water, give a phosphorescence color when exposed to ultraviolet light UV-resistant and low molecular weight (Reddy & Farid, 2006) melting point and decomposition ranges from 237-320 °C (Rustom, 1997).

Mycotoxins are secondary metabolic products that react chemically to abisdihydrodifuran or tetrahydrobisfuran combined with cyclopentanone or alacton (Jaimez *et al.*, 2000).

Aflatoxins are divided into two groups

a) Bisfuran-coumarin.

b) Bisfuran-coumarin-lactones.

(Rastogi *et al.*, 2001, 2001, Nakai *et al.*, 2008).

As a result of the poor storage of food and food products, human health will be affected, therefore the establishment of modern stores and careful handling of animals, their health and products in addition to control of fungi and its toxins, This study was designed to investigate the food associated fungi and presence of Aflatoxin B1 in the pasta and chips that are produced from wheat and corn according to the large consumption by children and adults.

## Materials & Methods:

### Sampling:

The samples were collected randomly from local markets of Basrah governorate in 20<sup>th</sup> of Oct. 2014, which were included chips (5 samples), popcorn (2 samples), and two samples of pasta. The samples were kept in refrigerator (4°C) for next laboratory experiments.

### The preparation of growing media

#### Potato dextrose agar (PDA)

The PDA (extract of 200g of potato, 20g of glucose, 20g of agar and the volume was completed to 1L with distilled water) the medium was supported by chloramphenicol (250 mg.l<sup>-1</sup>) then autoclaved at 121°C and 15 bar for 15min.

#### Coconut Agar

This medium was prepared according to Davis *et al.* (1967), by boiling 100g of coconut powder 300 ml of distilled water for 15 min, the mixture was filtered by several layers of sterilized gauze and the filtrate was equilibrated to 600 ml with distilled water. The medium was autoclaved at 121°C and 15 bar for 15 min.

#### Czapex solution      Czapek      Solution Concentrate

The solution was prepared according to Klich (2002) method: Sodium nitrate NaNO<sub>3</sub>, 30.0gm Potassium chloride KCl, 5.0 gm Magnesium hydroxide sulfate MgSO<sub>4</sub> .7H<sub>2</sub>O, 5.0 gm Hydrothermal sulphate FeSO<sub>4</sub> .7H<sub>2</sub>O, 0.1 gm Hydrogen sulphate ZnSO<sub>4</sub>.7H<sub>2</sub>O, 0.1 gm Copper sulphate CuSO<sub>4</sub> Cu<sub>5</sub>a, 0.05 gm, were dissolved in Distilled water and the solution equilibrated to 100ml. The mixture was kept in the refrigerator until use, with good continues shaking to dissolve the precipitated iron oxide.

### Preparation of Czapek medium (CZ)

The media was prepared by mixing 10 ml of Czapek concentrate with 1 gm of K<sub>2</sub>HPO<sub>4</sub>, 30.00 gm of Sucrose, 17.5 gm of Agar and the volume was completed to 1000 ml by Distilled water. The medium was autoclaved at 121°C and 15 bar for 15min.

### Isolation, Purification and diagnosis of the contaminating fungi

The primary isolation was performed by grinding 5 gm of the pasta or chips samples using mortar and pestle to a fine powder, serial dilutions (10<sup>-1</sup> to 10<sup>-6</sup>) were made, then the last three dilutions were obtained for isolation. The isolation was performed on PDA Petri plates in triplicates at 25 ± 2°C for 5-7 days. The fungal isolates were diagnosed morphologically by Dr. Abdulhafiz Al-Dabboun, Department of Marine Biology, Marine Sciences Centre according to Barnett & Hunter (1972). The isolates of *A. flavus* were purified then kept in a diagonal position at a 4°C until use.

### Calculation of the appearance and frequency percentages

The percentage of the appearance and frequency of each fungus was calculated according to the Gonzalez *et al.* (1995) equation:

$$\text{The appearance \%} = \frac{\text{Number of species or genres appearances} \times 100}{\text{Total number of samples}}$$

$$\text{The frequency \%} = \frac{\text{Number of species or genres colonies} \times 100}{\text{Total number of colonies}}$$

Table (1) Percentage of appearance and frequency of the isolated fungi.

Isolated fungi	appearance%	frequency %
<i>Aspergillus oryzae</i>	3.75	0.46
<i>Aspergillus alliaceus</i>	3.75	5.64
<i>Aspergillus candidus</i>	3.75	1.28
<i>Aspergillus fumigatus</i>	3.75	0.46
<i>Aspergillus sclerotiorum</i>	3.75	1.00
<i>Aspergillus flavus</i>	18.75	9.07
<i>Aspergillus niger</i>	27.50	10.69
<i>Alternaria alternate</i>	35.00	2.60
<i>Penicillium sp.</i>	43.75	7.15

### Detection of aflatoxin B1

#### UV light examination of aflatoxin B1 producing isolates of *A. flavus*

Eleven isolates of the *A. flavus* were grown in a 9 cm Petri dish containing coconut agar medium (3 replicates/isolate), then incubated in 27±1°C for five days. The cultures were examined under 365 nm wavelength of UV light to investigate possible ability to produce Aflatoxin (blue fluorescent colour), the examination was performed using the UV light device (Model UV, L21) (Yabe *et al.*, 1987).

#### Examination of the ability of *A. flavus* isolates to produce Aflatoxin isolates ability to produce aflatoxin B1

#### Ammonia vapor examination of aflatoxin B1 producing isolates of *A. flavus*

The aflatoxin production ability of *A. flavus* isolates determined using ammonia vapor test by growing the fungus on coconut agar

medium for seven days on 27±1°C, then treated with 20% ammonia solution by placing a filter paper in the lid of a petri dish and saturate it with the ammonia solution. The dishes were incubated upside at 27±1°C then examined after one hour and 24 hours to observe the pink colour density (Saito & Machida, 1999).

### Results & Discussion

#### Isolation Purification and diagnosis of the contaminating fungi

The isolation results (table 1; fig. 1) revealed the detection of nine species related to three main fungal genera *Aspergillus* spp., *Penicillium* sp., and *Alternaria* sp. Among isolated fungi, *Penicillium* sp. Showed the highest percent (43.75%) followed by *Alternaria alternata*, *A. niger* and *A. flavus*, *A. sclerotiorum*, *A. fumigatus*, *A. candidus*, *A. alliaceus* and *A. oryzae* (35.00, 27.50, 18.75, 3.75, 3.75, 3.75, 3.75 and 3.75%)

respectively, while the highest frequency percent was recorded by *A. niger* (10.96%) followed by *A. flavus*, *Pencillium* sp., *A. alliaceus*, *A. alternata*, *A. candidus*, *A. sclerotiorum*, *A. fumigatus* and *A. oryzae* (9.07, 7.15, 5.64, 2.60, 1.28, 1.00, 0.46 and 0.46 %) respectively. Among the isolated species, eleven *A. flavus* isolates (Puf1 A.f, Puf2 A.f, Indo1 A.f, Indo2 A.f, Indo1a A.f, Indo2a A.f, Indo2b A.f, Sha1 A.f, Sha2 A.f, Poch A.f, Far A.f) were targeted for next experiments (Table 2).

These results were corresponded with Qahtan (2002) results who isolated *A. flavus* from popcorn with other seven species of

stored and field fungi, which indicating that the storage conditions can encourage the growth of storage fungi especially the *Aspergilli* that leads to produces Aflatoxins like aflatoxin B1 and cyclopiazonic acid (Payne *et al.*,1998; Horn *et al.*, 2011; Alhaddad; 2013).

The UV test results (Table 2) showed that the examined isolates of *A. flavus* grouped into three main groups due to their ability to produce AFB1. The high production ability-group (++) were Puf1 A.f, Indo1 A.f, Indo2 A.f and Puf2 A.f; while the low production ability-group (+) were

**Table (2): Examination of *A. flavus*.**

Isolate name	UV test	Ammonia test	Isolation source
<b>Puf1 A.f</b>	++	++	<b>Chips</b>
<b>Indo1 A.f</b>	++	++	<b>Pasta</b>
<b>Indo2 A.f</b>	++	++	<b>Pasta</b>
<b>Puf2 A.f</b>	+	+	<b>Chips</b>
<b>Indo1a A.f</b>	+	+	<b>Pasta</b>
<b>Indo2a A.f</b>	+	+	<b>Pasta</b>
<b>Indo2b A.f</b>	+	+	<b>Pasta</b>
<b>Sha1 A.f</b>	+	+	<b>Chips</b>
<b>Poch A.f</b>	+	+	<b>Pop corn</b>
<b>Sha2 A.f</b>	–	–	<b>Chips</b>
<b>Far A.f</b>	–	–	<b>Chips</b>

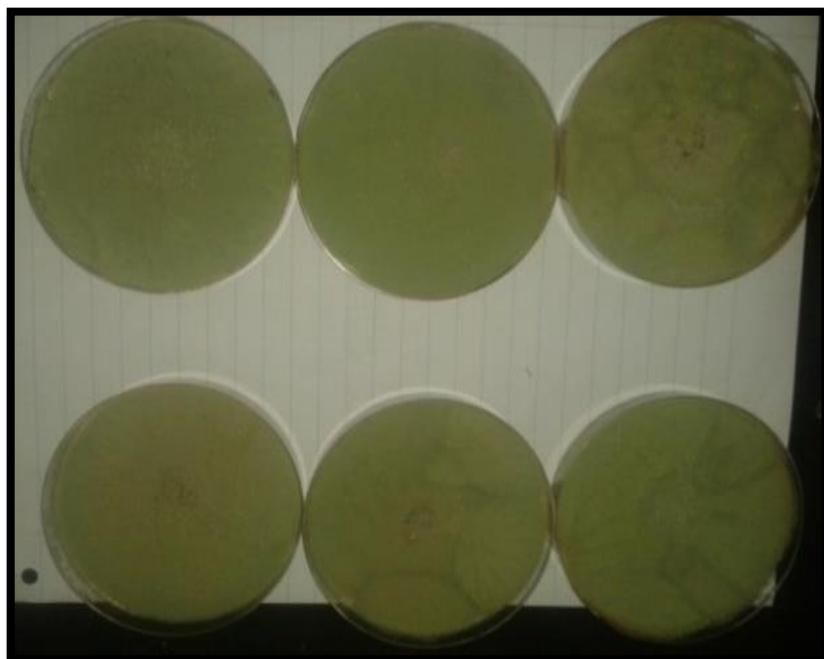


Fig. (1): *Aspergillus flavus* isolates on PDA medium.



Fig. (2): Ammonia test to examine *A.flavus* isolates ability to produce aflatoxin B1.

Indo1a A.f, Indo2a A.f and Indo2b A.f, and non-aflatoxigenic group were Sha1 A.f and Poch A.f. The results of UV test were confirmed by ammonia test (Fig. 2).

### Conclusions

Chips and pasta are contaminated with many fungi and fungi producing mycotoxins during processing and in poor storage conditions, In

addition to contamination with *Aspergillus flavus* producing aflatoxins.

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**Conflict of interest:** The authors declare that they have no conflict of interest.

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