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Isolation and identification of fungis in spices samples available in the local markets in Salah Al-Deen

KEY WORDS:

Spices, Aflatoxin Contamination, Fungi. Parasiticus, terreus, ochraceus, Alternariaand

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ABSTRACT

This study was conducted to estimation of fungal species and aflatoxin B1 in some types of Spices in Salah Al-deen governorate markets. In this study Six species of *Aspergillus* were detected which were *A. flavus, A. parasiticus, A. fumigatus, A. terreus, A. ochraceus* and *A. niger* and 5 genus of fungi were *Fusarium verticillioides*, Penicillium, Mucor, *Alternariaand Rhizopus* isolated on the three types of media used. The results show presence of the three types of mycotoxins in all samples. The concentrations of the aflatoxin B1 ranged between 1.05 - 7.90 ppm.

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INTRODUCTION

Spices is a type of the plants, such as the leave, bark, seed, fruit or root, which is used for coloring, flavoring, and even for preserving food, each spice has its unique chemical constituents which improve the flavor of the meal and create a specific sensation. In the Mediterranean and Middle East regions, spices are used daily for casual cooking to enhance the natural flavor of food (Dinizn *et al*, 2020). Spice is indigenous to India; it is valued for the spectacular nature of its burning, which is attributed to the alkaloid piperine (Nag and Chowdhury, 2020). Generally, Asia contributing to total global spice production is 95.1% followed by Africa, America and Europe (2.9%) (1.5%) (0.5%) respectively. Most of the spices in the Persian market are imported from abroad and might be contaminated with fungi. Mycotoxins are products by some of these fungi which are known to pose as potential health hazards for humans (Banerjee and Sarkar, 2003).

Most of the spices are used as ingredients in traditional foods or medicines or and as food supplements, High fungal count on dried spices (Hashem and Alamri, 2010). Aspergillus, Penicillium and Rhizopus were the most common moulds genera found. The presence of fungi on

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spices not only deteriorates the commodity and leads to loss of spice flavour but can cause mycotoxin contamination as well. The degree of spoilage and aflatoxin production by toxigenic Aspergillus flavus depend upon fungal strains, substrate, geographic area, climate, and plant culture system technique (Perrone *et al.*, 2014).

Because of their processing (harvesting techniques, drying, storage) and environmental conditions, spices are among the most contaminated food products with toxigenic moulds and mycotoxins, especially aflatoxigenic fungi and aflatoxins (AF) (Nurtjahja *et al*., 2019). In the EU, an acceptable level of aflatoxins for spices has been set at 0.70 - 3.69 µg/kg for total aflatoxins (Fundikira *et al.*, 2020). Aflatoxins contamination badly affect agriculture sector. Consequently, it may have bad impacts on main four pillars of food protection i.e. accessibility, availability, stability and utilization. Spices and dried fruits are more at danger to attack by aflatoxins due to unhygienic and bad storage conditions. Poor pre- and post-harvest conditions may cause contamination of spices and dried fruits. Careful drying, handling, transportation and storage are needed to avoid aflatoxin contamination in food commodities (Asghar *et al*., 2016).

Owing to the importance of this topic and its solid relation to human health and the nature of his food, a survey study on the contamination of the most used spices in our society by aflatoxins due to unhygienic and bad storage conditions to was determination to examine their extent of contamination and to focus light on the spices that are most vulnerable to the contamination by the aflatoxins which are considered among the most toxic pollutants and dangerous to human health if they are increase than the permissible level.

MATERIALS AND METHODS

Collection of samples

The spices used in this work were purchased from the local markets of Tikrit. Five of them were purchased packed in tightly closed boxes, which are (biryani spices, turmeric, dolma, fish, mixed spices) while the others were purchased uncovered in wooden boxes or bags inside or outside the stores, which are (chicken shawarma spices, kebbeh, burger meat, curry, yellow meat broth). Three samples for each spice studied herein were purchased from different places within the city and placed in polyethylene bags, then kept in the refrigerator at 4°C until they were used.

Isolate and identification of fungi

The fungal isolates were depending on classification key by observing growing on (CYA) Czapek Yeast Capek extract Agar, and Rose Bengal agar and PDA media, and the physical characteristics of the mycelia such as features (shape, size, color, and hyphae) by the compound microscope contain a digital camera and using a lactophenol cotton blue stained on slide mounted with small parts of mycelium (Pitt and Hocking, 2009; Samson *et al*., 2010; Gaddeyya *et al*., 2012).

Quantification of aflatoxin B1 by ELISA

The mentioned method was followed by a company Shenzhen Lvshiyuan Biotechnology Co. Ltd. China prepared for the materials and solutions used in extraction and determining the aflatoxin B1, fumonisin B1 and ochratoxin A using the ELISA method (Leszczynska *et al*., 2018). This method depends on mixing a specific amount of the complex of each poison-enzyme with a specific amount of the sample to be examined and mixed well and added to the titration plate covered with antibody surface. After incubation for a period of 15 minutes at room temperature, competition is between the toxin in the sample and the toxin associated with the enzyme with these bodies. Then the substance subject to the enzyme is added to the reaction solution and the reaction is stopped when the blue turns yellow. The optical intensity of the color of each slide was read using the spectrometer of the ELISA plates Bio_Tek Korean origin at a wave length 450 nm, in the central laboratory of Tikrit University.

RESULTS AND DISCUSSION

Occurrence of fungi in spices

In this study 6 species variety of Aspergillus were detected such as *A. flavus*, *A. parasiticus*, *A. fumigatus*, *A. terreus*, *A. ochraceus* and *A. niger* and 5 genus of fungi were isolated on the three types of media used, other genus *Mucor* spp., *Fusarium verticillioides*, *Penicillium* spp., And *Rhizopus* spp. (Table 1) (Figure 1). *A. flavus*, *A. parasiticus*, *A. ochraceus* and *Fusarium*

verticillioides found in all samples, other genus were isolate from some samples. Numerous studies have shown that fungi generating toxins, especially Aspergillus considerd one of the main pollutants in foods and fungi *A. flavus* and *A. niger* are the most isolated species (Oyewole *et al.*, 2020). Fungi with highest percentage occurrence on all the samples are *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus parasiticus*, *Aspergillus ochraceus*, Fusarium sp., *Rhizopus stolonifer*, yeast, and *Trichoderma koningii* (Jonathan *et al.*, 2016).

Table (1): Number of Fungi isolates in some types of Spices

No.	Fungi	Isolates No.
1	*A. flavus*	15
2	*A. parasiticus*	12
3	*A. fumigatus*	5
4	*A. terreus*	4
5	*A. ochraceus*	11
6	*A. niger*	8
7	*Mucor spp.*	3
8	*Fusarium verticillioides*	15
9	*Penicillium spp.*	6
10	*Rhizopus spp.*	3

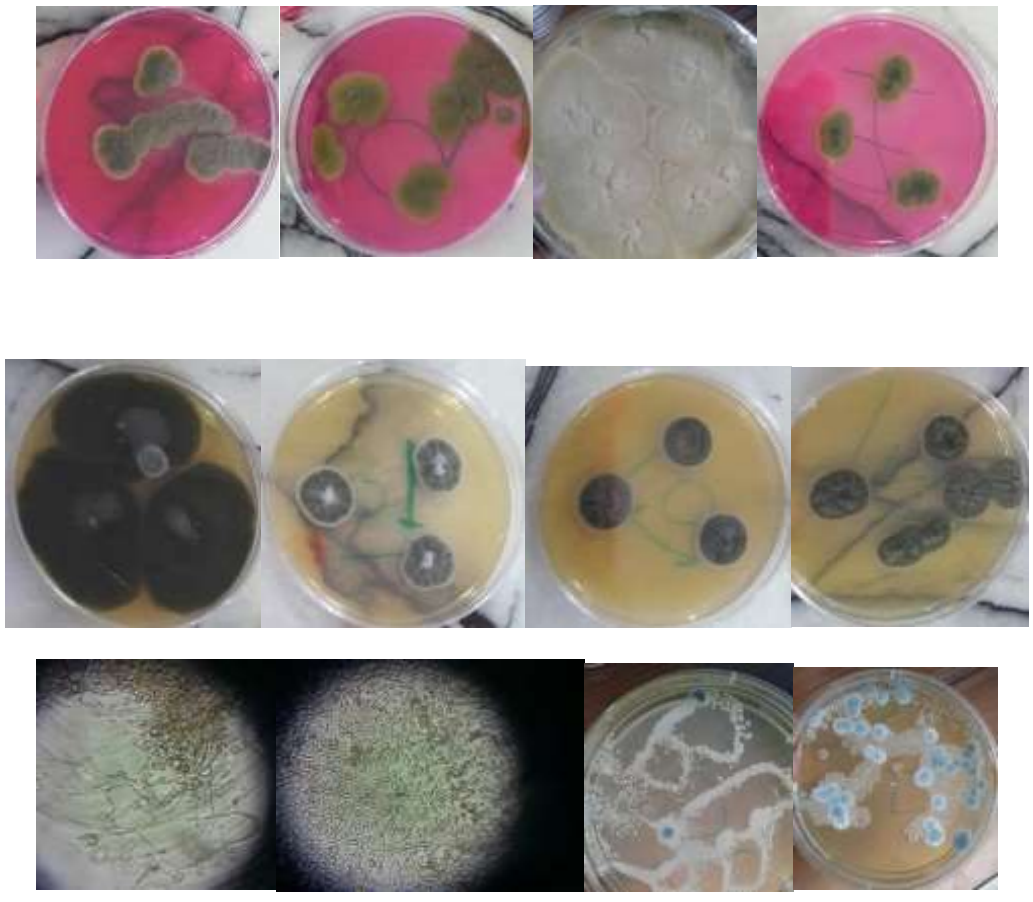

Figure (1): Isolate of fungi from spices

Ten different fungal genera and 16 species were isolated and identified as *Alternaria alternata*, *Aspergillus* spp., *Gliocladium* sp., *Hyalodendron diddeus*, *Memmoniella* sp., *Penicillium* spp., *Rhizopus* spp., *Syncephalastrum* sp., *Cladosporium lignicolum* and *Ulocladium botrytis* (Farid and Nareen, 2013). The reason for the emergence of fungal in foodstuffs is that the species possesses the ability to secrete a large number of enzymes that analyze foodstuffs that are used in nutrition and growth as well as increasing its spreading capacity, especially since some of its types can grow in a low moisture content as well as the relative density of the boards that produce them (Bouakline *et*

al., 2000). The commonly isolated fungi were species of *Aspergillus* (56.4%), *Penicillium* (25.1%), *Mucor* (12.8%) and *Eurotium* (5.7%) (Azzoune *et al.*, 2015).

Concentrations of mycotoxins in Spices samples

Table 2 show the concentrations of the aflatoxin B1 in some types of spices in Salah Al-Din Governorate markets. The concentrations of the aflatoxin B1 between (1.05 - 7.90) ppm. This may be due to infection with molds that produce toxins in the field or during storage, and provide theappropriate conditions for the production of these types of mycotoxins (Kong *et al.*, 2014).

These results are agreement with Jonathan *et al*. (2016) mentioned that aflatoxin B1 was found in all spices samples and it ranged between 3.85 to 28.03 µg/kg. Also aflatoxin B1 was found in all spices samples (Azzoune *et al*., 2016). And that these results obtained were higher than the allowable limit by the European Union, which is 2 µg / kg of aflatoxins, according to the standard specifications set by the Codex Committee (Mwalwayo and Thole, 2016). Zahra *et al*. (2018) detected aflatoxin B1 in 24 (26%) samples, the concentration between 23.99- 97.42 µg/kg in spices samples in Lahore – Pakistan. Zareshahrabad *et al*. (2020) showed that the concentration of aflatoxin in spices in Iran between 1.53 – 28.21 µg/kg. Maria *et al*. (2021) showed to the all samples of spices in São Paulo, Brazil contamination with aflatoxins at 0.10 - 48.67 ng/g. Concentration of aflatoxins detected in Spices from Morogoro Region, Tanzania at 0.2 - 164.9 µg/kg (Peter, 2021).

Table (2): The concentration of aflatoxin B1 in spices samples.

Samples	Conc. (ppm)
Biryani	6.14
Turmeric	7.90
Dolma	1.53
Fish	4.47
mixed spices	2.60
chicken shawarma spices	3.05
Kebbeh	1.05
burker meat	6.65
Curry	3.16
yellow meat broth	4.03

CONCLUSION

The current study represents for detecting mycotoxins AFB1by ELISA and isolate and identify fungi in some noodles samples. The results show to the all samples of noodles were contamination with aflatoxin B1 and Fungi *A. flavus*, *A. parasiticus*, *A. fumigatus*, *A. terreus*, *A. ochraceus* and *A. niger*, other genus *Mucor*, *Fusarium verticillioides*, *Penicillium*, *Rhizopus* and *Alternaria*.

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عزل وتشخيص الفطريات في عينات البهارات المتواجدة في الاسواق المحلية في صلاح الدين

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الكلمات المفتاحية:
توابل , التلوث بالافلاتوكسين , الفطريات

الخلاصة

اجري هذا البحث لتقدير انواع فطريات الافلاتوكسين B1 في بعض الانواع من البهارات الموجودة في اسواق صلاح الدين وهي Aspergillus . في هذا البحث تم الكشف عن ستة انواع من فطر A. flavus و A. parasiticus و A. fumigatus و A. terreus و A. ochraceus و A. niger و5 اجناس من الفطريات هي Fusarium verticillioides و Penicillium و Mucor و Alternariaand و Rhizopus المعزولة على الانواع الثلاثة من الاوساط المستخدمة في البحث . وقد اظهرت النتائج وجود الانواع الثلاثة B1 من السموم الفطرية في جميع العينات المدروسة . تراوحت تراكيز الافلاتوكسين بين 1.05-7.90 جزء بالمليون