Journal of Natural Sciences Research www.iiste.org
ISSN 2224-3186 (Paper) ISSN 2225-0921 (Online)
Vol.5, No.11, 2015

Study the Occurrence of Aflatoxins in some Crops and Dry Fruits in Iraqi Markets

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Abstract
This study was conducted to determine the fungal species and aflatoxin contaminations in crops and dried fruits samples from Iraqi markets after collected at one kilogram with 15 replicates from each of wheat, barley, corn, rice, peanut, figs, apricot and raisin in polyethylene packages. Samples were prepared by grinding and cultured on optimum medium for culturing of contamination molds, and extracted the samples to detect the aflatoxins present by used the HPLC system. The results were indicated that's all collected samples from the crops or dried fruits were contamination with mold species at different levels of counts from log 2.46 to 5.67 CFU/g at each wheat and peanut samples respectively. The results also indicated that *Aspergillus niger*, *A.flavus*, *A. paraciticus* and *Fusarium oxysporium.* was a dominant mold when compared with the other molds species. Aflatoxins analysis was showed as aflatoxin B1 only in concentrations ranges between 22.0-93.7ηg/g in dried apricot samples to 161.0-782.0 ng/g in peanut samples, and were not detected the other aflatoxin types in all collected samples.
Keywords: Aflatoxins; Crops; Dry Fruits; Iraqi Markets.

INTRODUCTION

Mycotoxins are secondary metabolites of molds that contaminate over than 25% of the human food supply (Moss, 2002).These reason was caused as the foods were become the most common source of mycotoxins exposure for the general public, and were occur in many agricultural commodities such as grains like corn, rice, wheat, coffee, peanuts and dried fruits such as fig, apricot, raisin, pistachio etc. (Hussaini, *et al.* 2007 and Ngoko, *et al.* 2008). Mycotoxins have the ability to resist milling and other industrial process (Peraica, *et al.* 1999 and Magan *et al.*2003).The most important kind of mycotoxin was the aflatoxins group which included aflatoxin B1(AFB1), B2 (AFB2), G1 (AFG1) and G2 (AFG2), the major aflatoxins were produced by *Aspergillus flavus* and *A. parasiticus* and the rare *A.nomius* that have a diversity of toxic compounds which affected on humans or animals health (Gong, *et al.* 2002,Bennett and Klich, 2003 and Bryden, 2007). These were a variety of well-characterized biologically and toxicologically (Williams, *et al.*, 2004 and Wagacha and Muthomi, 2008). Aflatoxins are among the most potent mutagenic and carcinogenic substances known has led to that being classified by the International Agency for Research on Cancer (IARC) as a Class 1 human carcinogen (IARC, 2002). They are associated with many chronic health risks, including the induction of cancer, immune suppression, and digestive, blood and nerve defects (Williams, *et al.* 2004,Bryden, 2007 and Muthomi, 2008).The severity, was depended on the mechanism of actions for each kind of aflatoxins, the extent of exposure, age and nutritional status (Gorelick, *et al.*, 1993).The danger of consuming foodstuffs contaminated with aflatoxin at levels above the regulatory limit was demonstrated in Zhuqing village in china at 2001(Wang, *et al.*, 2001), and again in 2004 in Kenya where 125 people were died following the consumption of homegrown maize containing high levels of aflatoxin (Azziz-Baumgartner, *et al.* 2004 and Lewis, *et al.*, 2005). Related to these effects, aflatoxins are regulated in the low parts per billion ranges in diet in most developed countries (Creppy, 2002 and Joseph, *et al.*, 2008) commonly range from 4-20 ng/g (FAO, 2004).

The aim of the study was to determine the total counts of molds colony in crops and dried fruits collected from Iraqi markets, then isolate and identified the most dominant species that contaminated the foods samples. Furthermore, detect the concentration range of aflatoxin B1 in collected samples.

MATERIALS AND METHODS

Samples Collections: A total of 120 samples were collected from random Iraqi markets which contained 15 samples at one kilogram from each wheat, barley, corn, rice, peanuts crops and dried fruits such as figs, apricot and raisin. Then situated in polymers packages. The samples then cleaned by removal the foreign materials if it's present (Roberts and Greenwood, 2003).

Fungal isolation and identification: All collected samples were prepared by grinding, then weighted 25 g from each sample to added to 225 ml of sterile saline solution (0.85%) in 250 ml erylnmaier flask and homogenized used orbital shaker for 5 minutes. Tenfold serial solutions were prepared, and one ml from the last tow dilution were poured into each petri dishes containing 15 ml from each malt extract agar, potato dextrose agar and rose bengal agar medium at triplicates for each ones. The cultural plate medium then incubated at 30° c for 5 to 7 days. Each fungal genus and species colonies were counted used colony counter (UVP, Germany), then different isolates were sub culturing on malt extract agar at 30 ° c for 5 days, and identified to species level according to

classification key (Samson, *et al.* 2000 and Winn*, et al.*, 2006).

Aflatoxins analysis: Fifty grams from dried fruits and crops samples were used to extract aflatoxins B1according to the methods in AOAC, (2002). used the chloroform and then with hexane for eluted aflatoxins from chromatographic column which prepared according AOAC, (2002), then evaporated by rotary evaporator system (Heidolph, Germany), until dryness, and storage in small screw glass container at 4◦ c until used in analysis. The quantification of aflatoxin B1 contents was determined used high performance liquid chromatography (HPLC, LC-10-Shimadzu-Japan) system according to Ali *et al*., (2005), with some modifications as follow: All extraction samples were dissolved in 1 ml of chloroform. AFB1 were determined in extracts in HPLC system which used pump 4015 and fluorescence detector at 366 and 418 nm, flow rate at 0.8 ml/ mint. 20 µl of the samples were injected according the procedure mention in Clara, *et al.*, (2002). All unknowns toxin was compared with the standard solution of AFB1 which provided by Sigma Company (USA).

Statistical analysis: Data were analyzed by the a nova analysis, using the general linear model of the Statically Analysis System (SAS Institute, 2001). Significant treatment differences were evaluated using Duncan's multiple-range test (Duncan, 1955). All statements of significance are based on the 0.01 level of probability. The design was at this model:

yij=µ+ti+eij{ i=1,2,3. ; J=1,2,3,4,and 5.}

RESULTS AND DISCUSSION

Total Counts of Fungal Contamination: The total colony counts of each fungal contaminations in different crops and dried fruits samples were shown in table 1. Fungi were detected as contaminated all collected samples at significantly different ($p<0.01$) in total counts of colony which were detected in arrange between log 2.46 to 5.67 CFU/g in wheat and peanuts samples respectively. While the total count of colony in dried fruits were arranged from log 3.48 to 4.58 CFU/g in apricot and fig samples respectively.

Table 1.The means total counts (CFU/g) of fungi in crops and dried fruits samples.

No. of samples	**Crops and dried fruits samples**	**Fungi total counts** (log CFU/g)
Crops		
1	**Wheat**	2.46^{d}±0.11
2	**Barley**	3.40^{c}±1.01
3	**Corn**	3.61^{c}±1.42
4	**Rice**	4.58^{b}±3.16
5	**Peanuts**	5.67^{a}±9.25
Dried fruits		
1	**Fig**	4.58^{b}±3.43
2	**Apricot**	3.48^{c}±1.07
3	**Raisin**	3.52^{c}±1.12

- Each number represents mean of three plates for 15 samples replications .
- Counts are expressed in log CFU/g , ±= Standard error.
- a-d:Values within columns with no common superscript differ significantly at 0.01 of probability.

Peanuts, corn and rice were appeared to be higher infections by Aspergillus, Penicillium and Fusarium genera which were frequently detected more than other fungal genera (table 2). *The* Aspergillus species were perform as the most species contaminated the samples collected and the most sequent species compared with the other species were appear as *Aspergillus niger, A. flavus, Fusarium oxysporium, Penicillium verrucosum, Alternaria alternata* and *Cladosporium herbarum.* Furthermore the other species of fungi which were detected in different samples from crops and dried fruits samples such as *Mucor plumbeus, Rhizopus oryzae and Absidia corymbifera.*

Table 2. Percentage of different fungi in crops and dried fruits samples.

Type of Molds	Crops				Dried fruits			
	Wheat	Barley	Corn	Rice	Peanuts	Fig	Apricot	Raisin
	%							
A.niger	11	3	5	19	39	30	17	10
A. flavus	-	15	26	19	39	22	-	20
A.parasiticus	20	-	17	7	26	-	24	-
A. fumigatus	-	-	13	10	11	2	-	-
A.ochraceus	17	28	-	8	-	-	-	18
P. verrucosum	-	5	-	17	7	6	-	-
F. oxysporium	12	7	26	13	-	-	-	14
M. plumbeus	-	2	-	-	6	9	12	-
A. alternata	9	-	2	3	4	15	12	6
A. corymbifera	-	5	-	-	5	-	12	-
C.herbarum	2	-	5	8	3	4	12	5
R. oryzae	-	5	1	7	-	-	12	9

-Each number represents mean of three plates for 15 samples replications.
Counts are expressed in log CFU/g, - Means not determined.

Aflatoxins occurrence: Aflatoxins was detected in all tested samples at a different concentrations. The results from table 3. were showed that concentration ranges were depended on the type of samples, for example the figs samples had highly concentrations range of aflatoxin B1 which was between 38.5-480 ηg/g while, the ranges in apricot as between 22-93.7ηg/gm. The peanut samples were also detected the aflatoxin B1 in highly concentrations and which was between 161-782ηg/gm and then frequently in the corn, barley, wheat and rice samples which arranged between 7.0-260, 19.3-259, 14-254 and 37.5-213ηg/g respectively, While the other types of aflatoxin such as B2, G1, and G2 was not founds in all tested samples.

The results in table 1. Indicated that a highly counts of molds in all kind of crops and fruits samples, and in the table 2. Showed that several species of molds were isolated from each samples, furthermore the aflatoxins concentration ranges were exceed the allowed levels in foods materials in Iraq or other countries standard which were limited as not exceed the 20 ppb in food samples. These results were agreed with Ngoko, *et al.*, (2008), Hussaini, *et al.,* (2007) and AbouDonia, (2008). The major fungal contamination sources of samples may be as the optimal conditions in fields or storage for growth and reproductive of fungi on grains and fruits kinds especially the temperature and humidity levels which were considered critical factors for molds growth, furthermore, the ingredients of each food materials may promotes of fungal growth rates (Magan *et al.*2003, Ulmer, et al., 2005 and Richard, 2007).

Table 3.The range concentration (ηg/g) of aflatoxin B1 detected in dried fruits and crops samples.

No. of samples	Samples Types	Aflatoxin B1 concentration ranges (ηg/g)
Crops types		
1	**Wheat**	14.0-254
2	**Barley**	19.3-259
3	**Corn**	7.0-260
4	**Rice**	37.5-213
5	**Peanuts**	161.0-782
Dried fruits types		
1	**Fig**	38.5-480
2	**Apricot**	**22.0-93.7**
3	**Raisin**	**29.5-270**

- Each number represents mean of five replicates.

The increased of molds growth rates almost causes aflatoxins production support by the molds species. In addition, to the diversity of ingredients in fig, peanut, raisin, corn, wheat and barley which were play an important roles in aflatoxins productions (Peraica, *et al*. 1999).

Acknowledgements: The authors would like to thank all member of the Research and Development Office in the Ministry of Higher Education and Scientific research in Republic of Iraq for donating supplies used

in these study.

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