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RESEARCH OPINIONS IN ANIMAL & VETERINARY SCIENCES

# Detection of Aflatoxin $B_1$ from feed and food from various market place in Iran

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## Abstract

A total of one hundred and fifty ready to use food thickeners were randomly collected from various markets in both urban and rural settings. Four (4) of the samples tested Achi (*Brachystegia eurycoma*), Akpalata (*Afzelia africana*), Ofor (*Detarium microcarpum*), and Ukpo (*Mucuna flagellipes*) were contaminated with fungal flora. The most common and prevalent fungi observed on incubated powdered form of food thickeners on media, were the *Aspergillus* group namely *Aspergillus flavus*, *A. parasiticus* and *A. niger*. Pure isolates of *A. flavus* and *A. parasiticus* from various food thickeners from open markets in Iran were screened for their potential to produce aflatoxin $B_1$ ($AFB_1$) on different agar media. Ultraviolet (UV) light, a standard procedure was used to differentiate the toxin from non-toxin form of *Aspergillus* sps. Further, aflatoxin quantification was done using TLC (Thin layer Chromatography) followed by fluorometry. *A. flavus* was more prevalent than *A. parasiticus* in all samples. Toxin and non-toxin isolates were grouped as per bright greenish-yellow fluorescence (BGYF) presumptive test under UV light. *A. flavus* and *A. parasiticus* produced $AFB_1$ on yeast extract sucrose (YES) and czapek yeast autolysate (CYA), though concentration of aflatoxin was higher on YES than CYA. The amounts of aflatoxin $B_1$ from *A. flavus* isolates ranged between 0.94 to 3.83 µg/g of agar and all positive *A. parasiticus* ranged from 0.22 – 2.87 µg/g of agar. Analysis of food thickeners also revealed a high incidence and alarming levels of naturally produced $AFB_1$. The levels of $AFB_1$ ranged between 4.0 and 95 µg/g in various food thickeners tested. That the presence of aflatoxin in food thickeners poses a potential health threat to consumers in this part of Iran and elsewhere is discussed.

**Keywords:** ready-to-use food thickeners, *Aspergillus* sps., $AFB_1$, substrate, TLC, Fluorometry

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## Introduction

Contamination of food and feed commodities by microbes (Fungus, bacteria) is main problem due to today's environmental condition. It is commonly found in any foodstuff or animal feed which can support fungal growth during growth, harvest, or storage, although the main production has been reported in grains, nuts, copra and cottonseeds. The problem of food and feed contamination with toxigenic moulds especially *Aspergillus* species is of current concern and has received a great deal of attention during the last three decades (Rustom 1997). These fungi are capable of growing on a great variety of food commodities and animal feed materials when the conditions of temperature, relative humidity and product moisture are favorable (Iamanaka et al., 2007; Rosi et al., 2007). This genus of fungi is known for some mycotoxins production as secondary metabolites which are very harmful to human and animals. Aflatoxin $B_1$ ($AFB_1$) is the most commonly occurring in this group and is known to contaminate agricultural commodities such as peanuts, corn, animal feed which is an important problem all through (CAST 2003). Mainly *A. flavus* and *A.parasiticus* produce aflatoxins $B_1$,$B_2$,$G_1$,$G_2$. The contamination of foods and animal feed with these mycotoxins is controlled worldwide by legal limits and depending on the toxicity of these mycotoxins, in the countries of the EU equal limits are valid for aflatoxins: namely 2 µg/kg for $AFB_1$ and 4µg/kg for all AF in total

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(Van Egmond 1995). Aflatoxins (AFs) are secondary metabolites produced by various fungal species and have the highest toxicity, among mycotoxins. Due to their toxicity including carcinogenic activity, Afs affect not only the health of humans and animals but also the economics of agriculture and food (Hwang et al., 2004). Recently, contamination of mycotoxins in food including imported ones has received much attention because of the increase in international food trade, due to new trade treaties.

Aflatoxins are toxic metabolites produced by certain fungi in/on foods and feeds by mould. They are probably the best known and most intensively researched mycotoxin in the world because of their toxic and carcinogenic potentials to humans and animals (Chu 2002). Aflatoxins have been associated with various diseases, such as aflatoxicosis, in livestock, domestic animals and humans throughout the world. The European Union (EU) has established demanding regulatory limits for controlling aflatoxins $B_1$, $B_2$, $G_1$ and $G_2$, in cereals, nuts, nut products and dried fruit, aflatoxin $M_1$ in milk, and ochratoxin A in cereals ( Gilbert and Anklam 2002). Aflatoxin contamination in food and feed is very important to human and animal health because the aflatoxins are toxic and carcinogenic.

There are also several methods to detect and quantify aflatoxin in food and feed. Several methods have already been proposed and reviewed for $AFB_1$ determination in food and agricultural products (CAST 2003; Kriska et al., 2005; Zheng et al., 2006). There have been several chromatographic methods previously used to determine contamination like TLC and HPLC (Sapsford et al., 2006). Solvent extraction of agricultural products has been suggested as an effective means of removing aflatoxins from mold-damaged commodities. Richard (1999) described the fluorometric analysis of aflatoxins in corn, corn meal, popcorn, rice, wheat, cottonseed and peanuts and found this method is quantitative, inexpensive and very efficient. Kastner et al. (2010) used HPLC method to analyse aflatoxin ,ochratoxin A and deyoxynivalenol (DON). $AFB_1$ concentrations of red ground pepper were analyzed by microtitre plate Enzyme linked immunosorbent assay (ELISA) (Ardic et al., 2008). Calleri et al. (2007) also used HPLC with fluorescence detection for $AFB_1$ determination. Several scientists Rosi et al. 2007; Tekinsen and Ucar, (2008) described $AFB_1$ by ELISA and HPLC Techniques. Dilara et al. (2002) described the comparative study of three different methods for the determination of aflatoxins in Tahini which were high-performance liquid chromatography (HPLC), fluorometry, and enzyme-linked immunosorbent assay (ELISA). An immunoaffinity column was used for cleanup and purification of extracts prior to detection by HPLC and fluorometry. The fluorometric determination method involving an immunoaffinity column cleanup step was found to be highly correlated with the HPLC method. Both methods were found to be effective due to their high recoveries and low variance for the prediction of total aflatoxin contamination in Tahini samples. The ELISA method, due to its high variation in replicates, was found to be applicable only as a screening method. This survey demonstrated the need for control of aflatoxin contamination of foodstuffs involving sesame seeds as an ingredient. Rosario et al. (1999) reported aflatoxin determination in corn and corn products by HPLC and Minicolumn method. The result obtained by them showed a good agreement between the minicolumn responses and the HPLC data. Therefore, the modified "lahar" minicolumn method is an efficient screening tool for aflatoxin detection which can be utilized in the aflatoxin monitoring of corn and its commercial products for quality improvement, particularly in regions where production and consumption of corn is high and limited aflatoxin analytical capability is available.

In our initial study, using traditional microscopy and DNA technique, the prevalence of *Aspergillus* species in ready-to-use food thickeners in South-East geo-political zone in Iran has been established ( Okwu 2008-2010). Present investigation aims at identification of a larger collection of food thickeners from open markets and selection of a suitable media for maximizing $AFB_1$ production by *A. flavus* and *A. parasiticus* strains and qualitative analysis of the same by TLC (Thin layer Chromatography) followed by quantitative analysis by fluorometry. In addition, samples were analyzed for level of $AFB_1$ contamination.

## Materials and Methods

### Isolation of *Aspergillus flavus* and *A. parasiticus* from food thickeners

Ready-to-use thickeners (Achi, Akpalata, Ofor and Ukpo) were obtained from several open markets from Iran. A total of one hundred and fifty (150) samples were collected with the aid of sterile polyethylene bags and transported to the laboratory aseptically for microbiological evaluations. Strains were named after the States and market place where samples were collected.

Samples were finely grounded with a dry Brawn coffee grinder. Powdered form of the above food thickeners were equally distributed on PDA plates and incubated for 7 days at room temperature (27 ± 2 °C). After incubation period, based on morphology and colony character, potential colonies of *A. flavus* and *A. parasiticus* were screened and identified. Both species were isolated from incubated samples and were further

purified individually by sub-culturing in PDA slants. They were then identified according to Raper and Fennell (1965) and Klich (2002). Pure identified cultures were stored at 4 °C during the duration of the study. Further, monoconidial colonies from pure cultures were checked under stereomicroscope (Leica) then transferred onto PDA plates and incubated at 25 ± 2 °C in the dark. Pure fungal cultures of *A. flavus* and *A. parasiticus* were used to test for $AFB_1$ production in different media.

**Extraction of $AFB_1$ from *A. flavus* and *A. parasiticus***

Petri dishes each containing agar media, yeast extract sucrose (YES) and czapek yeast autolysate (CYA) were inoculated with 1 mL of conidial suspension, from above, containing $10^5$ conidia. Three replications were maintained for each isolate for each media and incubated for 7 days at 25 ± 2 °C. Plates were observed daily for mycelial growth and aflatoxin production. After incubation period, cultures were subjected to the bright greenish-yellow fluorescence (BGYF) presumptive test under UV light at 360 nm, in which fluorescence indicates the presence of aflatoxin. The non- aflatoxin cultured plates were separated from the toxin producing ones based on the fluorescence.

The amounts of $AFB_1$, were qualitatively analyzed on thin-layer chromatography (TLC) plates under UV light. Aflatoxin $B_1$ was then extracted by grinding both medium separately in Waring blender using chloroform. Extraction and analysis of $AFB_1$ was done using methods described by Criseo et al. (2001) with slight modifications. The $AFB_1$ produced in media containing yeast extract sucrose (YES) and czapek yeast autolysate (CYA) was extracted separately. Moldy agar (20g) was placed into the blender containing 50 ml chloroform. The blender was run at a low speed for 2-3 min to homogenize the mixture. The mycelial mat was separated by passing through Whatman No. 1 filter paper. The filtrate was transferred to a separating funnel and the chloroform layer filtered through anhydrous Sodium sulphate. The extraction procedure was repeated twice using 50 ml of chloroform each time.

**Identification of $AFB_1$ on TLC from *A. flavus* and *A. parasiticus* extracts from food thickeners**

Extracts were combined and evaporated to dryness in a Rotavapor in $N_2$ chamber. A preliminary visual determination of $AFB_1$ was done by standard thin-layer chromatography (TLC) (20x20 cm, Merck, USA) and stored at 4 °C. The residue of each sample was re-dissolved in 10 µl chloroform and applied to the TLC plates. The solvents, toluol, ethylacetate and acetic acid (50: 30: 4 vol.) were used. The developed plates were viewed under longwave UV (365 nm) as blue spots and compared with standard aflatoxin (Biological Carolina, USA) spotted on the same plate. Two replicates were analyzed by spotting the crude extract of aflatoxins from each media.

**Quantification of $AFB_1$ by Fluorometer**

The quantitative estimation of $AFB_1$ was done by Fluorometer (TD-700 with standard PMT (P/N 7000-009). The fluorescent blue spot with $AFB_1$ was scraped and dissolved in 3 ml methanol and then filtered through a microfiber (0.45 mµ) filter. The clear solution was used to determine $AFB_1$ from *A. flavus* and *A. parasiticus,* respectively. The fluorometer was calibrated with the standard aflatoxin solution according to the User's instructions manual (Fischer Scientific). 2 ml of the diluted filtrate from above extraction was passed through the Aflatest Affinity column (AflaTest®, VICAM) in order to absorb all the AF produced and then the column was washed twice with 1ml portions of deionized water. AF was finally eluted with 1ml HPLC-grade methanol into a cuvet to which 1.0 ml Aflatest developer was added, thereafter, the corresponding fluorescence was recorded and the concentration of $AFB_1$ determined using standard calibration, from *A. flavus* and *A. parasiticus*, respectively.

**Preparation and Extraction of $AFB_1$ from food thickeners**

Samples were finely ground with a dry Brawn coffee grinder as above. Each sample was made into slurry by mixing with an equal amount of deionized water in a suitable container. The test portion (25 g) was placed in a Waring blender jar, followed by addition of 100 ml of 60 % (v/v) aqueous methanol/ water and 5 g NaCl (extraction solvent) and mixing for 5 mins. The homogenized sample was then centrifuged at 3000 rpm for approximately 5 mins. These portions contain pigments and lipids, were defattenned twice by extraction with 25-ml portions of hexane. The blender was run at a low speed for 2-3 mins to homogenize the mixture. The supernatant was filtered through a gravity filter paper (Whatman No. 1), and 10.0 ml of the filtrate was diluted in 40.0 ml deionized water. The diluted extract was filtered through a micro-fiber (0.45 mµ) filter, while keeping the filtrate in a filtration cup (modified, Asis et al., 2002). 3 ml of filtrate was pipetted into a 10-ml graduated cylinder. With protection from light, the extract was centrifuged to remove particulate matter and the supernatant recovered. Extract from each sample was evaporated to dryness in a Rotavapor in $N_2$ chamber. Visual determination of $AFB_1$ in each sample was done by standard thin-layer chromatography (TLC), followed by quantification using Flurometer as described above.

### Statistical analysis

The Statistical Presentation System Software (SPSS) of Microsoft (MS-Excel 03, complete Data analysis) was used to calculate the means, standard errors and standard deviations. All the experiments were repeated thrice.

## Results and Discussion

**$AFB_1$ production by *A. flavus* and *A. parasiticus* isolates**

Aflatoxins, highly toxic and carcinogenic compounds that frequently contaminate foods and feeds, are produced by several species in the genus. Aflatoxins, a group of mycotoxins mainly produced by *A. flavus* and *A. parasiticus*, have adverse health effects on humans and livestock that ingest aflatoxin-contaminated food products and feeds.

Aflatoxins, as secreted by toxigenic strains of *A. flavus* and *A. parasiticus*, are amongst the most carcinogenic, mutagenic and teratogenic substances found naturally in foods and feeds (Coulombe 1991; Ahmad et al., 1997) and are highly regulated in most countries. Although Iran is still a developing country, her National Agency For Food and Drug Administration and Control (NAFDAC) is working auspiciously to instituting strict regulation on food, feed and aflatoxin contamination by mould. There are reports from underdeveloped countries that aflatoxin is prevalent in food chain. From 1985 to 1994, over one hundred cases of mycotoxin food poisoning through consumption of contaminated food were reported in China every year (Annual Bulletin of Health Inspection, 1985-1994). In total, 471 food poisoning outbreaks occurred and 233 out of 6103 patients died. The case fatality was 3.82% which ranked the third among microbiological food poisoning. A study from 1997 to 2006 (Shih 2006)) revealed that 339 samples out of 1056 samples, including peanut candies, peanut butters, peanut meal, peanut rice soup and peanuts in Taiwan were tested positive for aflatoxins . With a detected rate of 32.1%. Among them, 65 samples contained aflatoxins above 15µg/kg and exceeded the action levels of Taiwan. Tabata (1998) reported aflatoxin contamination in food and food stuffs in Tokyo for 15 years during the period of 1982-1996 and detected aflatoxin in nuts, cereals, spices, beans and dairy products from commercial markets in Japan. Suprasert et al. (1999) studied mycotoxins contamination in food and feed in Bangkok and reported that aflatoxins were found in many kinds of food and feed such as peanut, corn, coix seed, rice, spice, milk and cheese. The level of aflatoxins contamination was not a serious problem, since it was within the regulation of Thailand limit (20ppb). Among 23 samples of foods and foodstuffs, only one sample from peanut was found to contain aflatoxin at level of 786 ppb, which was too high and the smell was rancid. The occurrence of aflatoxin $B_1$ and ochratoxin A in maize harvested in Vietnam between 1995-1996 was reported by Chau et al. (1997). Our investigation indicated that the food thickeners were contaminated by *A. flavus* and *A. parasiticus*, although *A. flavus* was more prevalent in Akpalata and Offor than Achi and Ukpo. Both toxin and non- toxin formers were present in all samples tested but most importantly, many aflatoxin-producing strains *A. flavus* and *A. parasiticus* were detected indicating that the food thickeners are a good substrate for aflatoxin contamination. Qualitative quantification of $AFB_1$ from *A flavus* isolates (Table 1) indicated that out of 20 isolates of *A. flavus* retrieved from various food thickeners, 15 isolates were qualified as positive, producing $AFB_1$ on TLC compared with standard at Rf 0.49. Similarly out of 20 isolates of *A. parasiticus* tested, only 10 produced $AFB_1$. Quantitative analysis by Fluorometer indicated that production of $AFB_1$ from all positive *A. flavus* isolates ranged from minimum 0.94 to 3.83 µg/g of agar and all positive isolates of *A. parasiticus* ranged from 0.22 – 2.87 µg/g of agar. Various researchers have reported various detection methods of aflatoxin from various foods and feed commodities. Kastner et al. (2010) used HPLC method to analyse Aflatoxin, Ochratoxin A and Deyoxynivalenol (DON). A total of 19 inoculum and five attiéké (a traditional Ivorian cassava product) samples, were investigated for aflatoxin contamination. The toxins AF $B_1$, $B_2$, $G_1$ and $G_2$ were recovered from five fortified cassava samples with rates of 83.0±27.5 %, which complies with EC recommendations (EC 2006). The quantification limit was set to 0.3 µg/kg based on the first calibration point which showed a signal-to-noise ration of >20:1 for aflatoxin $B_1$. In none of the samples were traces of aflatoxin $B_1$, $B_2$, $G_1$ or $G_2$ detected. $AFB_1$ concentrations of red ground pepper were analysed by microtitre plate. Enzyme linked immunosorbent assay (ELISA) method (Ardic et al., 2008) and revealed that seventy-two of the 75 ground deep-red pepper samples (96%) contained $AFB_1$ in the range of 0.11–24.7 µg/kg. Eleven (14.7%) samples were above the regulatory limits used in the European Union and in Turkey .Calleri (2007) also used HPLC with fluorescence detection for $AFB_1$ determination. Several scientists (Rosi et al., 2007; Tekinsen and Ucar 2008) described $AFM_1$ By ELISA and HPLC techniques. The separation and identification of aflatoxins and trichothecenes in submicrogram quantity by thin-layer chromatography/ fast atom bombardment (TLC/ FAB) mass spectrometry is reported by Tripathi et al. (1991). Mycotoxin contamination of feedstuffs used in Thailand was studied by analyzing aflatoxin $B_1$, zearalenone and deoxynivalenol in raw materials of feeds and complete feeds collected in 2000-2003 with TLC method (Taivgmunkhong et al., 2004).

**Table 1: Concentration of $AFB_1$ (µg/g of agar) produced by *A. flavus* and *A. parasiticus* isolates in food thickeners from various market place in East Iran.**

| *A. flavus** isolates from food thickeners | Concentration of AFB1 (µg/g of agar) | | *A. parasiticus** isolates from samples | Concentration of $AFB_1$ (µg/g of agar) Media | |
|---|---|---|---|---|---|
| Media | CYA** | YES** | | CYA | YES |
| Akpa1 | 1.88±0.015 | 2.51±0.258 | Akpa11 | 1.39±0.595 | 0.39±0.002 |
| Akap2 | 1.81±0.031 | 2.57±0.012 | Akap22 | 0.23±0.001 | 0.24±0.002 |
| Akap3 | 1.84±0.026 | 2.73±0.028 | Akap33 | - | - |
| Akpa 4 | - | - | Akpa 44 | - | - |
| Offor1 | 1.87±0.01 | 2.58±0.043 | Offor11 | 0.22±0.002 | 0.37±0.003 |
| Offor2 | 1.94±0.026 | 3.83±0.03 | Offor22 | 0.26±0.002 | 0.40±0.002 |
| Offor3 | 1.96±0.018 | 2.74±0.020 | Offor33 | - | - |
| Offor4 | 1.82±0.026 | 2.68±0.068 | Offor44 | - | - |
| Offor5 | - | - | Offor55 | - | - |
| Achi1 | 1.03±0.025 | 1.84±0.020 | Achi11 | 1.84±0.015 | 2.85±0.026 |
| Achi2 | 1.26±0.031 | 1.54±0.023 | Achi22 | - | - |
| Achi3 | 1.24±0.020 | 1.85±0.028 | Achi33 | 1.84±0.026 | 2.74±0.020 |
| Achi4 | - | - | Achi44 | - | - |
| Achi5 | - | - | Achi55 | - | - |
| Ukpo1 | 1.23±0.03 | 1.82±0.026 | Ukpo11 | 1.92±0.017 | 2.87±0.011 |
| Ukpo2 | 1.26±0.01 | 1.74±0.023 | Ukpo22 | 1.95±0.025 | 2.81±0.016 |
| Ukpo3 | 1.04±0.026 | 1.66±0.031 | Ukpo33 | 1.82±0.026 | 2.74±0.026 |
| Ukpo4 | 1.25±0.023 | 0.97±0.013 | Ukpo44 | - | - |
| Ukpo5 | 1.25±0.021 | 0.94±0.026 | Ukpo55 | 1.83±0.013 | 2.73±0.03 |
| Ukpo6 | - | - | Ukpo66 | - | - |

± Standard Error; *Isolates of *A. flavus* were coded after name of food thickeners collected from different open market place in Iran; **YES -yeast extract sucrose agar, CYA- czapek yeast autolysate

**Table 2: Intensity of A. *flavus* and *A. parasiticus* in food thickeners from various market place in Iran.**

| Name of food thickeners | No of samples | Markets place samples collected* | Presence of *A. flavus*** | Presence of *A. parasiticus*** | Concentration of $AFB_1$ in all food thickeners (range) µg/g |
|---|---|---|---|---|---|
| Akpalata | 4 | Umuahia (Isi-gate), Afor Nawfia, Ugwu-orie, Ogbete, Okigwe. | +++ | ++ | 8.5 – 95 |
| Offor | 5 | Umuahia (Isi-gate), Afor Nawfia, Ugwu-orie, Ogbete, Okigwe. | +++ | ++ | 8.0- 90 |
| Achi | 6 | Umuahia (Isi-gate), Afor Nawfia, Ugwu-orie, Ogbete, Okigwe. | ++ | +++ | 4.5-56 |
| Ukpo | 6 | Umuahia (Isi-gate), Afor Nawfia, Ugwu-orie, Ogbete, Okigwe. | ++ | +++ | 4.0- 50 |

* Food thickeners collected from different open market place in Iran; ** Degree of contamination by fungi: + Degree of contamination considered as low; ++ Degree of contamination considered as medium; +++ Degree of contamination considered as high; - No contamination.

In our investigation, all isolates grew on both on CYA and YES though aflatoxin production was significantly higher on YES than CYA, hence we observed that YES agar media is the better media for $AFB_1$ production by both *A. flavus* and *A.parasiticus*. Davis et al.(1966) studied aflatoxin production in YES media with modification and reported that cultural conditions influencing the production of aflatoxins $B_1$ and $G_1$ by *A. flavus* growing in a sucrose yeast extract liquid medium as stationary cultures and compares the toxin-producing ability of several isolates of *A. flavus* growing in YES medium and also found the YES medium is easy to prepare, relatively inexpensive, and is suitable for production of higher levels of aflatoxin than those reported for other media. For these reasons, YES medium appears to be suitable for both production of aflatoxin and for screening fungi for their ability to produce aflatoxins. Meimaroglou et al. (2009) used YES media and CZA media for aflatoxin production and studied the effect of various MeJA concentrations on growth of *A.parasiticus* and aflatoxin production.

**Detection of $AFB_1$ in food thickeners**

Our investigation revealed that food thickeners available to consumers in Iran are contaminated with mold with a dominance of *A. flavus* and *A. parasiticus* (Table 2). The analysis of natural occurrence of $AFB_1$ in food thickeners from open markets in this part of Iran further substantiate the probability of aflatoxin ingestion by our local people. The concentration ranged

from 4.0 to 95 µg/g of all sample tested. These findings provided evidence that there are aflatoxin problems in food thickeners which causes a potential threat to consumers' health. The high incidence of naturally produced aflatoxin in food thickeners illustrates the hazards with which this community is confronted. Contaminated food is the main vehicle for aflatoxin ingestion. In areas of the world with warm and humid climates such as sub-Saharan Africa and South East Asia, frequency of aflatoxin contamination of foods has been reported to be high (Shank et al., 1972; Peers et al., 1987; Yeh et al., 1989). In India Vasanthi et al. (1997) studied aflatoxin intake and reported that aflatoxin intake through the consumption of maize was assessed in 12 households in a rural population in southern India. This was based on the measurement of aflatoxin levels in cooked maize and the quantity of consumption of the maize.

The general public is concerned about food safety and the prevention of mycotoxin contamination in cereal grains, corn and nuts that enter the food chain. Therefore, rapid and accurate assays for mycotoxins are important to the grain industry, animal producers, overseas buyers and inspection agencies. Several methods have already been proposed and reviewed for $AFB_1$ determination in food and agricultural products (CAST 2003; Kriska et al., 2005; Zheng et al., 2006). However thin layer chromatography is still being widely used because it is simple and less expensive. Developing countries like Iran can easily adopt this method with very little cost to analyse food sample against contamination by aflatoxin producing mold. Further if quantification is necessary then HPLC, Gas chromatography and immunological technique may be adopted. In our case TLC was the method of choice because it was economical and fluorometry was easily available. Moreover, this is the first comprehensive report on the collection of food thickeners from various sources sold in open market in the South-east geo-political zone in Iran , identifying different strains of *A. flavus* and *A. parasiticus* and further selecting a suitable media for maximum $AFB_1$ production.

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