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OCCURENCE OF MICROORGANISMS IN CORN GRAINS STORED IN BIHOR COUNTY

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Abstract

Zea mays grains samples were collected randomly from three different agro-ecological areas of Bihor county, in order to identify the microorganisms depeloped on and in grains, to determine the incidence of fungi species and levels of mycotoxins producing by these fungi, during fall season. We have measured total count of bacteria and molds by inoculating specific dilutions of the samples suspension into various culturing media and incubating them for fixed periods at optimum temperatures. The fungi genera that we have identified in corn grains stored samples were: Penicillium 35.1%, Aspergillus 26.1%, Fusarium 24.3% and Rhizopus 14.5%. The presence of mycotoxins was determined by using thin layer chromatography (TLC). The data obtained from TLC revealed that the corn samples were contaminated by : Aflatoxin, Ochratoxin and Zearalelone.

Key words: mycotoxins, TLC, fungi

INTRODUCTION

According to FAO, the fungi produces secondary metabolites called mycotoxins, which are toxic. Among these, there are some important for food and feed contamination and human health, such as: fumonisins, aflatoxins, zearalenone, ochratoxins, deoxylevalenol (FAO, 2001).

Fusarium is a common contaminant of grains and it is prevalent in zones with a moderate climate. Some species of Fusarium genus are pathogens microorganisms specific for different plants, while other species grow saprophytically on dead plants (Krska R. et al, 2001). Mainly the species Fusarium verticillioides and Fusarium proliferatum are responsables for producing fumonisins in cereals (Yazar S., Omutag G. Z., 2008). Other species of Fusarium genum are capable to synthesize another toxin, called zearalenone: Fusarium culmorum, Fusarium graminearum, Fusarium moniliforme (Cadwell R. et al, 1970).

Some species of Penicillium and Aspergillus, in the temperate oe cold climate of some European countries from Balkan, produce a mycotoxin called ochratoxin A (OTA) which is considerate responsable from certain pathologies observed in humans. Different studies show that OTA is a nephrotoxic and possible carcinogenic substance (Czerwlecki L., 2001).

MATERIAL AND METHOD

We have collected a total number of 10 samples of stored corn grains, with different origin, derived from 3 agroecological areas of Bihor county, during fall season, when relative humidity was over 15%.

Total Bacterial Count Determination

Total Bacterial Count represents the total bacterial load in a given sample.

The method we have used for determining Total Bacterial Count (TBC**)** was Plate Count Procedure, wich constists of inoculating the surface of a Petri dish with Columbia agar, with a serial dilution of the sample suspension. The colony forming unit (cfu) was determined manually, through direct observation of cells in a specialized counting chamber slide viewed under phase contrast microscopy. The resulting colony counts were calculated as organisms per gram of corn grains (cfu/g).

The samples of corn grains were disintegrated in a standard diluent (peptone water 2%) to achieve 1/10 dilution, then were homogenized for 30 minutes. From this initial dilution, serial dilutions of 1/100 and 1/1000 were made. From each dilution, 1ml was transferred into two Petri plates. After that, 25 ml of Columbia agar were pouring in plates and they were incubated at 30^{o}C for 72 hours, in an incubator.

The number of colony forming units per gram (cfu/g) calculated using the formula:

cfu/g = no. of colonies × dilution factor

Total Fungi Count Determination

Total fungi count (TFC) was determined using the diluting plating technique on yeast extract glucose agar. The medium was sterilized and antibiotics (cloramphenicol) was added immediately to the agar in order to inhibit bacterial growth and it was transferred into Petri plates. The plates were incubated at optimum temperature 25^{o}C, for 5 days.

Fungi counts were determined using technique described by FDA's Bacteriological Analytical Manual (2001).

The fungal colonies were counted on the dilution agar plates, in order to determine colony forming unit per gram of sample.

Identification of fungi

The taxonomy of isolated fungal at the species level was based on conventional methods. These included physical macroscopic description of colonies on appropriate media (based on colony growth, texture, colour and pigment) and microscopic description of hyphae, phialides, conidiogenous cells, conidia and microconidia. The specialized literature used to make identifications included : Raper & Fennell (1965), Pitt J.L. (1979) and Burgess et al. (1994).

Sub-culturing of isolated colonies were inoculated in Petri plates with Yeast extract glucose chloramphenicol agar, Czapek yeast agar and Malt extract agar, for 14 days at 25°C, 37°C and 45°C, followed by the macroscopic and microscopic identification of fungal species using the keys described for Penicillium spp., Aspergillus spp., Fusarium spp., and Rhisopus spp.

Evaluation of mycotoxigenicity of fungal isolates

The presence of mycotoxins was determined by using thin layer chromatography (TLC).

The intensity of fluorescence for mycotoxins spots was estimated by using a florodensitometer.

The amounts of all mycotoxins were determined by comparing the densitometer reading of each sample with those of standard solutions using the following equation:

$$\frac{\mu g}{kg} = \frac{s.y.v}{x.w}$$

s= mycotoxin standard equal to unknown (µl)
y= concentration of standard mycotoxin (µg/ml)
v= dilution factor (µl)
x= sample extract giving fluorescent intensity equal to standard (µl)
w= weight of the original sample found in the final extract (g)

RESULTS AND DISSCUSIONS

The results we have obtained indicate that there is no significant difference between those 3 agro-ecological areas wherefrom corn grains had origin.The location of storages was also insignificant.

The percentage of contamination with fungi was medium and the incidence with mycotoxins has also medium values.

The results indicate that all corn grains samples were contaminated with Aflatoxins and the concentration of Aflatoxins ranged from 8 to 20 µg/kg with an average that is lower than the tolerance level.

From all 10 corn grains samples, 6 samples were positive for Ochratoxins and and the concentration ranged from 5 to 16 µg/kg.

Also 6 samples were positive for Zearalenone and the concentration ranged from 5 to 10 µg/kg.

Table 1. Values for total bacteria count determined in corn grains samples

Number of sample	TBC (cfu)
1	3,000,000
2	1,500,000
3	8,200,000
4	5,200,000
5	7,500,000
6	7,000,000
7	8,500,000
8	7,200,000
9	5,800,000
10	2,200,000

Table 2. Values for total fungi count determined in corn grains samples

Number of sample	TFC(cfu)
1	48,000
2	250,000
3	74,000
4	15,0000
5	160,000
6	200,000
7	160,000
8	260,000
9	130,000
10	86,000

Table 3. The incidence of molds in samples of corn grains stored

Number of sample	Penicillium spp %	Aspergillus spp %	Fusarium spp %	Rhisopus spp %
1	48	10	22	16
2	27	40	28	10
3	45	20	33	12
4	24	34	11	4
5	63	30	30	37
6	25	15	9	3
7	38	33	17	15
8	17	24	30	14
9	24	28	33	23
10	40	27	30	11

Table 4. Quantitative values determined for mycotoxins in samples of corn grains stored

Number of sample	AF µg/kg	OT µg/kg	ZEA µg/kg
1	10	0	5
2	11	0	0
3	9	0	5
4	17	12	8
5	18	11	0
6	20	10	0
7	8	16	0
8	8	5	7
9	20	14	9
10	10	0	10

CONCLUSIONS

The microflora of corn grains included many varieties of fungi and bacteria. The bacteria normally appeared to be involved in deterioration of stored grains, but they were incapable to penetrate corn grains. Thus, the deteriorating action in stored corn grains was principally due to pre-harvest fungal contamination of maize plant and through storage.

Corn grains contamination by fungi did not only reduced its quality through discolouring and decreasing its nutritional value, but also has generated mycotoxin production.

The study has showed that the presence of molds or their spores on or in corn grains does not necessarily mean that mycotoxins will always be produced.

Of the fungal spp. isolated, Penicillium was the most predominant and was succeeded by species of Aspergillus, Fusarium and Rhisopus.

Aspergillus genus was highly prevalent in samples from all 3 agro-ecological zones. Therefore, a risk of aflatoxin poisoning exists in the event that favorable conditions occur.

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