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Natural occurrence of aflatoxins in maize harvested in Serbia during 2009–2012

Jovana Kos*, Jasna Mastilović, Elizabet Janić Hajnal, Bojana Šarić

Institute of Food Technology, University of Novi Sad, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia

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ABSTRACT

The aim of the present study was to determine concentration of aflatoxins (AFs) B_1, B_2, G_1 and G_2 in 380 maize samples collected from different regions of Serbia during four years period and to analyze the influence of weather conditions on the AFs production.

Aflatoxins content was determined by direct competitive Enzyme Linked Immunosorbent Assay (ELISA) method.

In 180 maize samples analyzed in 2009–2011 period AFs were not detected. However weather condition changes in 2012 caused AFs presence in 137 (68.5%) of samples in the concentration range from 1.01 to 86.1 μg/kg with the mean level of 36.3 μg/kg.

The obtained results indicate great influence of hot and dry weather with prolonged drought during spring and summer 2012 on a contamination of maize with AFs.

Since the obtained results present the first national report of AFs maize contamination this study could be of a great interest for establishing monitoring programs of AFs in Serbia.

1. Introduction

Maize is one of the major crops grown in Serbia. About 40% of total planted area of field crops in Serbia is covered with maize. In the recent years Serbia was one of the largest maize producer and exporter in Europe with the approximate amount of six and two million tons per year, respectively. Maize is mainly used as a component of animal feed (80%) as well as for human consumption and starch production (Maslac, 2011, 2012). *Aspergillus* species are one of the most frequent contaminants of maize which can result in mycotoxin production during growth, harvest, storage, transport and processing (Bankole & Adebanjo, 2003).

Aflatoxins (AFs) are a group of mycotoxins produced as secondary metabolites mainly by three *Aspergillus* species including *Aspergillus flavus*, *Aspergillus parasiticus* and *Aspergillus nomius* (Greppy, 2002). Although, eighteen AFs have been identified, four of them have been found as contaminants in food and feed: B_1, B_2, G_1 and G_2 (Decastelli et al., 2007). Aflatoxins are highly toxic, mutagenic, teratogenic and carcinogenic compounds and International Agency for Research on Cancer included AFs as primary carcinogenic compounds (IARC, 2012). *Aspergillus* species can be found in soil, plant and animal remains, grains and seeds such as maize, peanuts, and tree nuts (Pitt, 2000). Toxins produced by *Aspergillus* species generally occur in agricultural products in tropical and subtropical regions where temperature and humidity are optimum for the growth of molds and production of the toxins (Rustom, 1997). Prolonged drought, high temperatures, substrate composition, storage time and conditions were reported as the main influence factors for fungal growth and AFs synthesis (Stack & Carlson, 2003, p. 43). Although crops contamination with AFs is a global phenomenon, tropical and subtropical areas are more susceptible to contamination than those in temperate regions (Hell, Cardwell, & Poehling, 2003).

Recent studies reported *Fusarium, Penicillium* and *Aspergillus* as the most frequent fungi contaminants of cereals, feedstuffs, vegetables and fruits under prevailing continental climate conditions of Serbia (Lević, Stanković, Bočarov-Stančić, Škrinjar, & Mašić, 2004). According to Jajić, Jurić, and Abramović (2008) cereals in Serbia are mainly infected by *Fusarium* species. It should be noted that data about *Aspergillus* toxins presence in agriculture products from Serbia are limited.

Altough Serbian regulations (Sl. Glasnik RS, 4/2010; Sl. Glasnik RS. 28/2011) for maximum allowed limits of AFs in maize intended for human and animal consumption were recently adopted and harmonized with European Union regulations (2002/32/EC, 2010/165/EC) monitoring program is still not prescribed. Due to the significant health risks associated with the presence of mycotoxins in food and feed it is important to establish control and monitoring

* Corresponding author. Tel.: +381 21 485 3837; fax: +381 21 450 725.
E-mail addresses: jovana.kos@fins.uns.ac.rs, jovanamatic5@gmail.com (J. Kos).

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programs as the sources for risk assessment. In the only published study of mycotoxins monitoring in Serbia, Škrbić, Malachova, Živančev, Veprikova, & Hajšlova (2011) reported presence of *Fusarium* toxins in wheat.

The data about AFs presence in maize has not been studied in Serbia as well as in neighboring countries. Hence, the aim of this first national report of AFs maize contamination was to analyze the natural occurrence of AFs in maize samples from 2009–2012 period and to provide insight into presence of AFs in Serbia.

2. Materials and method

2.1. Samples, kits and chemicals

The numbers of analyzed maize samples during 2009, 2010, 2011 and 2012 years were the following: 60, 60, 60 and 200, respectively.

The main maize growing areas in Serbia were included in the frame of the four-year monitoring program at the Institute of Food Technology, University of Novi Sad. Samples were collected and analyzed every year after harvest, from September to November. Sampling was performed according to EU requirements (2006/401/EC) in order to overcome irregular mycotoxins distribution among the crops and kernels. Particular numbers of incremental samples were combined in order to obtain aggregate samples of approximately 2–8 kg. Aggregate samples were homogenized and quartered to obtain a 500 g of laboratory samples which were kept in freezer at 4 °C until the analysis.

Determination of AFs were done by Enzyme Linked Immunosorbent Assay (ELISA) method using Quantitative AFs High Sensitivity Test kit and AFs Quantitative Test kit (Neogen Veratox®, Lansing, USA).

Chemicals used for ELISA analysis were distilled water (Millipore, BedFord, MA, USA) and methanol of analytical purity (Merck, Darmstad, Germany).

2.2. Sample extraction

500 g of each representative sample was ground to a 1 mm particle size using laboratory mill (Knifetec™ 1095 mill, Foss, Hoganas, Sweden). Subsamples of 5 g were extracted with 25 ml of methanol/water mixture (70:30, v/v) and shaken vigorously for three minutes on laboratory Griffin flask shaker (Griffin and George, Wembley, England). Extracts were filtered through a Whatman No. 1 filter paper (Whatman International Ltd., Maidstone, UK). The obtained filtrates were collected, vortexed (Vortex mixer, Velp Scientifica) and used for analysis.

2.3. Aflatoxin analysis by ELISA

AFs concentrations were determined according to the manufacturer's procedure. Free AFs in the samples and standards are allowed to compete with enzyme-labeled AFs (conjugates) for the antibody binding sites. After a wash step, substrate is added, which reacts with the bound conjugate to produce blue color. The intensity of the color is inversely proportional to the concentration of mycotoxin in the sample or standard. Intesity of the color in each well was measured at 650 nm in a microwell reader (Thermolabsystem, Thermo, Finland). The optical densities of the standards form the standard curve and the samples optical densities are plotted against the curve to calculate the exact concentration of mycotoxins.

At the beginning, all samples were analyzed with Quantitative AFs HS Test kit. Range of quantitation for this test kit is between 1 and 8 μg/kg. Samples with concentration of AFs higher than 8 μg/kg were additionally analyzed with AFs Quantitative Test kit (range of quantitation 5–50 μg/kg). Samples with content of AFs more than 50 μg/kg were analyzed again after dilution.

The analytical quality of the ELISA method was assured by the use of certified reference material as well as by participation in interlaboratory studies. Naturally contaminated maize with certified AFs content of 4.5 ± 0.5 μg/kg was used as certified reference material (CRM) for validation of Quantitative AFs High Sensitivity Test kit. CRM was supplied by Trilogy Analytical Laboratory (Trilogy® Reference Material, A-C-268, Washington, USA). Naturally contaminated maize sample from interlaboratory study with AFs mean value of 19.0 ± 4.18 μg/kg was used for determination of validation parameters of AFs Quantitative Test kit. The validation parameters (Table 1) were calculated and expressed using European Official Decision procedure (2002/657/EC) and their values were in accordance with recommendations given in Regulation 2006/401/EC. Further, the analytical quality of the ELISA method is assured by participation in five interlaboratory studies (LTSD-0015 report, Neogen Corporation, Technical Services Division, Natural Toxins, Lansing, USA).

2.4. Statistical analysis

Statistical analysis of variance was carried out by Duncan's multiple comparison tests using STATISTICA software version 10 (StatSoft Inc. 2011; USA). P values < 0.05 were regarded as significant.

3. Results and discussion

The results obtained in four years period indicate significant differences in occurrence of AFs in maize. In 180 samples analyzed during 2009–2011 period the presence of AFs was not detected.

Since weather conditions in 2012 were favorable for *Aspergillus* moulds growth, larger number of maize samples (200) was analyzed in comparison to previous years.

Aflatoxins occurrence in 68.5% of maize samples harvested in 2012 could be contributed to weather conditions favorable for mold growth and mycotoxins production. Extremely hot and dry conditions followed by drought were noted during maize growing season 2012 (Republic Hydrometeorological Service of Serbia, 2012).

Comparison of temperatures and precipitation for period of maize planting, growing and harvesting (April–September) in 2009–2012 and average values of these parameters in long-term period (1971–2000) is given in Table 2 (Republic Hydrometeorological Service of Serbia, 2009, 2010, 2011, 2012). The statistical analysis of variance between years showed significant difference for 2012 in terms of average temperatures and number of days with temperatures higher than 30 °C and 35 °C. Furthermore, 2011 and 2012 were characterized with significantly lower amount of precipitation in comparison to 2009 and 2010. It could be noted that different weather conditions during 2012 in

Table 1
Validation parameters of two ELISA methods for determination of AFs.

	Quantitative high sensitivity test	Quantitative test
LOD	0.50	1.40
LOQ	1.00	5.00
RSD_r	8.06	7.54
RSD_R	9.17	9.33
Recovery	103.2	96.5

LOD: limit of detection (μg/kg).
LOQ: limit of quantification (μg/kg).
RSD_r: relative standard deviation calculated under repeatability.
RSD_R: relative standard deviation calculated under reproducibility conditions.