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RESEARCH ARTICLE

Natural Incidence of Fungi and Mycotoxins on Corn Grains in Ibb (Yemen)

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ABSTRACT

Present study aimed to determine the moisture content, mycobiota and mycotoxins contents in the corn grains (*Zea mays* L.) at Ibb governorate in Yemen. For this purpose, a total of 30 corn samples were collected from the local shop and were processed using recommended protocols. The moisture content was 10.6 to 12.4 %, while total bacterial count was 1.2 x10^6 to 9.2 × 10^6 CFU. Total fungi count was 5.2 × 10^4 to 3 × 10^5 CFU, the most genera where *Aspergillus*, *Penicillium*, *Fusarium* and *Rhizopus*. On the other hand, Mycotoxin contents were, 7-22 µg Aflatoxins / kg corn grains, 5-18 Ochratoxin µg/kg corn grains and 4-11 µg Zearalenone / kg corn grains.

INTRODUCTION

Corn (*Zea mays* L.) is one of the most important cereal crops in the world as well as in Yemen. According to agriculture statistics in 2007-2008, corn production in the world reached to (788,112,128 tons) including only (86,000 tons) for Yemen with an agriculture areas of (41,000 Hectare), the imported corn to Yemen reached to (459,342 tons) meanwhile, exported corn from Yemen at 2007-2008 reached to (10,794 tons) (FAO, 2009). In most Yemen governorates like Ibb, corn is considered the second-cereal crop after wheat and it is very important because of its numerous uses. The micro-flora of cereal grains are comprised of wide varieties of fungi and bacteria. The bacteria normally appeared to be involved in deterioration of stored grains. Beside that the bacteria are incapable to penetrate cereal grains. Thus the deteriorating action in stored grains is principally due to pre-harvest fungal contamination (on plant) and through storage or during processing. The fungal contamination of cereal crops is affected by many factors including temperature, moisture content, a_w, storage periods etc. Fungi producing toxins in foods are classified into field fungi and storage fungi based on their ecological requirements for the growth. The fungi which attack cereal grains on plant and required 20-25% moisture content or a relative humidity of 70-90% for example; *Alternaria*, *Fusarium* and *Cladosporium* are referred as field fungi, while on the other hand the genera of fungi which required 13-18% moisture content like *Aspergillus*, *Penicillium* and *Fusarium* are storage fungi (Bankole, 1994).

Corn and some other cereals are providing an excellent media for fungi growth and mycotoxins contamination which attack either the plant in field or in cereal grains during the storage (pre- or post-harvested contaminations). Some of these pathogenic fungi produce mycotoxins which are considered secondary metabolites and it can cause diseases for both human and animals. Mycotoxins which are toxic secondary metabolites produced by certain genera of fungi. The disease which is caused by mycotoxins known as mycotoxicoses while the infection due to the fungi for human or animals know as mycoses (Nogaim, 2005, 2011).

So, this study focused on determination of moisture content, mycobiota and some Mycotoxins contents in the corn grains samples at Ibb governorate in Yemen.

MATERIALS AND METHODS

Sampling: The corn grains (*Zea mays* L.) samples (n = 30) were collected from 10 local shops in Ibb

governorate during fall session in October, 2008 when relative humidity was near about 50 to 55 %.

Media and chemicals: Standard plate count (SPC) agar, Malt extract agar, Czapek's agar and Potato dextrose agar (PDA), all from Oxoid Ltd, Basingstoke, UK, were obtained from local markets.

The standards of Aflatoxins (B_1, B_2, G_1 and G_2), Ochratoxin A and Zearalenone were obtained from Sigma, St. Lous, USA.

Acetonitrile for High Performance Liquid Chromatography was obtained from Sigma Chemical Company, P.O. 145508, St. Lous, USA.

Moisture contents of each sample were determined according to the standard method of AOAC (1995).

Microbiological examination: A total count of bacteria, yeast and molds were measured by inoculating specific dilutions of the samples suspension into various culturing media and incubating them for fixed periods at optimum temperatures. The resulting colony counts are then calculated as organisms per gram of corn grains CFU/g. Total viable bacterial counts were determined using the SPC agar according to the method recommended by the (FDA, Bacteriological Analytical Manual, 2001). Plates were incubated at 35 ◦C for 48h. Yeast and mold counts, were determined using the diluting plating technique described by (FDA, 2001) on malt extract agar medium. The medium was sterilized by autoclaving at 121 ◦C for 15 min. To inhibit bacterial growth, antibiotics or sterile tartaric acid solution was added immediately to the agar after it had been tempered before pouring into plates. The plates were incubated in the dark at 22–25 ◦C for 5 days.

Identification of fungi: All the fungal isolates were subjected to identification according to the procedures of (Nelson *et al.* 1983; Barnnett and Hunter 1972; Gilman, 1957). The numbers of *Aspergillus* species were classified according to the key published by (Raper and Fennell 1965), while for the identification of other fungal species the method described by Count *et al* (1954) was used.

Determination of Mycotoxins contents in Corn Grains: Aflatoxins, Ochratoxin A and Zearalenone were determined in corn grains samples according to the method described by Gallagher and Latch (1977) and we used multi-technique accordance AOAC (1995). For this purpose, 100 g of each corn grain samples was grounded, and was blended with 200 ml of methanol: water (8: 2 v/v) for 3 min. at a high speed. After filtration, using Whatman No. 2 filter paper, 50 ml of the filtrate were added to 50 ml of a cleanup solution having 150 g zinc sulphate, and 50 g phosphotungestic acid, in one litter of distilled water, in a 250 ml beaker and mixed using glass rod for 10 min. The mixture was again filtered through Whatman No. 4 filter paper and 75 ml of clear filtrate were shacked with 15 ml of benzene for one min. Layer of benzene was received into a small vial and evaporated to dryness and the residue was transferred to vial and evaporated off using a stream of nitrogen at a temperature below 60^{o}C. The dry film was used for the determinations of mycotoxins by thin layer chromatography (TLC).

Preparation of Mycotoxins Standards: For the preparation of Aflatoxins standards, the crystalline Aflatoxins (B_1, B_2, G_1 and G_2) were diluted in benzene-acetonitrile (98: 2 v/v) according to the method of AOAC (1995).

The concentration of the prepared solution was adjusted by using the spectrophotometer at wavelength of the maximum absorption closed to 350 nm and using the following equation:

$$\textbf{Aflatoxins (µg/mL)} = \frac{A \times MW \times 1000 \times CF}{E}$$

Where:

A=Absorbency at the wavelength of the maximum absorption

MW=Molecular weight of Aflatoxins B_1, B_2, G_1 and G_2

CF=Correction factor for particular instrument and cells

E=Absorbability of Aflatoxins in benzene-acetonitrile (98: 2 v/v)

The suitable concentration which used for TLC was 0.5 µg Aflatoxins /ml

Ochratoxin A and Zearalenone Standards: Ochratoxin A and Zearalenone were dissolved in benzene-acetic acid (99: 1 v/v) to obtain the required concentration by using spectrophotometer at a wavelength of maximum absorption closed to 317 nm by using the equation described above. The suitable concentration was 5 µg/ml of solvent.

Quantitative Analysis: The quantitative analysis was carried out according to the methods mentioned in AOAC (1995). The previously obtained extract was dissolved in 200 ml benzene-acetic acid (99: 1 v/v). Twenty µl from the extract of each standard of Aflatoxins (B_1, B_2, G_1 and G_2), Ochratoxin A and Zearalenone at different concentrations were spotted on TLC plate with the spots of samples .

The intensities of fluorescence of all mycotoxins spots were estimated by using a Fluoro-densitometer (Densitometer-TLC-100 Vitatron). The amounts of all mycotoxins were determined by comparing the densitometer reading of each sample with those of standard solutions using the following equation:

$$\mu g/kg = \frac{S.Y.V.}{X.W}$$

Where:

S=µl of mycotoxin standard equal to unknown.

Y=Concentration of standard mycotoxin in µg/ml.

V=Dilution factor (µl).

X=µl of sample extract giving fluorescent intensity equal to standard.

W=Weight in g. of the original sample found in the final extract.

RESULTS AND DISCUSSION

Microbiological analysis

It is well known that some fungi grow on and in corn grains and these fungi can cause the deterioration of stored grains. Therefore, the present work was started by determining some parameters such as moisture content, total bacteria plate count and total fungi count. After that, the isolation and identification of such fungal species was done by using Czapek's agar as a growing medium.

The data in Table 1 demonstrated that the moisture content was 10.6 to 12.4 % while total bacteria count was 1.2×10^6 to 9.2×10^6 CFU. Total fungi count was 5.2×10^4 to 3×10^5 CFU. Meanwhile these results were acceptable in such weather conditions of temperature and relative humidity in Ibb governorate, Yemen, i.e. at the same time of the year, in fall session. It is probably due to the poor handling methods used in corn grains storage in this area.

Fungi isolation on Czapek's agar medium

Data in Table (2) represents the isolated and grown fungi on Czapek's agar medium for corn grains obtained from various shops at Ibb Governorate collected in October 2008. The results in Table (2) showed the percentage of infected grains with fungal. Samples of corn grains stored in Ibb governorate were taken in October, 2008, and plated on Czapek's agar medium for 3 days at 28±2°C. The percentage of the infection is (the infected grains %). The number of the fungal colonies per 100 corn grains and percentage of the incidence of each fungal genus associated with 100 corn grains were determined. The results revealed that the number of fungal colonies is the same as the number of the infected grains per 100 grains (infection %) except in some instances where one infected grain produced more than one fungal colony.

Table 1: Moisture content, total count of bacteria and fungi

Place of Sample*	Moisture content %	Total bacterial Count (CFU)	Total fungi Count (CFU)
1	11.5%	4×10^6	5.2×10^4
2	11.1 %	1.2×10^6	2×10^5
3	11.8 %	8×10^6	7.2×10^4
4	10.6 %	4.9×10^6	1.3×10^5
5	12.4 %	7×10^6	1.1×10^5
6	11.5 %	6.5×10^6	2.3×10^5
7	11.4 %	9.2×10^6	1.2×10^5
8	11.3 %	8.5×10^6	3×10^5
9	11.7 %	5.1×10^6	1.5×10^5
10	10.9 %	1.7×10^6	9.1×10^4

* Total samples are 30, three samples from each place: this is the mean results.

Concerning fungal genera were found on and in corn grains. *Aspergillus*, *Fusarium*, *Penicillium* and *Rhizopus* were detected in all corn examined samples. However, the genus Rhizopus was not detected on (4 and 6 places). *Aspergillus* spp., which was considered as storage fungi, was the most dominant associated fungal species on the corn grains. While, *Penicillium* spp. and *Fusarium* spp., which was considered as field fungi, recorded the highest frequency of fungi isolated from the corn grains. However, the genus *Rhizopus* spp was found in some samples of corn grains, but it was not recorded in other samples. Similar results were mentioned by Fathi (1966, 1971), who isolated certain fungal species and genera in a high frequency from corn samples grown at northern parts of Delta but they were in a low frequency in the samples of southern parts of the country, Egypt.

The results revealed also that *Aspergillus* spp., *Fusarium* spp., *Penicillium* spp. and *Rhizopus* spp., were recorded at a high frequency among the total fungal species isolated from corn grains. Similar results were also reported by El-Kady et al. (1982), Amra (1988), Qahtan (2002) and Nogaim (2005, 2011).

Table 2: Genera of fungi on corn grains

Place of Sample*	% Infection	*Aspergillus*	*Fusarium*	*Penicillium*	*Rhizopus*
1	86%	12 %	26 %	50 %	12 %
2	88 %	30 %	30 %	22 %	18%
3	70 %	28 %	14 %	43 %	15 %
4	62 %	22 %	12%	66 %	0.0
5	79 %	10 %	10 %	40 %	40 %
6	72 %	38 %	27 %	35 %	0.0
7	80 %	30 %	35 %	17 %	18 %
8	93 %	36 %	29 %	27 %	8 %
9	90 %	18 %	36 %	26 %	20 %
10	75 %	37 %	24 %	25 %	14 %

* Total samples are 30, three samples from each place

Table 3: Survey of Mycotoxins in corn grains obtained from Ibb governorate, Yemen during October, 2008.

Place of Sample*	Aflatoxins (µg/kg)	Ochratoxins (µg/kg)	Zearalenone (µg/kg)
1	0.0	11	8.0
2	10	0.0	0.0
3	16	0.0	7.0
4	0.0	14	0.0
5	7.0	0.0	4.0
6	22	5.0	0.0
7	0.0	16	9.0
8	9.0	12	0.0
9	17	0.0	0.0
10	0.0	18	11

* Total samples are 30, three samples from each place:

The most common toxigenic fungi found in maize include species from the genera Aspergillus and Fusarium, mainly *Aspergillus flavus*, *Aspergillus parasiticus* and *Fusarium verticillioides, Aspergillus* species produce aflatoxins B_1, B_2, G_1 and G_2, which are considered to be involved in the etiology of human liver cancer as mentioned by (Rodriguez-Amaya, 2001).

Natural Occurrence of Some Mycotoxins in Corn Grains:

The data in Table (3) showed the natural occurrence of Mycotoxins in corn grains in 2008. The results indicated that (6) samples out of (30) were positive for Aflatoxins and Ochratoxins in corn grains, while only (5) samples out of (30) were positive for Zearalenone in corn grains. The concentrations of Aflatoxins ranged from 7 to 22 µg Aflatoxins/kg corn grains while Ochratoxins ranged from 5 to 18 µg Ochratoxin/kg corn grains. For Zearalenone, it ranged from 4 to 11 µg Zearalenone / kg corn grains.

These results are in a harmony with those recorded by several researchers; Atwa (1997) found that the levels of Aflatoxins B1, B2, G1 and G2 in the tested corn samples which were incident 33.3, 0.0, 40 and 10% for the samples collected from different countries, namely USA, Canada, Australia and Egypt, respectively. Rasti et al. (2000) also determined the level of contamination in imported and native corn [maize] with Aflatoxin B1 in central feed silos of Isfahan in Iran, the results showed that most of the samples were contaminated with Aflatoxin B1with a range of 0.0 to 9.9 µg/kg, and an average of 5.1 µg/kg, which was much lower than the tolerance level. These results agree with those of Hegazy (2000) who found that the highest Ochratoxin A contamination was observed in peanuts collected from Bolak El-Dakrour, which recorded 27.77 and 20.04 µg/kg for unsalted and salted peanuts, respectively. Silva and Vargas (2001) carried out a survey of Zearalenone (ZEA) in corn grains obtained from various regions of Brazil, and they analyzed 380 corn samples, where 30 samples 7.8%, were found to be contaminated with ZEA at a range of 46.7 to 719 µg/kg. In turn, Escobar and Sanchez-Regueiro (2002) found that 17.04 % of a total 4594 of the analyzed crops samples contained Aflatoxin B1, and the biggest percentages were in sorghum and groundnut with 83.3 and 40.4%, respectively. Maize, oat, wheat, and soya are fundamental raw ingredients in the elaboration of concentrates. Percentages of contamination with Aflatoxin B1 of 23.3, 10.7, 25, and 4.6 were found in maize, oat, wheat and soya, respectively. These results agreed with those of Qahtan (2002), Ebba (2003) and Nogaim (2005, 2011).

Conclusions

It can be concluded that the corn grains samples in Ibb governorate, (Yemen), had medium percentage of contamination by fungi and the occurrence of mycotoxins. There was no big or clear difference between the samples regarding the influence of the source of corn grains and the location of the storage.

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