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Quantitative Analysis of Mycotoxins Produced by Three Fungi in Dry Fruits in Saudi Arabia using High-Performance Liquid Chromatography

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The aim of this study was to determine and quantitative the mycotoxins produce by *Alternaria* and *Fusarium chlamydosporum* and *Penicillium* isolated from different dry fruits by using HPLC. The results showed that *F. chlamydosporum* one isolate from dry fig and tow isolates from raisins, Fusarium species can producing many mycotoxinauch as Fumonisin, Zearalenone, DON, T-2, Neosolaniol and HT-2. While, *Penicillium* one isolate identified as *P. italicum* that isolated from raisins and tow isolates *P. digitatum* can producing many mycotoxin such as penicillic acid, citreoviridin, citrinin and patulin. *Alternaria alternata* which isolated from date and raisins can produce mycotoxins Altenuene and Alternariol by concentration 49, 32 for Altenuene and 25 and 19 for Alternariol.

Key words: Mycotoxins, fungi, liquid chromatography, HPLC.

Mycotoxins are considered to be one of the most important contaminants in feeds and foods such as cereals, spices, coffee, nuts or dried fruits[1,2]. More than 25% of the agricultural production in the world contaminated with mycotoxins[3]. One mold species may be produce many different mycotoxins and/or the same mycotoxins as another species[4]. The diverse effects precipitated by these compounds are conventionally considered under the generic term "mycotoxicosis", and include distinct syndromes as well as non-specific conditions. Mycotoxin contamination of forages and cereals frequently occurs in the field following infection of plants with particular pathogenic fungi or with symbiotic endophytes. Contamination may be also occur during processing and storage of harvested products and feed whenever environmental conditions are appropriate for spoilage fungi.

Moisture content and ambient temperature are key determinants of fungal colonization and mycotoxins production. It is conventional to sub divided toxigenic fungi into field (or plant pathogenic) and storage (or saprophytic/spoilage) organisms. *Claviceps*, *Neotyphodium*, *Fusraium* and *Alternaria* are classical representatives of field fungi while *Aspergillus* and *Penicillium* exemplify storage organisms[5,6]. Mycotoxigenic species may be further distinguished on the basis of geographical prevalence, reflecting specific environmental requirements for growth and secondary metabolism. Thus, *Aspergillus flavus*, *A. parasiticus* and *A. ochraceus* readily proliferate

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under warm, humid conditions[7], while *Penicillium expansum* and *P. verrucosum* are essentially temperate fungi. The *Penicillium* mycotoxins occur widely in temperate foods, particularly cereal grains, *Fusarium* spp. are more ubiquitous, but even this genus contains toxigenic species that are almost exclusively associated with cereal from warm countries[8]. It is important to develop rapid, sensitive, and reproducible assays to detect the presence of mycotoxins. The accurate and rapid qualitative and quantitative analysis for mycotoxins has been topic of interest by many researchers. Different analytical methods having different sensitivity and accuracy which could be used for different purposes have been developed. The aim of our study is to determine the mycotoxins produce by *Fusarium chlamydosporum*, *Alternaria* and *Penicillium* and isolated from different dry fruits by use HPLC.

MATERIALS AND METHODS

Isolation, characterization and identification of mycoflora from the fruits

The fruits were collected from many markets in different areas in the Kingdom of Saudi Arabia which produce in many different countries. All the dry fruits used in this experiment were apparently free from physical damage and diseases. And make two groups from each Fruits, first group were surface disinfected by dipping with sodium hypochlorite (1%) for 3 minute and washed several times with sterilized water[9], and air, after that we put sterilized fruits on Petri-dish content Potato dextrose agar (PDA).

Quantitative analysis of fungal mycotoxins

Detection of Alternaria toxins production

Sample preparation

Flasks were made up containing 12.5 g of autoclaved polished rice at 40% moisture. Flasks were inoculated with agar plugs of one-week-old cultures of *Alternaria alternata*. Culture were incubated in the dark at 25±2°C for 21 days[10].

Extraction of *Alternaria* toxins

The method for the detection of *Alternaria* toxins in rice was described by Li *et al*., [10]. The culture was homogenized with 30 ml of methanol and filtered through a Whatman filter paper (no. 1). The filtrate was clarified with 60 ml of 20% ammonium sulphate. Culture filtrate was extracted three times with 10 ml of chloroform. The organic phases were combined, evaporated to dryness, and dissolved in 4 ml of methanol for AE, and AOH analysis by high-performance liquid chromatography (HPLC).

HPLC detection

The HPLC system consisted of a Shimadzu liquid chromatography (Shimadzu, Kyoto, Japan) equipped with a Shimadzu SPD-M10Avp UV photodiode array detector. The analytical column was ODS 4.6×250 mm 5 U. C18. Standards of AE and AOH were purchased from SIGMA Chemical Company (St. Louis, MO, USA). The mobile phase was methanol/water (80:20) containing 300 mg $ZnSO_4$-$H_2$0/1, for AE, AOH and AME. A flow rate of 0.7 ml/min was used. The wavelength for recording chromatograms was 258 nm for AE and AOH According to Scott and Kanhere[11].

Fusarium mycotoxins analysis

Fusarium mycotoxins (Fumonisin, HT-2, Zearalenone, T-2, Neosolaniol and DON) content was determined using the VICAM[12] method. The method was similar with all former toxins except the dilution buffer, developer and immunoaffinity column. Each isolate was grown in flask 100 ml on SMKY media. The incubation period was 7 days at 25±2°C .After blending on high speed for 1 min. with 5 g of sodium chloride. 20 ml of culture filtrate was added to 80 ml of methanol (HPLC grad) and filtered through a fluted filter paper. The extract (10 ml) was diluted with 40 ml of phosphate buffered saline (PBS)/0.1% Tween-20 wash buffer and filtered through a 1.0-Am microfiber filter. The diluted extract was passed through the column, which was washed with 10 ml of PBS/0.1% Tween-20 wash buffer followed by 10 ml of PBS. Fumonisins were eluted from the column with 1 ml HPLC grade methanol. A mixture of developer A and developer B (1 ml) was added to the elute collected in a cuvette that was placed in a fluorometer (VICAM Fluorometer Series 4, Watertown, USA) for fumonisin measurement.

RESULTS

Determination of mycotoxins produce by *Alternaria* sp isolated from different dry fruits

Data in Table 1. showed that fungi *Alternaria alternata* which isolated from date and

raisins can produce mcototins Altenuene and Alternariol by concentration 49, 32 for Altenuene and 25 and 19 for Alternariol.

Determination of mycotoxins produce by *F. chlamydosporum* isolated from different dry fruits

The results of isolation from dried fruits gave three isolates from species fungi *Fusarium chlamydosporum* one isolate from dry fig and tow isolates from raisins, *Fusarium* species can producing many mycotoxinauch as Fumonisin , Zearalenone, DON, T-2 , Neosolaniol and HT-2. Data in table 12 explain the isolate No 1 of *F. chlamydosporum* produce 650 µg of Fumonisin but isolates No 2 and 3 gave 400 and 550 µg. the three isolates of *F. chlamydosporum* were no significant different in produce mycotoxin Zearalenone (Table 2). on the other hand mycotoxin DON can produce by isolate No 3 which isolated from fig as a result of concentration 600 µg, while isolates No 1 and 2 were 100 µg and 155 µg receptively. The data showed that too there were non-significant difference in produce mycotoxin Neosolaniol, T-2 and HT-2.

Table 1. Determination of mycotoxins produce by *Alternaria*sp isolated from different dry fruits

Dried fruit	Isolates	Mycotoxins (µg)	
		altenuene	alternariol
Date	*A.alternate*	49	25
Raisins	*A.alternata*	32	19

Determination of mycotoxins produce by *Penicillium* species isolated from different dry fruits

The results of isolation from dried fruits gave three isolates from species fungi *Penicillium* one isolate identified as *P.italicum* that isolated from raisins and tow isolates *P.digitatum* No 2 from fig and No 3 from pomegranate *Penicillium* can producing many mycotoxinauch as penicillic acid, citreoviridin, citrinin and patulin the resulted showed that citrinin can produce by *Penicillium italicum* by concentration 6 µg other *P.digitatum* No 2 and 3 were 9 µg and 4 µg, the three isolates of *Penicillium* were non-significant in produce other mycotoins, (Table 3).

DISCUSSION

Using HPLC, we determined mycotoxins produce by *F. chlamydosporum* and found three isolates from these fungi, one isolate from dry fig and two isolates from raisins. *Fusarium* spp. can produce many mycotoxin such as Fumonisin, Zearalenone, DON, T-2, Neosolaniol and HT-2. This results in harmony with Naiker and Odhav[13] and Nor-Azliza *et al*.,[14].

Same method used with *Penicillium*, the results of isolation from dried fruits gave three isolates from species fungi *Penicillium* one isolate identified as *P. italicum* that isolated from raisins and two isolates *P. digitatum* from fig and pomegranate. *Penicillium* can produce many mycotoxin such as Penicillic acid, citreoviridin,

Table 2. Determination of mycotoxins produce by *F. chlamydosporum* isolated from different dry fruits

Dried fruit	Isolates	Mycotoxins (µg)					
		Fumonisin	Zearalenone	DON	T-2	Neosolaniol	HT-2
Raisins	*F. chlamydosporum*	650	75	100	4.0	2.3	0.9
Raisins	*F. chlamydosporum*	400	80	155	11.0	1.1	2.4
Fig	*F. chlamydosporum*	550	90	600	14.0	4.3	1.3

Table 3. Determination of mycotoxins produce by *Penicillium* species isolated from different dry fruits

Dried fruit	Isolates	Mycotoxins (µg)			
		Penicillic acid	citreoviridin	Citrinin	patulin
Raisins	*P. italicum*	650	75	100	4.0
Fig	*P. digitatum*	400	80	155	11.0
pomegranate	*P. digitatum*	550	90	600	14.0

Citrinin and Patulin[15]. The results showed that fungi *A. alternata* which isolated from date and raisins can produce mycotoxins Altenuene and Alternariol. This species found to be predominant compared to other fungi in study performed by Chulze *et al*., [16] in Argentina.

Formation of mycotoxin in dried fruit is mostly presented as a pre-harvest problem due to the fact that dried fruit are particularly susceptible to aflatoxin production and accumulation at the end of the maturation period, shriveled ripe stage during sun-drying on the tree, until the moisture loss reaches the point that prevents fungal growth[17,18,19]. Moreover, the nutritionally rich chemical composition of dried fruits, including high levels of sugars like glucose and fructose, lipoperoxides, amino acids like proline and asparagine and minerals like zinc, and environmental conditions such as temperatures, humidity and drought conditions, promote aflatoxin production[20-23]. The highly nutritious nature of the dried figs might also stimulate the production of OTA.

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