Towards managing and controlling food safety based on contamination with fungi and its mycotoxins

Afaf A Amin, Gulsen S Ahmed, Hoda H Abo Ghalia, Amera A Hamed

ABSTRACT

A significant portion of the agricultural produce in the countries and the world over become unfit for human consumption due to mycotoxins contamination of grains and cereals. The main toxic effects are carcinogenicity, genotoxicity, teratogenicity, nephrotoxicity, hepatotoxicity, reproductive disorders and immunosuppression. This study was done to identify the molds and aflatoxins that contaminate cereal–based baby foods and corn–based snack products. The most frequent fungal genera found in the samples were Aspergillus, Penicillium, Alternaria, Fusarium and Cladosporium with frequencies of 41, 16, 10, 8 and 3%, respectively. Additionally, the numbers of contaminated cereal–based baby foods samples with AFB1, B2, G1 and G2 were 14, 2, 6 and 4%. Also, 34, 14, 18 and 8% of corn-based snack samples respectively. Ten essential oils of (cinnamon, cumin, clove, fennel, garlic, lemon grass, marjoram, peppermint, rosemary and thyme) plants using in combating aflatoxigenic mold A. flavus growth and its aflatoxins production. The ten essential oils showed notable inhibitory effects on A. flavus growth and its aflatoxins production. Cinnamon and garlic essential oils caused complete inhibition to all types of aflatoxins at concentrations of 60 and 80µl respectively. There were some alterations produced by cinnamon, garlic and cumin essential oils at sub-lethal inhibitory concentrations such as abnormal cell shape, leakage of cell wall and the membranous organelles were disrupted.

Key words: Aflatoxins, fungi, cereal-derived products, essential oils, risk assessment

INTRODUCTION

Cereals and other crops are exposed to fungal attack in the field or during storage and this attack may result in mycotoxin contamination of the corps. The most commonly found of mycotoxins, namely aflatoxins, which carry potential risks for humans (Alpsoy, 2010). Aflatoxins are a group of structurally related toxin metabolites produced by many strains of Aspergillus flavus, Aspergillus parasiticus and Aspergillus nomius. Aflatoxin exposure has been linked to impaired growth (Gong et al., 2002), kwashiorkor (Coulter et al., 1986) and may also have a role in the modification of the etiology of hepatitis B (Turner et al., 2000) in African children. Aflatoxin B1 (AFB1) is one of the most potent toxic carcinogen, teratogen and mutagen and it is listed as a group I carcinogen by the International Agency for Research on Cancer (IARC) as it the cause of human primary hepatocellular carcinoma (IARC, 2002). The liver is the main target organ for aflatoxin toxicity and carcinogenicity.

The ranges of worldwide regulations for AFB1 and total aflatoxins were from 0 -30 µg kg–1 and from 0-50 µg kg–1, respectively (FAO, 2004). In the European Union, AFB1 and total aflatoxins levels in human commodities are regulated with Maximum Residue Levels (MRLs) that cannot be greater than 2 and 4 µg kg–1, respectively (European Commission (EC) Regulations, 2010).

Mycotoxins affecting ground nuts / pea nuts, cereals (maize, rice, sorghum, wheat, barley and oats), spices (black pepper, ginger and nutmeg) and chili are considered to be of greater significance world over for human beings. The exposure to levels of aflatoxins from nanograms to micrograms per day occurs through consumption of maize and peanuts, which are dietary staples in several tropical countries. Therefore, this study has been performed to evaluate the mycological quality and aflatoxins contamination based on analysis of AFB1, AFB2, AFG1 and AFG2 in one hundred randomly collected samples representatives of cereal–derived products. The risk for the consumers is also assessed. Thus, there is a need to search for
alternative approaches to store grains/cereals for human consumption without toxicity problems that are eco-friendly and not capital intensive. Plant extracts have antifungal and antitoxins properties under laboratory trials (El-Nagerabi et al., 2013).

**PROBLEM FORMULATION**

**Food Defense and Crisis Management:** The consequences of unsafe food can be serious and food safety management standards help organizations identify and control food safety hazards. As many of today's food products repeatedly cross national boundaries, International Standards are needed to ensure the safety of the global food supply chain.

Food safety management system (as HACCP, ISO 22000) specifies requirements for a food safety, where an organization in the food chain needs:

- To demonstrate its ability to control food safety hazards in order to ensure that food is safe at the time of human consumption.
- To plan, implement, operate, maintain and update a food safety management system aimed at providing products that, according to their intended use, are safe for the consumer.

**Developing a Food Defense Plan:**

- It is necessary to control and reduce any food safety hazards, must endorses the implementation of food safety management systems in retail and food service establishments combined with good basic sanitation, and other prerequisite programs.
- To effectively communicate food safety issues to their suppliers, customers and relevant interested parties in the food chain.
- To ensure that the organization conforms to its stated food safety policy, the safety management approach to food safety is based on a detailed examination of every stage in the production process for an individual food product, with an objective to identifying where and when hazards could occur.
- To design effective controls for each hazard:
  - Perform a Hazard Analysis
  - Decide on the Critical Control Points (CCPs)
  - Determine the Critical Limits
  - Establish Procedures to Monitor CCPs
  - Establish Corrective Actions
  - Establish Verification Procedures
  - Establish a Record Keeping System

**Crisis-management plan:** In the case of situations entailing direct or indirect risks to human health not provided for by the Regulations, Countries must establish a general crisis-management plan. As in the case of a serious risk, which cannot be dealt with under the existing provisions, must immediately set up a crisis unit, in which the Authority participates by providing scientific and technical support. The crisis unit is responsible for collecting and evaluating all relevant information and identifying the options available for preventing, eliminating or reducing the risk to human health.

Agriculture and health must move together. This means policymakers and researchers in both sectors will have to take off their blinders and look beyond the boundaries of their traditional areas of action for food safety management.

**MATERIALS AND METHODS**

**Samples collection:** One hundred cereal – derived samples, including 50 samples of cereal – based baby foods (for infants and young children) and 50 corn – based snacks samples, were randomly purchased from different supermarkets and small shops from Great Cairo, Egypt. The samples were purchased with intact package and analyzed before the expiration date.

- The cereal – based baby foods samples contain one or more of the following major ingredients: Wheat bran, barley, malt extract, sugar flakes of corn, cereals (wheat flour, rice flour, and corn flour), oats, dried fruits (date, raisin, orange and bananas), honey, chamomile, carrot, sugar, vegetable oil, vitamins, iron, traces of milk and minerals.
- The corn – based snacks samples, corn is the main component, in addition to different types of flavors including cheese, chili, pea nut, tomato, salt, ketchup and spices.

**Culture media:**

- Potato Dextrose Agar (PDA) (Difco™ Becton, (Jawetzet al., 2004)
- CzapekDox Liquid medium (modified) (OXOID) (Smith, 1960) Peptone, bacteriological (HIMEDIA, India) (Straka and Stokes 1957)

**Chemicals:**

- Standard aflatoxins, Carouse Chemical Company, Netherland.
- HPLC grade solvents and chemicals, Lab-scan Company, Egypt.
- Reagent kits that used in aflatoxins detection, Bio – diagnostic Company, Egypt.

**Investigated aflatoxins:** Four types of aflatoxins were investigated (B1, B2, G1 and G2).

**Essential oils:** Ten plants; cinnamon, cumin, clove, fennel, garlic, lemon grass, marjoram, peppermint, rosemary and thyme were purchased from the local market as medicinal plants and used for the preparation of essential oils by using glassware apparatus for steam distillation. The oils were dried over anhydrous sodium sulphate, stored in dark sterilized vial at 4°C until used (Association Official of Analytical Chemistry (A.O.A.C), 1995).

**Mycological analysis:** Ten g of each sample were ground and homogenized with 90 mL Peptone medium for 30 min on a horizontal shaker. Further serial dilutions to 10⁻³ were prepared.
One milliliter portions of these dilutions were transferred to sterile Potato dextrose agar medium and incubated at 22 - 25 °C for 5 days (Egyptian Standards 1793/2005). The number of colony-forming units per gram (cfu/g) was determined for each genus. All mycological analysis was done at Food Safety Department, Microbiology Unit, National Nutrition Institute, ministry of health, Cairo, Egypt.

Aspergillus flavus was found to be the most frequent mold isolated from the samples, so pure culture of the isolated A. flavus was subjected to identification at the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University (Cairo/ Egypt) to use it in further investigations. Stock culture of the identified A. flavus was maintained on slants of potato dextrose agar (PDA) medium, stored at 4 °C and subculture was made every two weeks.

Preparation of the spore suspension: Spores of A. flavus were harvested by adding mixture of 10 ml of sterile distilled water and 0.1% tween 80. The spore suspensions were aseptically filtered off the mycelia and adjusted with sterile distilled water to give a final spore concentration of approximately 10^6 spore/ml. The numbers of spores were counted using a haemocytometer, according to Atanda et al. (2007).

Determination of aflatoxins concentration in food samples by HPLC: The concentrations of aflatoxins (B1, B2, G1 and G2) in the collected samples were determined by High Performance Liquid Chromatography (HPLC) in the Central Laboratory of Residues analysis of Pesticides and Heavy Metals in Food /Agriculture Research Center (Ministry of Agriculture, Cairo, Egypt, according to the procedure of A.O.A.C (2003).

Estimation of daily intake (EDI) of aflatoxin B1 and total aflatoxins: Dietary intake is the tool for calculating the amount of ingested any toxin due to the consumption of contaminated food. Estimation of the daily intake (EDI) by using the following equation (Codex Alimentarius Commission, 2006):

\[
\text{EDI} = \text{Food consumption of the product} \times \text{mean of contamination} \\
\text{Average of body weight}^{***} \\
* \text{Food consumption of the product: Baby} = 30g, \text{adolescent and adult} = 50 g \\
** \text{Mean of contamination in samples by µg/kg} \\
*** \text{Average body weight: Baby} = 10 kg, \text{adolescent} = 40 kg \text{and adult} = 60 kg
\]

RESULTS AND DISCUSSION

Frequencies of different genera of fungi present in samples

From Figure 1, the samples of cereal- based baby foods and corn-based snacks are liable to infection with different fungi. Five genera of filamentous fungi were isolated from samples, Aspergillus, Penicillium, Fusarium, Alternaria and Cladosporium. These results were in agreement with Ismail et al., 2012, who found that Aspergillus, Penicillium, Fusarium and Cladosporium were the most common genera in baby food products that mainly made of cereal flour(s).

Aspergillus (36%) and Penicillium (12%) were the most prevalent genera. Aspergillusflavus was found in high percentage (16%) from the total Aspergillus spp. The manufacture of baby foods is based on dried cereals like corn, rice, millet, sorghum, wheat, oats and barley with various additional ingredients such as dried fruits or vegetables which are very suitable for growing these types of fungi. Fungi contaminating grains have been conventionally divided into two groups – field fungi and storage fungi. Field fungi are those that infect the crops throughout the vegetation phase of plants and they include plant pathogens such as Alternaria, Fusarium, Cladosporium, and Botrytis species. Their numbers gradually decrease during storage, and replaced by storage fungi of Aspergillus, Penicillium, Rhizopus and Mucor genera that infect...
grains after harvesting and during storage (Piotrowska et al., 2013). Among all the isolated fungal species, 93.2% belonged to the group of toxigenic fungi. Several toxin-producing Aspergillus species were reported to dominate on cereals, especially A. flavus. Also showed that Aspergillus was recovered in high Frequency, contaminating (46%) of corn - based snacks samples.

Corn is the main source of contamination in corn snacks plus some flavors or additives especially chili, spices and peanut. Rajasinghe et al., 2009, found that the aflatoxigenic A. flavus tobe dominant in spices during storage. The most important factors which influence the fungal growth and aflatoxins production are water availability and temperature. In Egypt, grains stored under high moisture / humidity (>14%) at warm temperature (>20° c) and/or inadequately dried can potentially become contaminated. Also initial growth of fungi in grains can form sufficient moisture from metabolism to allow further growth and mycotoxin formation, thus we must apply the food safety management system from farm to fork.

Monitoring of aflatoxins in cereal - based baby foods and corn - based snacks samples: Little information on the occurrence of aflatoxins on baby foods exist. The products screened in this study are generally commercialized in Egypt in small shops and supermarkets and consumed especially by children. Thus, more importance to their safety is needed. The data in Figure 2 concluded that 7 of 50 (14%) cereal – based baby foods samples were contaminated with aflatoxins B1 and its concentrations ranged from 0.54 to 2.22 µg/ kg, with a mean value of 1.38 µg / kg., the numbers of contaminated samples with B2, G1 and G2 were 1 (2%), 3 (6%) and 2(4%) with mean value of (0.5), (1.66) and (0.9) µg / kg, respectively.

![Figure 2: Number of contaminated cereal- based baby foods and corn-based snacks samples with aflatoxins B1, B2, G1 and G2.](image)

The high humidity and temperature in tropical and subtropical areas provide optimal conditions for toxin formation. Improper storage and less than optimal conditions during transportation, marketing and processing can also contribute to fungal growth and increase the risk of mycotoxin production (Sherif et al., 2009). Also 17 of 50 (34%) corn-based snacks samples, contained AFB1 in the range of 0.59 - 15.83 µg /kg with a mean value of 4.43 µg / kg and AFB2 was detected in seven samples (14%), its concentration ranged from 0.51 to 2.01 µg / kg with a mean value of 1.9 µg/kg. Sabbagh and Abedi-Tizaki, (2011) have shown that collected corn from south of Caspian Sea were contaminated by aflatoxin.

The most contaminated samples of corn – based snacks were those with chili, peanut and spices flavor than other flavor. Rajasinghe et al., 2009, found that the aflatoxigenic A. flavus to be dominant in spices during storage.

Maximum Residue Level (MRL) for aflatoxin B1 are seated in the regulations all over the world but no limits were seated for B2, G1 and G2. Infant's food must be free from any traces of aflatoxin B1 and total aflatoxins due to their dangerous effect on their growing. The European Maximum Level allowed for aflatoxin B1 in Processed cereal- based foods for infants and young children is 0.10 µg /kg (European Commission Regulation, 2010), so that in this case all cereal – based food samples which contaminated with aflatoxin B1 (7 samples) with arrange from 0.54 to 2.22 µg / kg are considered to be violated Because it over passed the European Maximum Level as showed in Table 1.

Thus according to the present study twelve samples of corn – based snacks surpassed the established limit (2 µg/kg) for AFB1 with a range from 2.26 to 15.83 µg/kg and with violation percentage 71 % and ten samples over passed the limit established for total aflatoxins (4 µg/kg) with a range from 4.18 to 28.37 µg/kg and with violation percentage 59 %. The carcinogenicity, mutagenicity and acute toxicity of AFB1 have been well documented, the IARC determined it to be a human carcinogen (group 1A) (Piotrowska et al., 2013). So a calculation of Estimated Daily Intake (EDI) is very important to lead up to assume the risk assessment of aflatoxin in consuming of the studied commodities.

**Estimation of the Daily Intake of AFB1 and total aflatoxins**

Exposure assessment is to identify the exposures that occur, or anticipated to occur, in human populations, risk of children from aflatoxin depends on the degree of exposure to and on the degree of hazard from individual toxins, A study (Sherif et al., 2009) investigated aflatoxin exposure in Egyptian children (n=50, aged 1-2.5 years) by assessing urinary aflatoxin metabolite. The Provisional Maximum Tolerable Daily Intake (PMTDI) which equal 1 ng AFB1 /kg body weight for adult and children without hepatitis B.

Data in Tables 2 and 3 represent the Estimation Daily Intake (EDI) of aflatoxin B1 and total aflatoxins for consumers in Egypt due to consuming corn based snakes and cereal based baby foods, which proved from this study that they were contaminated. Also showed that consumption of 30 g from the most contaminated sample (15.83 µg /kg) of corn – based snacks, by a child (20 kg) leads to an AFB1 daily intake about 23.75 ng AFB1 / kg bw/ day, that is more than 23 – fold higher than the PMTDI (1 ng /kg bw/day), which subject children to health risk, in addition, a consumption of 50 g by an adolescent
(50kg) and an adult (70kg) displayed a daily intake from about 11 to 15 fold higher than the PMTDI.

Table 1: Number of contaminated samples, mean, minimum, maximum, number of violated samples and percentages of violation of aflatoxins in samples of cereal-based baby foods and corn-based snacks.

<table>
<thead>
<tr>
<th>Samples</th>
<th>No. of contaminated samples</th>
<th>Mean (µg/kg)</th>
<th>Minimum (µg/kg)</th>
<th>Maximum (µg/kg)</th>
<th>MRL (µg/kg)</th>
<th>No. of violated samples</th>
<th>Violation %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cereals-based baby foods</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AFB1</td>
<td>7</td>
<td>1.38</td>
<td>0.54</td>
<td>2.22</td>
<td>0.1*</td>
<td>7</td>
<td>100</td>
</tr>
<tr>
<td>AFB2</td>
<td>1</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>AFG1</td>
<td>3</td>
<td>1.66</td>
<td>0.8</td>
<td>2.98</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>AFG2</td>
<td>2</td>
<td>0.9</td>
<td>0.6</td>
<td>1.2</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>7</td>
<td>2.42</td>
<td>0.54</td>
<td>6.9</td>
<td>0.1</td>
<td>7</td>
<td>100</td>
</tr>
<tr>
<td>Corn-based snacks</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AFB1</td>
<td>17</td>
<td>4.43</td>
<td>0.59</td>
<td>15.83</td>
<td>2**</td>
<td>12</td>
<td>71</td>
</tr>
<tr>
<td>AFB2</td>
<td>7</td>
<td>1.9</td>
<td>0.51</td>
<td>2.01</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>AFG1</td>
<td>9</td>
<td>3.74</td>
<td>1.1</td>
<td>8.14</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>AFG2</td>
<td>4</td>
<td>1.6</td>
<td>0.92</td>
<td>2.42</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
<td>7.22</td>
<td>0.59</td>
<td>28.37</td>
<td>4</td>
<td>10</td>
<td>59</td>
</tr>
</tbody>
</table>

* EU MRL allowed for aflatoxin B1 in Processed cereal-based foods for infants and young children is 0.10 µg / kg.
** EU MRL in human commodities for aflatoxin B1 is 2 µg/kg and total aflatoxins 4µg/kg.

Table 2: Estimated Daily Intake (EDI) of aflatoxin B1 and total aflatoxins by children, adult and adolescent consuming corn-based snacks:

<table>
<thead>
<tr>
<th>Toxin</th>
<th>Concentration of toxin µg/kg</th>
<th>Consumption (g/person/day)*</th>
<th>Mean Body weight (bw)***</th>
<th>EDI (ng/kg b.w./day)*****</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFB1</td>
<td>More contaminated sample 15.83 ug/kg</td>
<td>Children 30 g</td>
<td>Children 20 kg</td>
<td>23.75</td>
</tr>
<tr>
<td></td>
<td>Less contaminated sample 0.59 ug/kg</td>
<td>Children 30 g</td>
<td>Children 20 kg</td>
<td>0.88</td>
</tr>
<tr>
<td>Total aflatoxin</td>
<td>More contaminated sample 28.37 ug/kg</td>
<td>Children 30 g</td>
<td>Children 20 kg</td>
<td>42.5</td>
</tr>
<tr>
<td></td>
<td>Less contaminated sample 0.59 ug/kg</td>
<td>Children 30 g</td>
<td>Children 20 kg</td>
<td>0.88</td>
</tr>
</tbody>
</table>

*Estimated as the less daily consumption (Content packaging of the product).
**WHO (1983)  ***EDI (ng /kg bw./day ) Calculated for each toxin

Table (3): Estimated daily intake (EDI) of aflatoxin B1 and total aflatoxins by babies consuming cereals-based food.

<table>
<thead>
<tr>
<th>Toxin</th>
<th>Concentration of toxin µg/kg</th>
<th>Consumption (g/baby /day) *</th>
<th>Mean Body weight (bw)***</th>
<th>EDI (ng/kg b.w./day)*****</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aflatoxin B1</td>
<td>High contaminated sample 2.22 ug/kg</td>
<td>30 g</td>
<td>Baby (6 months) 6 kg</td>
<td>11.1</td>
</tr>
<tr>
<td></td>
<td>less contaminated sample 0.54 ug/kg</td>
<td>30 g</td>
<td>Baby (6 months) 6 kg</td>
<td>2.7</td>
</tr>
<tr>
<td>Total aflatoxin</td>
<td>High contaminated sample 6.9 ug/kg</td>
<td>30 g</td>
<td>Baby (6 months) 6 kg</td>
<td>34.5</td>
</tr>
<tr>
<td></td>
<td>Less contaminated sample 0.54 ug/kg</td>
<td>30 g</td>
<td>Baby (6 months) 6 kg</td>
<td>2.7</td>
</tr>
</tbody>
</table>

*Estimated as the less daily consumption (Content packaging of the product).
**WHO (1983)  ***EDI (ng /kg bw./day ) Calculated for each toxin
Similarly, there is a significant risk for babies (6, 12 and 24 months) who occasionally consumes a cereal – based food (30 g per day) where, an AFB daily intake is from 6.66 to 11.1 ng/kg bw/day, that is more than 6 to 11 fold higher than the PMTDI, while the consumption of less contaminated sample, the daily intake is about 2 fold higher than PMTDI.

Australia Market Basket Survey (1992) reported that the mean dietary intakes of total aflatoxin are 0.15 ng/kg bw per day for Australians, EU Scientific Cooperation on Questions Relating to Food Projects (SCOOP), 1996 showed that the dietary exposure of Europeans to AFB1 ranged from 0.03 to 1.28 ng/kg bw per day.

Results of this study explain the reason behind outbreak of children diseases especially cancer and liver diseases.

Antimicrobial activity of essential oils on Aspergillus flavus growth:

Antifungal activity assay: Ten essential oils were tested for their antifungal effect on the growth of A. flavus isolate on potato dextrose agar media, using agar well diffusion method at concentrations 10 and 20 µl for each essential oil. The inhibition of A. flavus growth was expressed as inhibitory zones (Mean) and measured in (mm) diameter as illustrated in Figure 3.

![Figure 3: The antifungal activity of different essential oils on Aspergillus flavus growth expressed as inhibition zone diameter (mm).](image)

As showed in Figure 3, the three essential oils cinnamon, garlic and cumin were particularly effective against A. flavus growth exhibiting high diameters of growth inhibition (44.7, 42.3 and 32.7 mm) respectively, at a dose of 10 µl. Mohmmadpour et al. (2012) indicated that Cuminum cyminum L. (cumin) has good antifungal activity against A. flavus, A. parasiticus and A. niger. Othman and AL-Delamiy (2012) found that garlic (Allium sativum) extract is more effective as anti- Aspergillus spp. agent than black cumin extract.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) of the more efficient 3 essential oils against A. flavus: Table 4 provides (MIC) and (MFC) values and MIC/ MFC ratio, results revealed that the oils tested exhibited different degrees of antifungal activity against toxigenic A. flavus. The maximum antymycotic activity was shown by cinnamon followed by garlic and cumin essential oil. Cinnamon essential oil exhibited the strongest antifungal activity, with MIC and MFC values of 0.05 µl/ml for each, when cinnamon essential oil was added to A. flavus culture medium, development was affected from the concentration of 0.005 µl/mL. Also, Wahegaonkar and Shirurkar (2013) reported that garlic is the most effective in inhibiting spore germination of different fungi. While, cumin essential oil exhibited relatively moderate activity with an MIC value of 0.38 µl/ml and 0.66 µl/ml as MFC value. Mohmmadpour et al. (2012) reported that C. cyminum L. essential oil had substantial antifungal activity against A. flavus PICC-AF39, Aspergillusflavus PICC-AF24. C. cyminumL oil killed more than 60% of the spores of the tested Aspergillus species within 12 hours.

![Figure 4: The percentage of inhibitory effect of cinnamon essential oil on aflatoxins B1, B2, G1 and G2 production by A. flavus in Czapek medium.](image)

Table 4: MIC and MFC of the more efficient 3 essential oils against A. flavus growth.

<table>
<thead>
<tr>
<th>Essential oils</th>
<th>MIC (µl/ml)</th>
<th>MFC (µl/ml)</th>
<th>MFC/MIC ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cinnamon</td>
<td>0.05</td>
<td>0.05</td>
<td>1 (microbicidal)</td>
</tr>
<tr>
<td>Garlic</td>
<td>0.11</td>
<td>0.22</td>
<td>Greater than 1 (microbiostatic)</td>
</tr>
<tr>
<td>Cumin</td>
<td>0.38</td>
<td>0.66</td>
<td>Greater than 1 (microbiostatic)</td>
</tr>
</tbody>
</table>

Karouet al. (2005) stated that, if the MFC/MIC ratio is greater than 1, it is considered as microbiostatic, while ratio equal or smaller than 1 is considered as microbicidal. From the obtained results we may conclude that cinnamon essential oil has fungicidal nature on A. flavus, garlic and cumin essential oil have a fungi static nature on A. flavus.

Effect of different concentrations of essential oils on aflatoxins Production by Aspergillusflavus in Czapek liquid media: The biosynthesis of aflatoxins can be inhibited by essential oils (EOs) extracted from herbal, medicinal and aromatic plants which are toxic to the fungal growth and aflatoxin production of A. flavus and A. parasiticus and this has been suggested by many researchers.
Cinnamon essential oil: Figure 4 demonstrate the effect of different concentrations (10, 20, 40, 60, 80 µl / 50 ml media) of cinnamon essential oil on aflatoxins production. The concentration of aflatoxins B₁, B₂, G₁ and G₂ in control sample were 541, 219, 292.3, and 174.7 µg/50 ml media, respectively. The most effect observed with concentration (20µl /50 ml media) aflatoxins concentrations B₁, B₂, G₁ and G₂ were 1.77, 2.43, 4.73 and 1.1 µg/50 ml media, respectively, which meaning it reduced by (99.7%), (98.9%), (98.9%) and (99.4%), respectively. Moreover, the highest inhibition percentage of aflatoxins production (100%) was at concentration (40µl /50 ml media) of cinnamon essential oil. Montes-Belmont and Carvajal (1998) used the essential oils of Cinnamomum zeylanicum for the protection of stored maize grains against A. flavus, it has significant antifungal activity against A. flavus in culture media and a complete inhibition of A. flavus growth was observed at 200 ppm of the essential oil.

Also, Figure 5 illustrated that treated concentrations (40, 60, 80 µl / 50 ml media) of cinnamon essential oil gave clear reduction in the mycelium dry weight, it dramatically depressed to 0.09, 0.01, 0.0 g, with inhibition percentages of (96.1%), (99.6%) and (100%), respectively.

![Inhibition Percentage % of mycelium dry weight](image1)

Cinnamon essential oil concentrations (µl/50 ml medium)

Figure 5: The percentage of inhibitory effect of cinnamon essential oil (EO) on dry weight of mycelia of Aspergillusflavus in Czapek medium.

Garlic essential oil: The greatest inhibition (Figure 6) for the four types of aflatoxins was observed at (20µl EO /50 ml media), at concentrations (40, 60, 80 µl EO / 50 ml media) there were a complete inhibition and no aflatoxins detected, the inhibition percentage was 100% for all types of aflatoxins. Allicin, thiosulfonate and other compounds of garlic showed fungistatic activity against A.spp. such as A. flavus, A. fumigatus, A. terreus and P. chrysogenum (Kumar and Jain, 2010).

Also, Figure 7 illustrated that the dry weight in treated media with different concentrations were reduced. This mean there was a great effect of garlic essential oil on the mycelium growth of A.flavus and this result was in agreement with Wahegaonkar and Shirurkar (2013) who reported that garlic is most effective in inhibiting spore germination of different fungi.

![Inhibition Percentage % of mycelium dry weight](image2)

Garlic essential oil concentrations (µl/50 ml medium)

Figure 7: The percentage of inhibitory effect of garlic essential oil on dry weight of mycelia of Aspergillusflavus in Czapek medium.

Cumin essential oil: The concentration (10µl EO /50 ml media) gave clear reduction in the aflatoxins concentration B₁, B₂, G₁ and G₂ (Figure 8).

The complete inhibition of aflatoxin production (100%) was at (80 µl EO /50 ml media). Singh and Upadhayay (1991) showed that Cuminaldehyde, an active principle from the oil of Cuminum cerium Linn, has antifungitoxic activity against A. flavus.

![Inhibition Percentage % of aflatoxins concentrations](image3)

Figure 8: The percentage of inhibitory effect of cumin essential oil on aflatoxinsB₁, B₂, G₁ and G₂ production by A. flavus in Czapek medium.
The lowest mycelium dry weight was obtained at concentration 80 µl EO /50 ml media, as showed in Figure 9. This means that the increasing concentrations of cumin essential oil caused a significant reduction in the aflatoxin concentration and in the mycelium growth of Aspergillus flavus. Karbinet al. (2009) found that Cuminum cyminum essential oils possess a remarkable inhibiting the growth of Aspergillus flavus.

![Graph showing inhibition percentage of mycelium dry weight](image)

Cumin essential oil concentrations (µl/50 ml medium)

Figure 9: The percentage of inhibitory effect of cumin essential oil on dry weight of mycelia of Aspergillus flavus in Czapek medium.

Examination under the Transmission Electron Microscope (TEM)

Stained sections were examined with a Transmission Electron Microscope (JEOL 1010, Japan) at the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University.

![Micrographs of mycelial T.S. of A. flavus under normal conditions (untreated) and under essential oils treatment.](image)

Figure 10: TEM Micrographs of mycelial T.S. of A. flavus under normal conditions (untreated) and under essential oils treatment.

(A) Untreated mycelia (control) (X= 25000),
(B) Cinnamon oil-treated mycelia (X= 25000),
(C) Garlic oil-treated mycelia (X= 25000),
(D) Cumin oil-treated (X= 25000), mycelia

Figure 10 & 11 shows that the cell membrane is a very important target of the essential oil components, the surfaces of the hyphae and conidia treated by oils became rough in contrast to the control group. The cell wall (CW) exhibited degenerative changes; it was disrupted and became rough and villi form. Subsequently, the cell wall became very thin and even seemed to disappear in some hyphae. The membrane-disruptive activity of essential oil components may be closely associated with the interference with enzymatic reactions of the membrane, such as respiratory electron transport, proton transport, and coupled phosphorylation steps (Khosravi et al., 2011). The oils passed not only through the cell wall but also through the plasma membrane and then interacted with membranous structures of the cytoplasmic organelles. The marked action of oil components might have conferred lipophilic properties and the ability to penetrate the plasma membrane (Knobloch et al., 1989). It is believed that lipophilic properties of oils may assist in penetration of cell membrane and, accumulation of polysaccharides in stress condition may lead to rupture of plasmalema in fungal cells.

![Micrographs of A. flavus spores under normal conditions (untreated) and under essential oils treatment.](image)

Figure 11: TEM Micrographs of A. flavus spores under normal conditions (untreated) and under different essential oils treatment.

(A) Untreated spore (control) (X= 25000),
(B) Cinnamon oil-treated spore (X= 25000),
(C) Garlic oil-treated spore (X= 25000) and
(D) Cumin oil-treated spore (X= 25000).

A. flavus spores treated with the essential oils were illustrated, the normal morphology of the fungal conidia was disturbed, moreover, the conidial cell wall was found to possess a sheath (sh) with spinuloseechinulations, while the cytoplasmic membrane was shrunk, and their materials concentrated at the center of conidia. Additionally there was partial disappearance of cell wall and the cytoplasm had small pigmented deposits and vacuoles (V). Fernanda et al. (2012) reported that strong antimicrobial activity could be correlated with essential oils containing high percentage of monoterpenes, eugenol, cinnamic aldehyde, and thymol. Finally there was disorganization of cytoplasmic contents accompanied by intensive degradation and lysis of the nucleus and mitochondria.
CONCLUSION AND RECOMMENDATION

The current results revealed that cereal – based baby foods and corn – based snacks were contaminated by fungi. Many of these fungi are capable of producing mycotoxins. Contamination of such foods (especially those for babies) is a matter of health hazard for human consumption. However their safety can be insured and improved greatly by using quality raw materials. It was noticed that the contamination occurs for cereal grains before, during or after harvesting, during drying process, or even during food production and this contamination could be also due to long-term storage, marketing under non-hygienic conditions of the food products. So, we suggest that monitoring fungal contaminations as well as mycotoxins should be carried out periodically and the procedures to prevent mould contamination should be developed. Due to health and economic consideration, natural plant essential oils may provide an alternative method to protect food from fungal contamination. So, this study aims at evaluate the use of essential oils from cinnamon, garlic and cumin as antifungal agents to be suitable for applications on the food industry. They can be used as growth inhibitors of *Aspergillusflavus* and its aflatoxins production. The main reason for their suitability is their natural origin, which consumers find comforting and low risk.

REFERENCES


