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Isolation and identification of fungi associated with some Libyan foods

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Cereal and its products can be contaminated with fungi in the field, during drying, processing, transportation and subsequent storage, which may lead to secretion of mycotoxins under favourable condition. The aim of this study was the isolation and identification of some fungi associated with four kinds of Libyan food products of different trademarks. Twenty four (24) samples of couscous, macaroni, wheat flour and rice regularly used for human consumption by Libyan family were collected from local markets in the city of Alzawia, west of Tripoli, Libya. The results reveal isolation of 113 isolates belonging to nine genera: *Penicillium*, *Aspergillus, Fusarium, Paecilomyces, Alternaria, Rhizopus*, *Mucor, Scopulariopsis* and *Cladosporum.* Approximately 24 species were identified to belong to those isolated genera, several of which are known as main producer of mycotoxins especially *A. flavus* which are known to produce aflatoxins, *Aspergillus niger, Aspergillus carbonarious*, *Penicillium chrysogenum* and *Penicillium verrucosum* known to produce ochratoxin and *Fusarium oxysporum* and *Fusarium chlamydosporum* known to produce fumonisins and trichothecenes. Certainly, the occurrence of such types of mycotoxins can pose a health threatening risk for the consumer of those food items. Presence of these fungi in food products could be due to lack of good agriculture and food manufacturing practices throughout the food chain.

Key words: Couscous, macaroni, wheat flour, rice, fungi, Libya.

INTRODUCTION

Cereals and derived products represent an important nutrient source for mankind world-wide. In addition they are the most important dietary food for North African populations (Riba et al., 2010). Wheat is the most important small-grain cereal crop in the world with a total production of almost 700 million tonnes per year (Anonymous, 2010). Unfortunately, cereals are naturally contaminated with fungi in the field, during drying, processing, transportation and subsequent storage and it may be difficult to completely prevent mycotoxins formation in contaminated commodities, particularly those that are produced in tropical and subtropical climates, in countries where high temperature and humidity promote the growth and proliferation of fungi (Kumar et al., 2008).

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Thus, they are often colonised by fungi, including species from the genus *Aspergillus*, *Penicillium* and *Fusarium*, which cause significant reductions in crop yield, quality and safety due to their ability to produce mycotoxins. Mycotoxins commonly occurring in cereals and cereal products include zearalenone, fumonisins, trichothecenes (as deoxynivalenol and T2-HT2), ochratoxin and aflatoxins (Miller, 2008). It was reported that 25-50% of harvested world crops have been contaminated with mycotoxins (Ricciardi et al., 2012). A number of surveys have been carried out to identify a general pattern of toxigenic fungi and mycotoxins contamination in crops that are dried prone to contamination. In a research work by (Shaker et al., 2013) for isolation and identification of some fungi that produce mycotoxins in 80 different food samples that included rice in Iraqi market showed presence of *A. niger, A. parasiticus, A. veriscolar, A. ochraceus, P. citirinum, P. verrucom, Mucor* spp. and *Rhizopus. stolonifer.* In study by Belkacem-Hanfi et al. (2013) using a total of 127 durum wheat samples intended for food production that were collected during 2010-2011-2012 season in Tunisia indicated the presence of a total of 6035 postharvest fungi strains. The most pre-dominant post-harvest fungi genera isolates were *Alternaria* (28%), *Fusarium* (19%), *Penicillium* (19%), *Aspergillus* (14%), *Rhizopus* (7%), *Mucor* (6%) and other fungi (6%). The study also revealed that all stored grains were highly contaminated by field fungi and storage fungi. A recent study in Egypt revealed the occurrence of toxigenic fungi in 10 samples of wheat and 9 samples of rice from markets in central delta provinces. The researchers reported isolation of eight fungal genera that belongs to *Aspergillus*, *Fusarium*, *Penicillium*, *Mucor*, *Rhizopus*, *Trichoderma*, *Alternaria* and *Cladosporium* (El-Shanshoury et al., 2014). Similarly, in India, a study for the fungal associated with 36 samples of stored rice showed isolation of 96 isolates belonging to 16 genera of *Aspergillus* (50%), *Penicillium* (25%) and *Fusarium* (25%) (Sawane and Sawane, 2014). Evaluation of fungal contamination of consumed wheat flour for 89 samples carried by Rezazadeh et al. (2013) in Tabriz, Iran, reported that 31.5% of the samples contaminated with fungal genera belong to *Aspergillus* (50%), *Mucor* (7%), *Penicillium* (3.5%), *Acremonium* (14.5%), *Cladosporium* (3.5%) and *Alternaria* (3.5%). Survey for isolation and identification of fungi were conducted in two well-known markets in Ogun state-Nigeria on January 2014 using a total of 21 samples of different kinds and trade marks (Ezekiel and Sombie, 2014). The study revealed that 11 samples (52.4%) contaminated with fungi belong to genera of *Aspergillus* (56.3%), *Fusarium* (6.3%), *Penicillium* (6.3%) and other fungi (31.3%). In North African countries, foods that are most susceptible to contamination by fungi are locally produced or imported cereals, such as wheat. This crop is usually staple in dry Mediterranean regions of North Africa, where its consumption is in the form of couscous, pasta, macaroni, and bread. The mycobiota of wheat and wheat products was found to be dominated by *Aspergillus, Penicillium* and *Fusarium* species (Riba et al., 2008). The aim of this study was to isolate and identify some fungi associated with several major food products destined for human consumption and regularly used by most of the Libyan families.

MATERIALS AND METHODS

Food samples

Twenty four (24) food samples of several kinds and trade marks were randomly collected few days before analysis from different markets and bakery places in the area of Alzawia city with valid expiration date for consumption. They consist of 6 samples of wheat flour (National production), 6 samples of couscous (National and imported production), 6 samples of rice (Imported production) and 6 samples of macaroni (National and imported production).

Isolation of fungi

Isolation of fungi from rice and macaroni were carried out based on method described by Samson et al. (2010). Food samples were initially subjected for surface sterilization with 0.2% sodium hypochlorite solution for 2 min and rinsed three times with sterile distilled water and excess water on the sample was mopped using sterile filter paper. The samples were directly placed on Potato dextrose agar (PDA) media supplemented with chloramphenicol (in triplicates) at a plating rate of 5 pieces per plate and incubated at 25°C for 5 - 7 days. Each fungal colony obtained was then sub cultured on PDA and incubated at 25°C for 5 - 7 days for subsequent characterization and taxonomic identification. In case of wheat flour and couscous samples, dilution plating technique was used by taking10 gof each sample and added to 90 ml of sterilized distilled water. This mixture was then shaken and 1 ml aliquots were spread on the surface of PDA media supplemented with chloromphenicol (in triplicates), and incubated at 25°C for 5 - 7 days. Each fungal colony obtained was then subcultured on PDA and incubated at 25°C for 5 to 7 days for subsequent characterization and taxonomic identification.

Identification of fungi

Isolated fungi were identified on the basis of their micro and macro-morphological characteristics using standard taxonomic key used prevoiusly (Samson et al., 2010; Pitt, 1979; Pitt and Hocking, 2009; Raper and Fennel, 1965; Ellis et al., 2007).

RESULTS AND DISCUSSION

The results of this study indicate the isolation of 113 fungal strains that were found to belong to 9 genera of *Penicillium, Aspergillus, Fusarium, Paecilomyces, Alternaria, Rhizopus*, *Mucor, Scopulariopsis* and *Cladosporum,* covering 24 species (Table 1) and the most common genera isolated were *Penicillium* and *Aspergillus* (Table 2). Some of these genera are known to produce mycotoxins in food products such as aflatoxins, ochratoxin, trichothecenes (as deoxynivalenol

Table 1. Isolation and identification of fungi associated with some Libyan foods.

Genus	Species	Food samples			
		Couscous	Macaroni	Wheat flour	Rice
Penicillium	*P. citirinum*	+	+	-	-
	P. corylophilum	+	+	+	+
	P. conescens	-	+	+	-
	P. vinaceum	-	-	+	+
	P. duclauxii	-	-	-	+
	P. expansum	-	-	+	+
	P. verrucosum	-	+	+	-
	P. chrysogenum	-	+	+	-
Aspergillus	*A. flavus*	+	+	+	+
	A. carbonarious	+	+	+	+
	A. candidus	-	-	-	+
	A. niger	-	-	+	+
	A. ustus	-	+	-	-
	A. veriscolar	-	+	+	-
	A. fumigatus	+	-	-	-
Fusarium	*F. chlamydosporum*	-	+	+	-
	F. oxysporum	-	-	+	-
Paecilomyces	*P. lilacinus*	+	-	-	-
	P. variotil	+	-	-	+
Alternaria	*A.alternata*	+	+	-	-
Rhizopus	*R. stolonifer*	-	-	+	+
Mucor	*M. hiemalts*	+	-	-	-
Scopulariopsis	*S. brevicaulis*	-	-	-	+
Cladosporium	*C. cladosporioides*	-	-	-	+

+, Presence of fungi; **-,** absence of fungi.

Table 2. Percent (%) of isolated and identified fungi associated with some Libyan foods

Fungal genera isolated	%
Penicillium	33.33
Aspergillus	29.16
Fusarium	8.33
Paecilomyces	8.33
Alternaria	4.17
Rhizopus	4.17
Mucor	4.17
Scopulariopsis	4.17
Cladosporium	4.17

and T2-HT2) and fumonisins which could pose a risk for consumer health. The distribution of the above stated genera in the 4 food items under study is shown in Table 1. As could be seen from the table, some species were detected in all food items, while some were present in only one or two items. For instance, *A. flavus* and *A. carbonius* were detected in all food items, while the other *Aspergillus* species were present in only 1 or 2 items. Similar findings could be seen for the rest of the isolated fungal species. That was true for *Fusarium, Alternaria, Rhizopus*, *Scopulariopsis*, *Paecilomyces* and *Cladosporium.*

Results of this study are in conformity with several studies around the world. In a study for the presence of toxigenic fungi associated with 48 samples of marketed rice grain collected from different markets in Uganda revealed presence of 8 *Penicillium* species including *P. chrysogenum* and *P. citrinum* which are considered as ochratoxin A producers, and presence of 8 *Fusarium* species included *F. solani, F. graminearum, F. oxysporum* and *F. verticillioides* which are able to produce fumonisins and trichothecenes (as deoxynivalenol and T2-HT2), and presence of 12 *Aspergillus* species that include *A. flavu* and *A. parasiticus*, which are able to produce aflatoxins and *A. ochraceus* and *A. niger* strains known to produce ochratoxin A (Taligoola et al., 2010). A total of 125 samples of winter and spring wheat harvested in 2009, 2010 and 2011collected in southern Sweden (Lindblad et al., 2013) and the isolation and identification result showed that *F. poae* and *F. avenaceum* were present in almost all samples, other common *Fusarium* species were *F. graminearum* and *F. culmorum,* present in more than 70% of samples. Mycological survey carried out (Embaby et al., 2012) on freshly harvested wheat grains from the main production regions in Egypt resulted in eight fungal genera isolates and identified as: *Alternaria* (36.9%)*, Aspergillus* (12.4%)*, Drechslera* (1.3%)*, Epicoccum* (0.7%)*, Fusarium* (5.2%)*, Mucor* (0.2%)*, Penicillium* (18.3%) and *Rhizopus* (25.0%).

Mycotoxins analysis revealed that 3 fungal isolates were reported to produce aflatoxins and one of *F. moniliforme* isolate was able to produce fumonisin B_1. A study of fungal mycoflora and mycotoxins for 88 polished rice samples harvested in 2002 intended for human consumption were obtained from several grain wholesale markets in Seoul, Korea (Park et al., 2005): isolation of 63 strains belong to genera of *Aspergillus*, *Penicillum* and *Fusarium* and 18 strains have the ability to produce aflatoxins, ochratoxin, fumonisin B_1, deoxynivalenol, nivalenol and zearalenone. Research work to explore fungi and mycotoxins associated with rice grains during storage for 25 rice samples collected from different locations of district Mandi in India revealed that all the samples were found to be contaminated with one or more fungal genera of *Aspergillus* (41.6%), *Fusarium* (8.3%), *Penicillium* (16.6%) and other genera (41.5%). In addition, aflatoxins B_1 and B_2 were detected in 72% of the total samples used in the study. The researchers stated that the presence of these toxigenic fungi and aflatoxins poses a risk for consumer's health and it is necessary to check the rice grains prior to final distribution for public use (Gautam et al., 2012). The mycotoxins contamination of 123 samples of imported rice and its producing fungi in Zabol, (Iran) investigated by Amanloo et al. (2014), showed that 34 (27.6%) of the samples contaminated with *A. flavus, A. parasiticus*, *A. niger, A. fumigatus,* and fungal genera of *Penicillium, Fusarium* and *Rhizopus,* and presence of aflatoxins.

Presence of several species of possible toxigenic fungi reported in this study is an alarming indictor for the possibility of the presence of mycotoxins in food samples. Incidence of ochratoxin A in rice used for human consumption for 100 samples in Morocco showed that 26 (26%) samples contain ochratoxin A at concentration range from 0.08-47 ng/g (Juan et al., 2008). In Algeria, 108 samples of wheat and its derived products, intended for human consumption, were collected during pre-harvest in a state of storage and from flour and semolina mills by (Riba et al., 2010) from two regions: during the seasons of 2004 and 2006 the results of isolation and identification revealed that 150 isolates belong to *Aspergillus* genera (64.5%) representing 144 strains of *A. flavus* and 6 strain of *A. tamarii*, also the result showed presence of aflatoxins B_1 in 56.6% of the wheat samples and derived products (flour, semolina and bran) with concentration levels ranging from 0.13 to 37.42 ng/g. In a study by Raiola et al. (2012) for 27 samples of Italian commercial pasta indicated presence of ochratoxin A in 26 (96.30%) samples and deoxynivalenol in 22 (81.48%) samples.

In conclusion, presence of different species of fungi associated with food products used in this study could represent a serious problem by secretion of different kinds of mycotoxins which could affect human health, and all relevant authorities should work together in reducing the risk of contamination and risk of mycotoxins exposure from consumption of cereals and derived products. In fact, it is important to obtain assurance that the products are safe and of high quality. Food inspection during the food chain production plays an important role in food control. High risk products, such as with mycotoxins contaminated foodstuffs, shall be subject to an increased level of official control.

Also in the same time, immediate action should be taken to insure the application of good agriculture and manufacturing practices to prevent the fungal contamination and growth in cereals and cereal products during the entire food chain by using different methods to minimize fungal infection, including seed treatment, bio control and possibly cropping sequence. Other crop production practices that can be managed to reduce mycotoxins may include irrigation, fertilization and plant density. Another potential strategy to reduce mycotoxins contamination is to grow cultivars resistant to fungal invasion and subsequent mycotoxins production and final and most important factor after the above mention practice is to control the storage condition such as moisture and temperature of the row material and final product.

Conflict of interests

The authors did not declare any conflict of interest.

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