Detecting Contamination with Mycotoxins in Local Maize Species Used in Food and Feed

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Abstract

This study was conducted to detect contamination with mycotoxins aflatoxin and fumnisin in local corn species used in food and feed, 30 samples of local yellow corn were collected from the markets of Baghdad, 15 samples of corn intended for human consumption and 15 samples of corn intended for consumption as animal feed during spring, summer and autumn seasons from the year 2020, 5 samples per season, The moisture content of the samples was measured and ELISA technique was used to detect the concentrations of mycotoxins in them. The results showed significant differences at (P0.05) between humidity levels and mycotoxin concentrations for different seasons of the year, the high rates of humidity in maize samples intended for food and feed in the spring season recorded 14.34% and 17.22%, respectively, it is higher than the acceptable limits in the Iraqi standard for corn 12-13%, while the highest concentrations of aflatoxin and fumininin in corn for food in the spring were 9.64 and 742 ug / kg, respectively, so is corn for fodder 17.21 and 961.6ug / kg, and the lowest concentrations of mycotoxins for corn in food for the summer were 8.32 for aflatoxin, 353.8 ug / kg for fumnisine, and for feed 11.15 ug / kg for aflatoxin, the lowest concentrations of fumnizine for fodder were recorded in autumn 608 ug / kg, Most of the concentrations of these two fungal toxins were within the acceptable limits in the European and American standards, except for samples C₁ and C₃ of maize used for food in the spring, it was higher than the acceptable limits, reaching 10.87 and 10.75 ug / kg, respectively.

Key words: corn, mycotoxins.

Introduction

The yellow corn crop is considered one of the strategic crops in the world and ranks third in production after the wheat and rice crops, yellow corn is distinguished from other cereal crops by the ratio of carotenoids and its content of oils, starch and protein, which makes it of high nutritional value for human and animal consumption alike [1, 2], corn crop production is exposed to many diseases or contamination with pathogens, whether in the field or during harvest and storage, perhaps the most important and most dangerous problem associated with the production of this crop is contamination with many types of bacteria and fungi [3], the toxin-producing fungi species belonging to the genera Aspergillus, Fusarium, Rhizopus, Alternaria and Penicillium are among the most pollutants present during production processes in the maize crop, as the environmental conditions in which the crop is grown, in addition to what the grains represent in a suitable nutrient medium, allows the growth and development of these fungi and thus the production of mycotoxins and the contamination of corn grains with them [4, 5, 6], in spite of the number of mycotoxins exceeding three hundred species, aflatoxins, phomonzines, and trichotenoids are the most dangerous, widespread and

threatening mycotoxins compounds with their biological effects on humans and animals, and their devastating economic impacts on farmers and grain producers around the world, this issue is not limited to one society without the other, but it differs in proportions between developed countries than in developing countries that lack the factors of proper agricultural production [7, 8, 9], studies indicate that it is almost impossible for yellow corn crops to be free from contamination with one of the mycotoxin compounds, or to avoid contamination with them, this prompted many developed countries in cereal production and research centers to adopt various strategies to reduce the effect of grain contamination with pathogens and the spread of mycotoxins in these strategic crops [10]. In Iraq, the maize crop is grown in the spring and autumn seasons, and some studies have indicated that many fungi are associated with them, including those related to mycotoxins, specifically aflatoxins and fumnisins, for the corn crop intended for human consumption or used in the production of feed, especially poultry feed [11, 12], the risk of the presence of fungi contaminating the crop grown in autumn increases due to its ripeness coinciding with this season, which is characterized by low temperatures and high relative humidity in the air in addition to the high moisture content of yellow corn kernels, this provides an ideal condition for the growth and spread of various types of fungi and thus the production of mycotoxins that pose an underlying threat to human and animal health, directly or indirectly. Some mycotoxins, such as aflatoxins, are stable under various conditions of storage and transportation of temperatures and humidity, so they are among the toxins that are difficult to remove or reduce their negative effects on human and animal health, they accumulate inside the tissues and organs of the body and cause serious health problems such as acute hepatitis, weak immunity, severe effects on the nervous system, impaired development of fetuses, various types of cancer, especially liver cancer [13, 14, 15], in view of the seriousness of these toxins and their health and economic effects on humans and animals, this study aimed to uncover contamination with mycotoxins (aflatoxin and fumnizin) in local corn types used in food and feed, which were collected from wholesale markets in Baghdad.

Materials and methods

Samples collection

30 samples were collected from local maize grains (15 samples of maize classified for human consumption by 5 samples for each season of spring, summer and autumn and 15 samples of maize classified for use as animal feed by 5 samples for each season of spring, summer and autumn), samples were collected randomly from wholesale stores in the city of Baghdad, at a rate of 250 g per sample during the year 2020, and placed in clean plastic containers and the moisture content of it was assessed upon its arrival to the laboratory and kept at a temperature of -20 ° C until the completion of the rest of the tests.

Moisture content estimation

The moisture content of the samples was determined using the method presented in A.O.A.C., 2005 [16] by placing 2 g of each sample in a vessel of known weight then it was inserted into an electric oven at a temperature of 125 m for 4 hours and the vessels were cooled and weighed, the process was repeated until the weight was fixed, the percentage of moisture

content was calculated through the weight difference of the corn sample before and after drying in the oven.

Prepare samples for mycotoxin assessment

25 g of each sample of corn was weighed and placed in an electric oven at a temperature of 80 °C for 24 hours and subsequently milled separately using a laboratory mill and sifted with a fine-punched sieve to obtain 5 g of flour, mycotoxins were extracted by placing each milled sample in a clean glass beaker and adding 25 ml of methanol 70% to it and stirring the sample for 3 minutes to ensure that it is mixed well, each sample was filtered using (Whatman No. 1) filter paper to obtain an extract of not less than 5 ml for the purpose of completing the examination for the determination of mycotoxins (aflatoxin and fuminizine) and according to the method recommended by the company that supplied the measuring kit for those toxins using the ELISA technique.

Determination of mycotoxin concentrations using the ELISA technique

The concentrations of mycotoxins (aflatoxin and fumnizine) were measured in extracts of corn samples with Enzyme-Linked Immuno Sorbant Assay (ELISA) using a kit for aflatoxin and fumnisine, a special kit for total aflatoxin and fumnisin quantitation which was obtained from Veratox Neogen of the United States, according to the method mentioned in [17].

Statistical analysis

The statistical program SAS (2012) [18] was used in data analysis to study the effect of the seasons on the studied traits according to a complete random design (CRD). The significant differences between the averages were compared with the Lest Significant Difference-LSD test.

Results and discussion

Table 1 show the moisture content and toxins concentrations of aflatoxin and fumnisine in the local corn samples classified for human consumption, the results showed that there were significant differences at the level of (P \le 0.05) in the moisture content of the local corn samples between the spring, summer and autumn seasons, it recorded rates of 14.37%, 9.42% and 10.35%, respectively, for those seasons, most of the samples taken in the spring, summer and autumn seasons recorded humidity ratios within the acceptable limits in the Iraqi standard specification [19], which determined the moisture content of stored yellow corn to be no more than 12-13%, with the exception of samples C₁, C₃ and C₄ in the spring, they recorded high humidity levels of 15.25%, 18.72% and 14.91% respectively, the difference in the moisture content of the corn samples is due to the different climatic conditions of the sites where the crop is grown, the different storage method, and the degree of maturity of the crop at harvest [20], Al-Warshan [13] indicated that the average humidity of the Iraqi corn crop when harvested in the spring ranged between 16.2-19.5%, while the ratio ranged between 14.3-18.5 in Syrian corn stored in closed warehouses in the spring [21], the increase in humidity in the local corn crop is due to the fact that many farmers, traders or marketers of this crop store it in inappropriate locations and improper storage conditions such as unfinished floors or rooms without tight ceilings, or the use of polyethylene covers to protect the crop from moisture, animals and insects, which leads to an increase in the humidity inside the store, and this leads

to encouraging the growth and spread of many fungi, and thus the grains are exposed to poisoning with various toxins produced by these fungi [22, 12].

Table 1: Moisture content and toxins concentrations of aflatoxin and fumnizine in local corn samples classified for human consumption.

Season	Total	Sample	Moistur	Averag	Total	Averag	Total	Averag		
	Sample	S	e %	e	Aflatoxi	e	Fumonisi	e		
	S				n (µ		n			
					g/kg)		(μ g/kg)			
		C_1	15.25		10.87		785			
		C_2	11.14		9.02		674			
Spring	5	C_3	18.72	14.37	10.75	9.64	823	742.4		
		C_4	14.91		9.33		787			
		C_5	11.85		8.23		643			
		C_6	9.33		7.74		403			
		C_7	9.25		8.85		552			
Summe	5	C_8	10.38	9.42	9.05	8.32	432	453.8		
v		C ₉	9.17		7.27		437			
r		C ₁₀	8.98		8.68		445			
		C ₁₁	10.54		9.15		527			
		C ₁₂	10.18		8.73		538			
Autum	5	C ₁₃	9.85	10.35	8.56	8.67	405	500.6		
n		C_{14}	9.87		7.15		412			
		C ₁₅	11.32		9.75		621			
LSD				2.916 *		1.454		84.372		
value						NS		*		
* (P≤0.05).										

By referring to Table 1, we note that the local yellow corn samples C₁ and C₃ for the spring season recorded aflatoxin concentrations of 10.87 and 10.75 µ g / kg, which are higher than the permissible limits in the Codex Standard [23] and the European Standard [24], which determined the aflatoxin concentration in yellow corn intended for human consumption to be no more than 10 μ g / kg, and no significant differences were found between the levels of aflatoxin concentrations in the spring, summer and autumn seasons, which amounted to 9.64, 8.32 and 8.76 μ g / kg, respectively, it is evident that there was a clear correlation between the humidity level in most of the corn samples and the aflatoxin concentration, which was within acceptable limits, AL-Rawi [25] attributed the existence of variation in aflatoxin concentrations in corn for many reasons, including the severity of infection in the field, the length of the storage period, the temperature, the relative humidity of the atmosphere, and the moisture content of the samples, although the humidity of most of the samples under study recorded concentrations lower than the acceptable limits, so as not to encourage the infection of the grains with fungi, but it greatly helps in the process of producing toxins if the grains were mainly infected in the field before harvesting, this is consistent with what Magan 2016 [26] stated that high temperature, humidity factor, and previous infection with fungi of corn crop during growth in the field helps in a significant way in contamination of grains with high

levels of aflatoxin, Alhamadani [27] and Al-Rawi [25] recorded high levels of aflatoxin contamination in the local corn crop, ranging between 12.4 - 41.2 and 38.1 - 58.2 μ g / kg, respectively in the two studies, while Mutiga [28] indicated that aflatoxin concentrations in maize samples in southern Kenya ranged between 2.4 - 9.2 μ g / kg.

The results of detection of Fumonisin concentrations in the local corn samples designated for human consumption (Table, 1) showed significant differences at the level of ($P \le 0.05$) among the rates of this toxin during the spring, summer and winter seasons, which were 742.4, 543.8 and 500.6 µ g / kg respectively for those seasons with an LSD of 84.372, it should be noted that the concentrations of fumnizine toxins for all samples were within the permissible limits in the European [24] and American [29] standards, which specified that its concentration does not exceed 3000 µg / kg in corn intended for human consumption, the results indicated by the studies of [17] in Malawi and [21] in Syria came close to what this study found in terms of the concentration of fumnizine in stored yellow corn in the summer, as the two studies separately recorded a concentration rate of this toxin of 388.04 and 670 µ g. / kg, respectively, while a study of Daniel and Bernard [17] found a higher concentration of toxin in maize stored in spring, which was $3551.92 \mu g / kg$, studies [30, 17] suggested that the increase in the concentration of fumnizine in the spring season is due to the high level of humidity in the air and moderate temperatures, which encourages the fungi to grow and produce toxins, Hemmad et. al. [31] indicated in their study of stored Iraqi corn that high temperatures and humidity encourage the production of fumnizine toxins by the fungus Fusarium and the chance of the yellow corn crop becoming infected with this fungus increases when it is damaged by pests and insects.

The results of the moisture test for local corn used for animal feed (Table, 2) showed significant differences at the level ($P \le 0.05$) in the humidity ratio between the spring, summer and autumn seasons, it has been observed that there is a significant increase in humidity, especially in the spring, as the rate is 17.22%, followed by 14.75% in the spring and 13.12% in the summer, the humidity percentage for most of the samples was higher than the acceptable limits in the Iraqi Standard [19] which defined it as 12-13% for stored yellow corn, except for the sample F_{10} in the summer, which recorded an acceptable rate of 11.85%, Ali [12] confirmed in her study that local corn classified for use as animal feed is generally stored openly after harvesting with simple coverage by polyethylene sheets, which exposes it to weather conditions and the possibility of an increase in humidity, especially in the spring and winter seasons, as it recorded high levels of humidity that reached 18.94% in March and 21.54% in January, the results of studies carried out by Garcia-Diaz [30] in Spain and Nyangi [6] in Tanzania on maize used for fodder were higher than the results of this study in that the humidity increased in forage maize stored in open warehouses in the winter was 26.87% and 23.91%, respectively.

Table 2, shows the concentrations of aflatoxin and fumnizine toxins in the local corn samples classified as forage and according to the spring, summer and autumn seasons, the results show that there are significant differences between the concentrations of these toxins in the different seasons of the year, despite the fact that most of them are within the acceptable limits for these toxins in the feed, as defined by the European [24] and American standards

[29] with 20 μ g / kg for aflatoxin and 4000 μ g / kg for fumnizine, the levels of aflatoxin concentrations in spring, summer and autumn were 17.21, 11.15 and 11.93 μ g / kg, respectively, as well as fuminisin 961.6, 736 and 608 μ g / kg, respectively, for those seasons, studies [33] in Rwanda and [21] in Syria recorded rates of aflatoxin in fodder for the spring

Table 2: moisture content, aflatoxin and fumnizine concentrations in local corn samples classified as forage.

Season	Total	Sample	Moistur	Averag	Total	Averag	Total	Avera		
	Sample	S	e %	e	Aflatoxi	e	Fumonis	ge		
	S				n (µ		in			
					g/kg)		(μ g/kg)			
		F_1	18.55		17.57		957			
		F_2	17.78		17.72		988			
Spring	5	F_3	15.23	17.22	16.23	17.21	951	961.6		
		F_4	16.43		16.97		925			
		F_5	18.09		17.55		987			
		F_6	14.73		13.28		964			
		F_7	12.24		10.98		875			
Summ	5	F_8	14.05	13.12	10.95	11.15	623	736		
er		F_9	12.71		10.42		621			
		F_{10}	11.85		10.12		597			
		F_{11}	14.51		12.24		526			
		F_{12}	13.78		11.63		544			
Autum	5	F_{13}	15.85	14.75	13.18	11.93	619	608		
n		F_{14}	13.74		10.81		687			
		F ₁₅	14.87		11.78		664			
LSD				3.061 *		3.866 *		102.52		
value								*		
* (P≤0.05).										

season were 18.57 and 19.65 µ g / kg, respectively, and for the summer season, 14.35 and 15.46 μ g / kg, respectively, the proportion of fumnisine was high in the fodder for the spring, summer and autumn seasons 3658.82, 1496.25 and 5902.14 µ g / kg, respectively, in the study that Garcia-Diaz [32] conducted on forage corn in Spain, while the percentage of fumnisine was lower in study [33], reaching 905.27, 1761.93, and 2653.48 µ g / kg, respectively, for those seasons, likewise, in study [6] it was 1273.32 µ g / kg, and the concentrations of fumnisine were very low and within acceptable limits ranged between 141 - 363 µ g / kg in the study that was conducted on yellow corn produced in Croatia [34], this was attributed to the lack of high temperatures that encourage the growth of fungi despite the high humidity in the spring, Battilani et. al. [4] indicated in their study of yellow corn that covered large parts of Europe that temperatures exceeding 20 ° C and humidity of more than 14% encourage the growth of fungi producing mycotoxins, therefore, the northern European regions, where the low temperatures recorded a significant decrease in the production of aflatoxin and fumnisine, amounting to 6 μ g / kg and 67 μ g / kg, respectively, while high concentrations of these toxins were recorded in maize crops destined for human consumption, as well as fodder in southern European regions, where the temperatures and humidity are relatively high, especially in the Mediterranean countries, the concentrations of aflatoxin reached 31 µ g / kg and fuminizine 5186.65 μ g / kg.

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