Toxigenic Molds in Different Grains from Albania

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Abstract**—***Mycotoxins are secondary metabolites produced by molds, produced by the organism under certain stress conditions, such as drough and high temperatures in field, or high humidity levels in storage facilities. It is well known that their presence may result in hazardous implications in the humans and animals health. The aim of this study was to analyze the presence of mycotoxigenic moulds in cereals harvested in Albania. Microbiological evaluation was accomplished in different media for moulds. Despite the fact that method is conventional, presenting approximate values, it helps to a judgement on the mycotoxigenic molds and other microorganisms.*

The concentration of microorganisms varied 0.5-260 x 10³ cfu/g in wheat and 80-200 x 10^4 cfu/g in maize. After the identification and classification was found presence of mainly Ascomycete classes; especially Aspergillus, Penicillium and Fusarium species. In conlusion, after comparing the finding with interantional standards was concluded that wheat comodity was within standards, while some of maize samples passed the allowed levels.

Keywords— Albania, Maize, Toxigenic molds, Wheat.

I. INTRODUCTION

The aim of this study was to evaluate the presence of mycotoxigenic fungis in different crops harvested in Albania. The evaluation of the diversity of filamentous molds and investigation of their presence were conducted in wheat and maize samples.

Cereals and cereal products constitute the staple food in the diet of the Albanian population. Their parallel use as to human consumption as well as livestock feed, implicates the importance of food and feed safety issue on cereal grains and cereal products. Microorganisms contaminating the cereal grains may originate from air, soil, water, different living organisms, storage and shipping, as well as processing stages. Many factors, part of the environment, influence microbial contamination, including rainfall, drought, humidity, temperature, sunlight, frost, soil conditions, wind, insect, bird and rodent activity, harvesting equipment, use of chemicals in production versus organic production, storage and handling, and moisture control (Heredia et al. 2009).

According to the stage of fungal contamination, frequently they are grouped into two categories, field fungi and storage fungi (Miller, 1995). The first group invades the grain before harvesting, in field, in climatic conditions when the moisture content during harvesting period vary 18 to 30%, whereas storage fungi, considered as post-harvest invaders, infect grain when they have lower moisture contents (14 to 16%). Field fungi consist primarily of species of *Alternaria*, *Cladosporium*, *Fusarium*, and *Helminthosporium*, while storage fungi include species of *Eurotium*, *Aspergillus*, *Penicillium*, and *Mucor* (Riba *et al*., 2008).

Finally the fungal growth may affect the grain's quality and have several negative consequences such as appearance alteration, technological properties modification, fungal infections or allergies development, and finally economic losses (Bennett et al. 2003).

Mycotoxigenic fungi presence in grain comodities in certain climatic conditions may indicate for mycotoxin presence, which may have hazardous implications in the humans and animals health. Within a given species, their impact on health is influenced by age, sex, weight, diet, exposure to infectious agents, and the co-occurrence with other mycotoxins through synergic effect manifestation and pharmacologically active substances (Milicevic et al., 2010). The majority of mycotoxins are grouped, according to their toxic activity, under chronic conditions as mutagenic, carcinogenic or teratogenic; while according to site of action they are grouped in: hemo-, hepato-, nephro-, dermato-, neuro- or immunotoxins (Niessen, 2007).

II. METHOD OF STUDY

Cereals samples were obtained in sterile condition from different regions of the country during harvesting year of 2016. In this study were analyzed 15 samples (6 winter wheat samples and 13 maize samples). An amount of 0.5 kg samples were obtained in order to prepare the sample average with diagonal division.

2.1 Fungal Isolation and Identification

Examination of the contamination to the samples on molds was based on a slightly modified method of the European Feed Microbiology Organization (EFMO) (VDLUFA, 2007). To 20 g of each sample, 180 ml of 0.5% peptone water was added. The mixture was homogenized using a linear shaker for 20 min and then diluted to final concentration of 10^{-2}, 10^{-3} and 10^{-4} (VDLUFA, 2007). 1 ml aliquots of each dilution were spread (in duplicates) on the surface of solid medium. The composition of the medium was (per litre) as follows: 40 g malt extract, 2 g glucose, 1 ml Marlophen 810®, 60 mg Bengal Rose, 60 mg oxytetracycline-HCl (OTC), 12 g agar, 1000 ml distilled water. Plates were incubated for 3 days at 27°C in the dark and normal atmosphere. Afterwards, plates were stored at room temperature for another 3 days. Finally, the colonies were counted and the results were expressed as average colony forming units in thousands per gram of sample (10^3 CFU/g) using the following formula.

$$N = \frac{\Sigma C}{V \times n \times d} \quad (1)$$

N = number of colony-forming units per gram of sample (CFU/g)
ΣC = sum of all colonies of the count plates
V = volume of the dilutions pipetted in the count plates in ml
n = number of count plates that can be evaluated
d = dilution factor

Taxonomic identifications of different genera of moulds were made visually and where applicable, by means of a magnifying glass or a stereomicroscope. Closer characterization was possible using a light-optical microscope.

III. RESULTS AND DISCUSSION

The microorganisms, isolated from wheat samples are shown in Table 1, while the results in the maize samples are shown in Table 2, the evidence on molds distribution in the analyzed samples.

TABLE 1
THE PRESENCE OF TOXIGENIC MOLDS IN WHEAT (IN X 10^3 CFU/G)

	Alternaria spp.	*Fusarium spp.*	*Cladosporium spp.*	*Penicillium spp.*	*Aspergillus spp.*
Sample 1	1	0.5	0.5	n.d	n.d
Sample 2	n.d	1	n.d	3.5	2
Sample 3	n.d	n.d	n.d	1	1
Sample 4	0.5	5	n.d	3	1
Sample 5	n.d	1	n.d	10	34
Sample 6	n.d	0.5	1	1	3
Sample 7	n.d	40	n.d	210	10

n.d.= not detected

The leading contaminants among fungi in the wheat samples were *Aspergillus* spp. and *Fusarium* spp. Which were detected almost in all samples (77.7%) followed by *Penicillium* spp. (66.6%). *Alternaria* spp. and *Cladosporium spp.* were lowest contaminated only 22.2 %. The wheat sample with the highest number of moulds was sample 7 (260 x 10^3).

TABLE 2
TOXIGENIC MOULDS PRESENCE IN MAIZE SAMPLES (IN X 10^4CFU/G)

	Alternaria spp.	*Fusarium spp.*	*Cladosporium spp.*	*Penicillium spp.*	*Aspergillus spp.*
Sample 1	n.d	2.5	n.d	10	26
Sample 2	n.d	10	n.d	40	140
Sample 3	n.d	8	n.d	12	80
Sample 4	n.d	100	n.d	5	24
Sample 5	n.d	4	n.d	80	14
Sample 6	n.d	82	n.d	11	20
Sample 7	n.d	n.d	1	100	100
Sample 8	25	n.d	n.d	120	32
Sample 9	n.d	11.5	3.5	30.5	2

n.d.= not detected

The general means of fungi showed that *Penicillium* spp. (92.3%%) and *Fusarium* spp. (84.6%), followed by *Aspergillus* spp. (76.9%) were the most frequently isolated genera, while *Alternaria spp*. and *Mucor spp*. were the least frequently isolated genera (7.7%) from maize samples.

The maize sample with the highest number of moulds was sample 7 (200×10^4) .

It is well known, that environmental conditions have great influence on the development and spread of moulds and consequently on the production of mycotoxins. Water stress, temperature stress and insect damage of a host plant are, under field conditions, the major determining factors of mould infestation and toxin production. With stored grain, factors which are likely to affect mycotoxin formation include moisture content and the composition of the substrate, environmental temperature, exposure time, damage to seeds, oxygen availability, carbon dioxide concentrations, fungal abundance, prevalence of toxic strains, spore loads, microbial interaction and invertebrate vectors, particularly insects. Spoilage, fungal growth and mycotoxin formation result from the complex interactions of these factors (Santin, 2005).

IV. CONCLUSIONS

In this research, moulds, identification and classification, we revealed a high frequency of the genus *Aspergillus*, *Penicillium* and *Fusarium*. The concentration of microorganisms vary 0.5-34 x 10^3 cfu/g in wheat samples and vary 80-200 x 10^4 cfu/g in the maize samples. In conlusion, wheat samples which have been analyzed were within standards, while maize samples were not within standards. Additional studies incorporating analysis of toxin potential are needed to more fully assess the importance of species and contamination of cereals, in order to protect the population from risks associated with mycotoxin contamination.

REFERENCES

[1] Bennett, J.W., Klich, M. (2003). Mycotoxins. Clinical Microbiology Review 16:497-516.

[2] Heredia, N., Wesley, I. and Garcia, S. (2009). Microbiologically Safe Foods, USA.

[3] Milicevic, D.R., Škrinjar, M. and Baltic, T. (2010). Real and Perceived Risks for Mycotoxin Contamination in Foods and Feeds. Challenges for Food Safety Control.Toxins, 2: 572-592.

[4] Miller, J.D. (1995). Fungi and Mycotoxins in Grains. Implications for Stored Product Research. Journal Stored Product Research 31:1-16.

[5] Niessen, L. (2007). PCR-Based Diagnosis and Quantification of Mycotoxin Producing Fungi. International Journal of Food Microbiology, 119:38-46.

[6] Riba, A., Mokrane, S., Mathieu, F., Lebrihi, A. and Sabaou, N. (2008). Mycoflora and Ochratoxin A Producing Strains of Aspergillus in Algerian Wheat. International Journal of Food Microbiology, 122: 85–92.

[7] Schmidt, H. L., Bucher, E., Spicher, G. (1981). Microbial Content Determination of Bacteria, Molds and Yeasts in Feed. Nutrient Media and Methodology. Agricultural Research, 34(4): 242-50.

[8] Smith, M.T., Yarrow, D. (1996). Identification of the Common Food Borne Fungi. In R. A. Samson, E. S. Hoekstra, J. C. Frisvald, O. Filtenborg, Introduction to Food-Borne Fungi 3-232.

[9] VDLUFA (2007). Standard Operation Procedure for the Enumeration of Micro-organisms Using Solid Culture Media. In VDLUFA Method Book III, Suppl. 7 (ch. 28.1.1., 14 p). Darmstadt: VDLUFA.