



Detection of the amount of contamination of some foods manufactured with deadly fungal toxins

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Abstract

About 40 isolates backed to 17 species of fungus belonging to 11 genera as well as sterile fungus were isolated from five types of processed foods (corn chips, tomato paste, pasta, milk powder, canned Peas) Of these, Most of the isolates isolated during the study were deficient fungi (Deuteromycotina) and fungus fungi (Zygomycotina). *Aspergillus* fungal species recorded a clear appearance and achieved the highest frequency ratios. 13 were due to the fungus that has the ability to produce toxins, the most famous is the fungus *Aspergillus flavus*, which has the ability to produce dangerous aflatoxin toxicity and revealed its ability to produce toxins through the use of coconut agar. The results of the statistical analysis using the square (X²) showed that the total number of fungal isolates produced by aflatoxin (P <0.05) was significantly different depending on the type of food.

Keywords: fungal toxins, contamination, biological activity, Deuteromycotina

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INTRODUCTION

Mycotoxins are defined as a group of biological compounds produced by a group of fungi that have the ability to produce secondary metabolites when grown in an environment suitable for their production. These products are bioactive compounds, which are non-immune toxins. Of the components that drive the body to form antibodies to it, most of which are toxic to humans, animals, plants and microorganisms (Ismail, 2014). Fungicides reach human and animal food through contamination of food and food with their fungi. This is called direct contamination. The food encourages fungus to grow either during different production stages or during transport during storage (EFSA, 2009). Mycotoxins are classified according to the type of damage they cause: Mycotoxins are classified according to the type of damage they cause: Hepatotoxins (these are toxins that affect, damage, or cause cancer in the liver, such as Aflatoxins), Nephrotoxins (they are fungal toxins that affect the kidneys and cause kidney cancer and kidney failure, such as citrinin toxins), Genototoxins (these are toxins that affect the reproductive system and cause infertility in most cases, such as Zeralenone toxins) and Gastrointestinal toxins (they are toxins that affect the digestive tract, such as Trichothecine (Ismail, 2014). Aflatoxins is one of the secondary metabolic compounds produced by two fungi belonging to the genus *Aspergillus*: *A. flavus* and *A. parasiticus*. Or Chronic (EFSA, 2007). Aflatoxins belong to the group of dior furanomarine (Difurancomarine) and are

characterized by a strong flare emitted fluorescence when exposed to long waves of ultraviolet radiation, and with this property, it is possible to detect the presence of these dangerous compounds at very low levels of 5.0 ng or less, as They are colourless or white crystals that dissolve in medium-polar solvents such as chloroform and methanol, and their solubility in water is limited to about 20–10 mg (Jubouri and Ibrahim, 1998). These toxins were discovered in 1961 after a previously unknown condition in England that led to the death of more than 100,000 young turkeys in a short period of time after ingesting pistachio powder imported from Brazil, called Turkey disease (EFSA, 2009). Aflatoxins are among the most dangerous fungal toxins on poultry health, especially that fungi that grow in the field on many types of feed stocks consisting of poultry feeds of corn, wheat, barley and soybeans are a major source of contamination of these grains with aflatoxins (Villar and Carson, 2004). The present study aimed to show the extent of contamination of food with fungi, especially producing dangerous toxins and to detect the extent of the impact of these toxins on public health.

MATERIALS AND METHODS

Food samples: Food samples were obtained from the local markets of Nasiriyah, southern Iraq.

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Table 1. Fungi isolated from various foodstuffs

Isolated fungi	Tomato paste	Number of isolates	Canned peas	Number of isolates	pasta	Number of isolates	Corn Chips	Number of isolates	Powdered milk	Number of isolates
<i>Absidia corymbifer</i>	+	3	+	1	-	-	+	2	+	2
<i>Acremonium kiliense</i>	+	2	-	-	-	-	-	-	+	6
<i>Alternaria alternata</i>	+	5	+	1	+	4	+	2	-	-
<i>A.chlamyospora</i>	+	2	+	2	-	-	-	-	-	-
<i>A.phragmospora</i>	-	-	+	4	+	5	+	3	-	-
<i>A.radicina</i>	+	3	-	-	+	2	+	4	-	-
<i>Aspergillus flavus</i>	+	3	+	2	+	2	+	5	+	3
<i>A.fumigatus</i>	+	5	+	1	+	3	+	2	+	2
<i>A.niger</i>	+	3	+	1	+	2	-	-	+	3
<i>A.ochraceum</i>	+	2	-	-	-	-	-	-	+	2
<i>Auerobasidium pulluans</i>	+	1	+	2	-	-	+	4	-	-
<i>Cladosporium cladosporioides</i>	-	-	-	-	-	-	+	3	+	2
<i>Cladosporium</i>	+	5	-	-	-	-	+	5	-	-
<i>Curvularia lunata</i>	+	2	-	-	-	-	-	-	+	1
<i>Fusarium sp.</i>	+	3	+	2	-	-	-	-	-	-
<i>Humicola fuscoatra</i>	+	2	-	-	-	-	+	4	-	-
<i>Mucor sp.</i>	+	3	+	1	-	-	+	2	-	-
<i>Nigrospora oryzaeom.</i>	-	-	+	1	-	-	-	-	-	-
<i>Penicillium sp.</i>	+	5	-	-	-	-	+	1	+	2
<i>Rhizopus stolonifer</i>	+	2	-	-	+	2	+	2	+	2
<i>lignicola Pesante Scytalidium</i>	+	2	-	-	+	2	-	-	-	-
Sterile Mycelia	+	1	+	2	+	2	+	2	+	2
<i>Ulocladium atrum</i>	+	5	-	-	-	-	+	2	-	-

$\chi^2=5.070$ $p<0.05$ $\chi^2_{\text{tabulated}}=4.8$

Culture media used in fungi isolation: For the purpose of isolating the fungi found in food samples, two types of media were used: Potato-dextrose agar medium (Potato-dextrose agar) and Sabouraud dextrose agar medium (Sabouraud dextrose agar). The autoclave was sterilized at 121 ° C and pressed 15 lb / Ing 2 for a quarter of an hour (Linera and Ewel, 1984) after 250 mg of Chloramphenicol antibiotic was added to inhibit bacterial growth. After sterilizing the media, it was poured with sterile Petri dishes and left to harden. Each foodstuff (tomato paste, powdered milk, chips) was suspended directly and every two grams were added to each substance for 10 ml of distilled water. The solid foodstuffs (pasta, canned peas) were thoroughly crushed before the suspension was prepared and the suspension was prepared in the same way as the previous ones. Each suspension inside the hood was prepared after UV sterilization to ensure no contamination from the external environment. Then the food samples were transplanted to the previously prepared Petri dishes by taking 1 ml of each suspension with a sterile medical syringe and placed on the medium and then spread it inside the dish by a spreader, Then the dishes were bent inside the incubator and at a temperature of 25 and then the dishes were left inside the incubator for 5 days after which was diagnosed Fungal colonies by applying a drop of lactophenol dye onto a glass slide and taking part of the colony by needle medical syringe Mix it well with the dye, then slide the slide cover and examine the model under a 40x light microscope. The following sources were used in the diagnosis of isolated fungi: (Domsch et al. 1980; Klich and Pitt 1988 Pitt and Hocking, 1997; Klich 2002).

Purification and grow thing of *A. flavus* isolates on coconut agar: After the diagnosis of fungal colonies the colonies of the fungus *Aspergillus flavus* were purified

Through the cultivation of new pre-prepared Petri dishes and incubation of new dishes in the same way as the previous and after the end of the lapping period the dishes were extracted from the incubator and then developed *Aspergillus flavus* on the coconut agar Which was prepared in vitro according to the method described by Davis et al. (1987), 100 g of coconut grated was mixed with 300 ml hot distilled water in an electric mixer, filtered through a damp cloth In addition, 250 mg/ of the antibiotic Chloramphenicol was added to inhibit bacterial growth. The medium was used to develop *A. flavus* and detect its ability to produce aflatoxins.

Detection of the ability of *A. flavus* isolates to produce aflatoxins: The ammonia vapor method was used for this purpose. The test was performed as reported by Satio and Machida (1999) to test isolates for *A. Flavus* purified on the production of aflatoxin toxins, by taking part of the edge of the colony to be tested and developed on the center of the PDA at the age of 5 days to the center of coconut a car, and then incubated dishes at 25 degrees for five days, and after the emergence of fungal growth on the medium, Test the feasibility of these isolates on the production of fungal toxins by ammonia vapor by placing filter papers moistened with 20% liquid ammonia solution in the dish lid, and the results were recorded by changing the color of the colony base from white to pink, which indicates the isolation of aflatoxin toxins after an hour. Cuddling after 24 hours.

RESULTS AND DISCUSSION

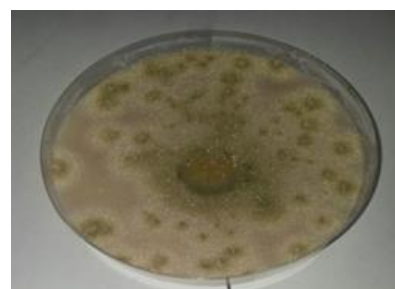
The results of the present study as shown in **Table 1** showed a distinct fungal diversity, where the number of fungal species isolated from corn gypsum (14) species of fungi as well as sterile fungal (sterile mycelia), and was observed to increase this diversity of fungal, was

Table 2. The ability of *A. flavus* isolates to produce aflatoxin toxins

Symbol of isolate	Source of isolate	Ability of isolates to product aflatoxins
AF 1	Pasta	+
AF2	Tomato paste	+
AF3	Tomato paste	+
AF4	Peas	+
AF5	Powdered milk	-
AF6	Peas	+
AF7	Corn chips	-
AF8	Pasta	+
AF9	Corn chips	+
AF10	Tomato paste	+
AF11	Powderd milk	+
AF12	Pasta	-
AF13	Peas	+
AF14	Corn chips	+
AF15	Powderd milk	-
AF16	Pasta	-
AF17	Tomato paste	+
AF18	Tomato paste	+
AF19	Peas	+
AF20	Corn chips	-
AF21	Pasta	+
AF22	Powdered milk	+
AF23	Tomato paste	+
AF 24	Pasta	+
AF25	Peas	+

the number of fungal species isolated from Tomato paste (19) species in addition to sterile fungal, but the number of fungal species isolated from the pasta, (8) types of fungi as well as sterile fungal and reached 10 fungal types for both milk powder and canned peas in addition to Sterile fungal. It is noticed that the number of isolated fungal species formed more than the number of isolates isolated from tomato paste amounted to 59 isolates, while the number of isolates isolated from corn chips was 43 isolates and 24 fungal isolates were isolated from pasta and 26 isolates were isolated from milk powder and 20 isolates were isolated from canned peas. The results of the statistical analysis showed that the number of isolates was significantly affected by the different nutrients as they showed significant differences using kai square ($p < 0.05$) χ^2 depending on the nutrient superiority of tomato paste over corn chips, pasta, powdered milk and canned peas. The χ^2 Promises that increase in the number of isolated species and the increase in the number of fungal isolates that were isolated from tomato paste is due to the increase in water content of tomato paste in addition to the nutrients contained in it if both are encouraging the growth of fungi, and the gradient was observed according to water content were followed by canned peas and then pasta and milk powder And corn chips (Mark,2016).

Isolated fungal species varied during the study in the number of fungal species belonging to each race, both

**Fig. 1.** Fungal isolate producing mycotoxins**Fig. 2.** Fungal isolate non-producing mycotoxins

fungi *Aspergillus* and *Alternaria* outperformed the rest of the other races, each isolated four species, the species belonging to the genus *Aspergillus* are *A. flavus* and *A.fumigatus* and An Alternative species *A. alternata*, *A.chlamydospora*, *A.phragmospora* and *A.radicina* were isolated from the genus *Alternaria*. The genus *Cladosporium* was isolated from two species, while one isolate the rest of the fungi from tomato paste, and the results of the present study are consistent with the results of previous studies as indicated (Kunwar (1989) that *Aspergillus*, *Alternaria*, *Cladosporium*, *Muco* and *Rhizopus* are the most important fungi associated with food. 25 fungi isolates were tested for *A. Flavus* to detect its ability to produce aflatoxin using coconut a car medium. It was found that the total number of isolates produced was 19 (76%) while the number of non-productive isolates was 6 (24%). *A. flavus* and being one of the most important types of fungi capable of producing mycotoxins, especially aflatoxins B1, B2 when growing on food (EFSA, 2007).

CONCLUSION

The results of the statistical analysis using the square (χ^2) showed that the total number of fungal isolates produced by aflatoxin ($P < 0.05$) was significantly different depending on the type of food.

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