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Isolation and identification of fungus in spices samples available in local markets in Salah al-Deen

ABSTRACT

This study was conducted to the isolation and identification of spicesborne fungi and Estimation of natural occurrence of aflatoxin B1 at different areas in local markets in Tikrit city. Fungi were isolated from spices during this study's period. Six types of *Aspergillus* were detected: *Aspergillus flavus*, *Aspergillus parasiticus*, *Aspergillus fumigatus*, *Aspergillus terreus*, *Aspergillus ochraceus* and *Aspergillus niger* and Five different genera of fungi were isolated: *Fusarium moniliforme*, *Mucor* spp., *Penicillium* spp., *Rhizopus* spp. and *Alternaria* isolation on the three types of media: Czapek Yeast extract Agar, Rose Bengal agar and PDA. The results show the aflatoxin B1 concentrations between 1.05 - 7.90 part per million by using ELISA method.

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INTRODUCTION

Spices have been used as food preservation and fragrances as protective for most canned foods. There are about 30 kinds of spices used in cooking, including black pepper, curry, cumin and turmeric, the most famous in the world. In addition to cooking, in many other areas, the paperless part of the plant is often used, such as buds or roots, such as in ginger or grains, such as black pepper and cumin (Dimicet al., 1995). The most common spices are such as 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. In the Mediterranean and Middle East regions, spices are used daily for casual cooking to enhance the natural flavor of food (Diniz et al., 2020). Spice is indigenous to India is valued for spectacular nature of burning, which is attribute to alkaloid piperine (Nag and Chowdhury, 2020). Generally Asia contributing to total global spice production is followed by Africa, America and Europe (2.9%) (1.5%) (0.5%) respectively. Most of

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the spices in the market are contaminated with fungi and Mycotoxins are products by some fungi which known to duo health hazards for humans (Banerjee and Sarkar, 2003).

Aspergillus, *Penicillium* and *Rhizopus* the most molds found on spices not deteriorates and duo to loss of spice the flavor. (Perrone *et al*, 2014).

Spices are the contaminated food products with toxigenic molds and mycotoxins, especially fungi and aflatoxins because some process such as harvesting, drying, storage and environmental conditions (Nurtjahja *et al.*, 2019). The EU as acceptable rang of aflatoxins for spices between 0.70 - 3.69 µg/kg for total aflatoxins (Fundikira *et al.*, 2020). Spices are danger to attack by aflatoxins to bad storage conditions and Poor pre- and post-harvest conditions cause contamination (Asghar *et al*, 2016).

Owing to importance of topic and 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 to was determination to examine the contamination and to focus light on the spices that are most vulner able to contamination by aflatoxins which considered the toxic pollutants and dangerous to human health they increase the permissible level.

MATERIALS AND METHODS

Samples collection

Samples of spices in this study used were collected from markets of Tikrit city. All types were collected packed in closed boxes, which are (biryani spices, turmeric, dolma, fish, mixed spices, chicken shawarma spices, kebbeh, burger meat, curry and yellow meat broth). Three samples for spice were purchased from different places within city and placed in polyethylene bags, and kept in refrigerator at 4°C until used.

Isolate and identification of fungi

The fungal isolates depend on classification key by growing on PDA, Czapek Yeast extract Agar and Rose Bengal agar media. The physical characteristics of mycelia such as size, color, shape and ic test by compound microscope is contain digital camera and slide are stained by lactophenol cotton blue (Pitt and Hocking, 2009 : Samson *et al*, 2010 : Gaddeyya *et al*, 2012).

Quantification of aflatoxin B1 by ELISA

For determination of aflatoxin B1 is used the company Shenzhen Lvshiyuan Biotechnology Co. Ltd. China method, extraction and determining the aflatoxin B1 by ELISA system plates (BioTek) Korean at 450 nm wave length, in the central laboratory of Tikrit University. (Leszczynska *et al*, 2018). Method depending on mixing the complex of poison-enzyme with the sample, incubation at room temperature, competition between the toxin in the sample and the toxin with enzyme.

RESULTS AND DISCUSSION

Occurrence of fungi in spices

Five species of *Aspergillus* was detected *A. fumigatus*, *A. parasiticus*, *A. flavus*, *A. niger* and *A. ochraceus* and som genus of fungi isolate such as *Fusarium moniliforme*, *Mucor* spp., *Rhizopus* spp. and *Penicillium* spp. (Table 1) (Figure 1). Some genus of fungi are found on spices samples such as *Aspergillus parasiticus*, *Aspergillus ochraceus*, *A. flavus* and *F. moniliforme*. Some studies show that fungi production of toxins especially *Aspergillus* (Oyewole *et al*, 2020). Fungi occurrence on the spices samples such as *Aspergillus ochraceus*, *Aspergillus parasiticus*, *Fusarium* spp., *Rhizopus stolonifer*, , *Aspergillus niger*, *Aspergillus flavus* yeast and *Trichoderma koningii* (Jonathan *et al*, 2016).

Table (1): Fungi isolates from all samples of Spices

Samples	Fungi species	Isolates
biryani spices	<i>A. flavus</i> , <i>A. ochraceus</i>	7, 8
turmeric	<i>A. parasiticus</i> , <i>Rhizopus spp.</i>	4, 8
dolma	<i>A. fumigatus</i> , <i>A. ochraceus</i>	3, 2
fish	<i>A. flavus</i> , <i>F. verticillioides</i>	3, 1
mixed spices	<i>A. ochraceus</i>	11
chicken shawarma spices	<i>A. niger</i> , <i>Rhizopus spp.</i>	5, 3
Kebbeh	<i>Mucor spp.</i> , <i>Rhizopus spp.</i>	1, 2
burger meat	<i>Fusarium verticillioides</i>	15
Curry	<i>Penicillium spp.</i> , <i>F. verticillioides</i>	4, 2
yellow meat broth	<i>Rhizopus spp.</i>	3

Figure 1: Isolate of fungi from spices

Different of fungal and sixteen species are isolated and identified *Gliocladium spp.*, *Aspergillus spp.*, *Alternaria alternata*, *Hyalodendron diddeus*, *Rhizopus spp.* and *Penicillium spp.* (Farid and Nareen, 2013). The fungi isolated from species such as *Penicillium*, *Mucor* and *Aspergillus* (56.4, 25.1, 12.8 and 5.7) % respectively (Azzoune *et al*, 2015).

Aflatoxin B1 Concentrations in Spices samples

The results in Table 2 shown to the aflatoxin B1 concentrations in spices types of Salah El-Din markets between 1.05 - 7.90 ppm. This may be pollution with fungi and its production aflatoxins (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 $\mu\text{g/kg}$. Also aflatoxin B1 was found in all spices samples (Azzoune *et al*, 2016). Zahra *et al*, (2018) detected aflatoxin B1 in 24 (26%) samples, the concentration between 23.99-97.42 $\mu\text{g/kg}$ 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 $\mu\text{g/kg}$. (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 $\mu\text{g/kg}$ (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

In this study detected the fungi in all samples of spices such as *Aspergillus terreus*, *Aspergillus flavus*, *Aspergillus parasiticus*, *Aspergillus fumigatus*, *Aspergillus ochraceus*, *F. moniliforme* and *Aspergillus niger*. Other genus *Mucor*, *Rhizopus*, *Penicillium*, and *Alternaria*. So the results are shoe to the aflatoxin B1 in all samples of spices.

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

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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* و 5 اجناس من الفطريات هي *Fusarium verticillioides* و *Penicillium* و *Mucor* و *Alternaria* 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.

الكلمات المفتاحية:

توابل , التلوث بالافلاتوكسين , الفطريات .