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Fumonisins in Serbian Corn: Long-time Assessment under Actual Climate Change Conditions

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Maize samples collected in Serbia during a period of seven years were investigated on the presence of fumonisins. Concentration of fumonisins was determined by validated direct competitive Enzyme Linked Immunosorbent Assay method. This method was in accordance with European Union requirements, therefore accredited and performed in the Serbian accredited official laboratory. Summary analysis of all obtained results revealed fumonisins contamination in 82% of the total of 291 maize samples with average contamination being 1.515 mg/kg. An increase in the percentage of contaminated samples (from 51 to 100%), as well as an increase in mean fumonisin concentration in positive samples (from 0.227 to 3.281 mg/kg) and median values in positive samples (from 0.070 to 2.140 mg/kg) was noticed during the observation period. Although in Serbia there is no regular control of fumonisins in corn for animal feeding, long-term results indicate their wide distribution in this grain. Since the data on climate elements show change in temperature and precipitation in relation to multiannual average on the territory of Serbia, further research on the effects of climate change on the development of mold, the appearance of insects and the production of toxins are necessary in order to check the resistance of currently grown hybrids in the territory of Serbia on fungal growth and fumonisins production.

Keywords maize, fumonisins, climate change, Serbia

Abbreviations: FUMs – fumonisins; FAO – Food and Agriculture Organization of the United Nations; WHO – World Health Organization; IARC – International Agency for Research on Cancer; EC – European Commission; ELISA – Enzyme Linked Immunosorbent Assay; RHSS – Republic Hydrometeorological Service of Serbia

Introduction

Fumonisins (FUMs) are mycotoxins known as common contaminant of cereals, mostly maize and maize-based products (FAO/WHO 2011). FUMs are primarily produced by molds of the genus *Fusarium*, FUMs B series being the most prevalent members of a family of toxins, which are directly associated with a range of pathogenic effects. Since

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the presence of FUMs in maize has been statistically linked with cases of human esophageal carcinoma in South Africa and China, International Agency for Research on Cancer categorized *Fusarium moniliforme* toxins as potentially carcinogenic for humans (agents of the class 2B), similar to the ochratoxin A (IARC 2002).

The presence of FUMs in raw maize is determined by climatic conditions before and during harvest as well as by storage conditions. Investigations of the effects of diverse factors revealed that FUMs production are predominantly influenced by geographic locality and weather conditions (daily precipitation, maximum and minimum daily temperatures, relative humidity), insects, and, finally, by the maize hybrid itself (De la Campa et al. 2005). Global monitoring data on FUMs content in maize point out the importance of identifying their presence and emphasize the risks from toxicity of these mycotoxins (FAO/WHO 2011).

Before imposing the European Regulation (EC 2006a; 2007), only several countries had relevant national legislation on maximum permitted FUM levels. Subsequently, regulations on diverse contaminants including mycotoxins as well as FUMs have been published in the Official Journal of the European Union (EU). Thus, maximum permitted levels of particular contaminants in food have been laid down in the Commission Regulation No 1126/2007 (EC 2007) was adopted amending Regulation No 1881/2006 as regards *Fusarium* toxins in maize and maize products. Maximum permitted FUM contents in maize and maize-based products for human nutrition in Serbia (Serbian Regulation 2010; 2014a) are harmonized with EU Regulation. The recommendation of European Commission of August 17, 2006 (EC 2006a) encompasses maximum recommended values for deoxynivalenole, zearalenone, ochratoxin A, fumonisin B_1, and fumonisin B_2 in feed. The Regulation on the amendment of the Regulation on the quality of feed (Serbian Regulation 2014b) partly harmonizes the legislation in this field with the EU; however, FUMs have still not been included.

In spite the fact that maize is the most prevalent grain crop produced in Serbia because of favorable climatic conditions and fertile soil (Table 1) and is mainly used for animal feeding, maximum permitted FUM levels have been not prescribed for neither maize used for animal feeding nor in feed-mixtures. Almost the half of the total maize production in Serbia originates from individual producers, who face the problem of both somewhat lower yield and appropriate drying and storage, that is, adequate protection of the quality of harvested maize in view of mycological and mycotoxicological contamination. In spite of abundant reports on the presence of FUMs in maize and wheat (Jakšić et al. 2012; Kos et al. 2014), as well as frequent isolation of molds of the genus *Fusarium* and other

Table 1. Maize production in Serbia (average 2005–2014)

	Maize		
	Area harvested (ha)	Production (t)	Yield per ha (t)
Republic of Serbia	1,157,677	6,059,670	5.2
Vojvodina	651,188	3,924,337	6.3

Source: Statistic Office of the Republic of Serbia 2014.

fusarium mycotoxins from grains and feed samples (Jajić et al. 2008), the presence of FUMs in feed is mostly not subject to control in Serbia because of the lack of relevant legislation.

Considering that FUMs in feed have not been addressed in Serbian legislative, while regulation of their contents in food for human consumption has been laid down as late as 2010, the information on grain-crops (especially maize) contamination with these mycotoxins is still very scant. In that respect, there is a critical need for investigating the presence and contents of FUMs in Serbia. The analysis of large number of samples was performed with an aim of obtaining a more realistic insight in the occurrence of this mycotoxin in maize. Having in mind that FUMs are common contaminants of maize, more precise understanding of environmental conditions favoring FUMs accumulation in maize grain is indispensable. Thus, the objective of this study was the long-term investigation of the FUMs concentration in maize during the production years characteristic by change of climatic factors in relation to the long-term average in Serbia.

Materials and Methods

Samples and sampling

Maize samples analyzed for FUM contents are collected in the period 2005–2014, immediately after harvest, as well as upon storage. The samples mainly originated from different localities in Vojvodina Province, but also from other regions of the Republic of Serbia. The sampling was performed directly on the field, after harvesting and immediately before storage in the silos (from September 1 to November 1), and these samples are labeled with B. Samples that were sampled after storage were collected between November 1 and September 1 of the following calendar year, and are marked with A.

Sample preparation and analytical method

Immediately upon sampling, 1000 g of each sample was prepared by grinding in a laboratory mill in such a way that >93% passes through a 0.8 mm sieve. The samples were than homogenized by mixing, packed in plastic bags and stored at –20 °C, and allowed to reach room temperature prior to analysis.

Determination of total FUMs level using ELISA method was performed applying *ELISA Ridascreen® Fumonisin R3401* kits (R-Biopharm, Darmstadt, Germany). The analytical quality of the ELISA method was assured by the use of certified reference materials as well as by participation in interlaboratory studies. For validation and analytical quality of the ELISA method, there were used naturally contaminated maize reference materials with certified FUMs content of 0.7 ± 0.1 mg/kg and 4.0 ± 0.1 mg/kg (TR-F100, lot #F-C-439, and lot #F-C-441, Trilogy Analytical Laboratory, Washington, USA). The validation parameters were calculated, and their values were in accordance with recommendations given in EU Regulation 2006/401 (EC 2006b). Laboratory limit of detection was 0.025 mg/kg, and determination 0.050 mg/kg. The limit of detection was determined by the series of 6 repeated blind probe measurements and calculated as the sum of the average value of the blind probe and 3 standard deviations. Quantification limit was determined as the sum of the average value and 10 standard deviations. Relative standard deviation calculated under repeatability was 8%, while relative standard deviation calculated under reproducibility conditions was 10%. The recovery rate was 99%. Furthermore, the analytical quality of the ELISA method was assured by participation in proficiency testing scheme (Central Institute for Supervising and Testing in Agriculture, Czech Republic, 2014).

Statistics

Statistical analysis was performed using the PAST software package, version 3.25, Oslo, Norway. Statistical data analysis included the following: univariate analysis (descriptive statistics), and two sample *t*-test (unequal variances).

Results

Fumonisin contents in maize in Serbia

Presenting the long term data for all investigated samples is the best way to provide insights on FUMs contamination of maize in Serbia. The results of our analysis of FUM contents in 2005-harvest maize as well as for the period 2009–2014 are displayed in Table 2.

Presented results revealed that in only 16 out of 291 analyzed samples of raw maize concentration of FUMs exceeded maximum permitted levels (4 mg/kg) laid down in relevant regulations for human consumption (Serbian Regulation 2014a; EC 2007). It is to be emphasized that all samples analyzed in the framework of this investigation were intended for feed. Given that maximum permitted level of FUMs in maize for animal feeding are pretty high, being 60 mg/kg, all investigated samples were in accordance with EU recommendations (EC 2006a). However, the maximum levels for certain species of animals are more stringent. That is how in feedingstuffs for pig, horses, rabits and pets limit is 5 mg/kg, for fish 10 mg/kg, for poultry, calves, lambs and kids it is 20 mg/kg, while adult ruminants and mink have high tolerance, to even 50 mg/kg. Although the tolerable level of fumonisin in corn is high, given its significant participation in mixtures, there is a risk of contamination of complete feed (Jakšić et al. 2018). The amounts of 5 mg/kg can cause pulmonary edema (Dilkin, 2010), however, the impact of low FUMs concentrations in feed on pig health should not be neglected. Pigs are very sensitive to FUM, and it should be considered the effects of chronic exposure to such low dietary levels. Exposure to an average concentration of FUMs in naturally contaminated feed had no effect on pig health but did affect the digestive microbiota balance, with *Salmonella* exposure amplifying this phenomenon (Burel et al. 2013). In addition to the negative impact on pig health, feed contaminated with FUMs has an impact on economic losses. More than 0.1 mg/kg FUMs in feed had affect on lower weght gain, while concentrations from 1 mg/kg of toxin have negative impact on meat quality (Rotter et al. 1994).

Table 2. Fumonisin contents in maize in 2005 and in the period 2009−2014

Year	Sampling time	No. of samples	No. of positive (% of positive) samples	No. of inappropriate[a] (% of inappropriate) samples	Global total mean (mg/kg)[b]		Content in positive samples (mg/kg)		
					Lower bound	Upper bound	Average	Range	Median
2005	B	35	18 (51)	–	0.117	0.129	0.227	0.034−1.04	0.070
	A	35	21 (60)	–	0.248	0.348	0.413	0.031−3.30	0.202
	B+A	70	39 (56)	–	0.182	0.293	0.327	0.031−3.30	0.129
2009[c]	B	34	17 (50)	–	0.176	0.188	0.350	0.030−1.52	0.229
2010[d]	A	24	24 (100)	1 (4)	1.084	1.084	1.084	0.060−12.80	0.216
2011	A	17	15 (88)	1 (6)	1.316	1.319	1.492	0.117−13.22	0.689
2012	B	9	9 (100)	4 (44)	6.767	6.767	6.767	0.351−20.34	3.630
	A	69	67 (97)	4 (6)	1.256	1.255	1.256	0.044−10.46	1.120
	B+A	78	76 (97)	8 (10)	2.171	2.172	2.229	0.044−20.34	1.165
2013	B	12	11 (98)	1 (8)	1.166	1.165	1.197	0.031−4.22	0.421
	A	35	35 (100)	–	0.397	0.397	0.397	0.045−1.90	0.276
	B+A	47	46 (98)	1 (2)	0.657	0.658	0.672	0.031−4.22	0.309
2014	A	21	21 (100)	5 (24)	3.281	3.281	3.281	0.449−10.20	2.140

A – After storage.
B – Before storage.
[a] Content of FUMs ≥4 mg/kg.
[b] Two scenarios were considered when calculating the mean values; samples in which the concentration was below the limit of detection (0.025 mg/kg) were assumed to have a value of zero (lower-bound estimates) or the limit itself (upper-bound estimates) (FAO/WHO 2011).
[c] Jakšić et al. 2011.
[d] Jakšić et al. 2012.

Analyzing the number of samples in which FUMs were detected, an increase in the percentage of contaminated samples (from 51 to 100%) was noticed during the observation period. Also, an increase in mean FUM concentration in positive samples (from 0.227 to 3.281 mg/kg) as well as median values in positive samples (from 0.070 to 2.140 mg/kg) can be observed, with the exception of 2013. These differences are conditioned by agro-meteorological conditions, and it should also be taken into account that some samples came from regular control and some were associated with certain animal health problems. Differences in measured concentrations of toxins in samples before and after storage were tested by using the Welch's *t*-test, since the population variances are unequal. The results of the statistical analysis showed that there was no significant difference in the concentration of FUMs in the samples before and after storage. For all three observed production years (2005, 2012, and 2013), there was no significant difference at the 0.05 level. As already noted, the weather has effect on the production of fumonisins in maize on field. This research did not include a detailed climate analysis of each locality from which the samples came. However, since the samples originate from a relatively small geographical area, we analyzed the general change in climate elements and agrometeorological data provided by Republic Hydrometeorological Service of Serbia (RHSS) for the analyzed production years on territory of Serbia (Table 3).

It can be seen that during the observed period there were production years with an important changes in temperature and precipitation compared to the perennial average. However, given the observed time period, we can discuss about the impact of environmental factors and the response of corn hybrids on the potential conditions of contamination by mold and toxins, rather than on climate change. Certainly, this research will be a

Table 3. Pronounced changes in climatic factors relative to the perennial average (1961–1990) in Serbia during the observed period (RHSS 2004–2014)

Year	General characteristic of vegetation period	Deviation of the mean annual temperature	Deviation of the mean precipitation during the growing season	Impact on agricultural production
2005	Humid	+0.3 °C	+20%	Favorable conditions for growth and maturation
2009	Average	+1.0–1.4 °C	+10%	Interfering corn silking, and pollen dusting
2010	Warm and humid	+1.2 °C	+40%	Negative impact of precipitation surplus
2011	Warm and dry	+1.2 °C	–17%	Lower quality, lower yield, higher activity of plant pests
2012	Extreme dry	+1.0–1.4 °C	–75%	Lower quality, lower yield, higher activity of plant pests, *Fusarium* infection
2013	Warm	+1.4–1.6 °C	average	Lower quality, higher activity of plant pests
2014	Warm and extreme humid	+1.8 °C	+100–200%	Yield above average, widespread plant diseases

contribution to the analysis of climate change after a longer period of time. In addition, a large influence on the contamination has grown on different maize hybrids. Different resistance of cultivated hybrids to toxin producing fungi (Krnjaja et al. 2016) is an additional cause of differences in FUMs contamination. However, the increase of the resistance level will surely lessen the environmental effect, so resistance stabilizes low fumonisin concentration better than any other mean. An effective control of insect damage is also inevitable.

Discussion

As aforementioned, there are not many reports on investigation either of FUMs or FB1 content in maize in Serbia. Stanković et al. (2011) detected FUMs in 70.7% of 203 analyzed maize samples in the period 2007–2009. The concentration of FB_1 varied within a range 0.75‒4.9 mg/kg with an average value 1.226 mg/kg. The authors suggested that variations in FUM contents are due to different resistance of the plants as well as stress conditions associated with drought and insect attacks during investigation period. Namely, the highest prevalence of positive samples (80.1%) was recorded in 2007 characterized by high temperatures and draught. Kos et al. (2014) established contamination of FUMs ranging from 0.500 to 3.020 mg/kg in all 90 analyzed samples of 2012-harvest maize. The authors concluded that production of FUMs was favored by hot and dry weather conditions. Similar researches were conducted in our neighboring countries, e.g. Croatia, Hungary, Romania, and Bulgaria. The data from 1992 revealed FUMs contamination in Croatia (58% positive for FB1 and 21% for FB_2), and Romania (50% positive for FB_1 and 17% for FB_2) (Doko et al. 1995). However, the reported FUM contents were lower as compared with those obtained in Serbia, as indicated above. Namely, the average FB_1 levels in positive samples in Croatia were 0.020 mg/kg (range 0.010–0.060 mg/kg) and in Romania 0.010 mg/kg (range 0.010–0.020 mg/kg), whereas FB_2 content was the same in both countries being 0.010 mg/kg. However, according to the reports of Jurjević et al. (1999) and Domijan et al. (2005) the frequency of FUMs contamination ($FB_1 + FB_2$) in 1996, 1997, and 2002 in Croatia was significantly higher, being 99%, 93%, and 100%, respectively. The average contents of total FUMs in positive samples from 1996, 1997, and 2002 were 0.645, 0.134, and 0.460 mg/kg FB_1, respectively, whilst three of 49 investigated samples were positive for FB_2 at concentrations 0.068, 0.109, and 3.084 mg/kg. According to maize analysis from 2007 (Šegvić Klarić et al. 2009), the presence of FUMs was confirmed in 27% samples, with average concentration of 3.690 mg/kg. In 1997, in maize products analyzed in Hungary (Varga et al. 2004), FUMs were detected in 67% samples yet with the very low contamination levels (0.016-0.058 mg/kg). The analysis of maize for FUMs contamination was performed in Bulgaria considering the climatic conditions (Manova and Mladenova 2009), and FUMs were detected in 94.7% of samples ranging between 0.249 and 4.050 mg/kg. According to the data from 2001, 50% of samples contained 0.03–6.56 mg/kg FUMs (Vrabcheva et al. 2002). The investigation of the presence of fusarium mycotoxins in cereals in Croatia performed by Pleadin et al. (2013) revealed that maize was the most contaminated cereal, and the highest percentage of all analyzed cereal samples was contaminated with deoxynivalenol followed by zearalenone, FUMs, and T2. However, the highest percentage of maize samples was contaminated with FUMs (90%) at average concentration of 1.756 mg/kg. All samples were 2011-harvest, a year characterized by warm and dry weather as compared to 2010 (90% positive samples with average concentration 4.509 mg/kg) characterized by cold and humid weather conditions. Thus, the authors attribute generally lower FUMs production in 2011 to the climatic conditions (Pleadin et al. 2012). There are many different studies on FUMs in maize worldwide. Italy and Brazil, as significant producers of maize, with a climate conducive to FUM production, pays considerable attention to control. Recent data show concentrations comparable to our research. In Brazil, 0.4 to 9.1 mg/kg were measured in maize in 2012 (Silva et al. 2017), and in Italy between 2006 and 2008, mean values at different locations were from 4.8 to 10.9 mg/kg (Berardo et al. 2011). On the other hand, data from USA show significantly higher contamination than in Europe, and an average concentration of 37.97 mg/kg was measured in Arkansas (Abbas et al. 2006).

The results (Table 2) were compared with the results presented in studies of FAO/WHO (2011) obtained by summarizing the results of global analysis of 7,060 samples. The percentage of positive samples in Serbia (according to our investigation) was higher being 85% as compared to 76%. However, the average FUMs concentration in Serbia is lower (1.262 mg/kg as compared to 1.625 mg/kg, when considering upper limit). According to the presented review of global occurrence of FUMs, it is obvious that climatic conditions are greatly responsible for the formation of *Fusarium monilifome* and *F. proliferatum*, and hence the occurrence of FUMs in maize. Variations in the levels of maize contamination are likely to be associated with agricultural factors and, partly, with variable sensitivity of particular crops to *Fusarium* species interrelated with climatic conditions. Besides, the applied methods for FUMs analysis should be taken into consideration given the differences in detection limits for particular techniques and hence different results on the percentage of contaminated samples.

The obtained results indicated high incidence of FUMs contamination of maize samples, yet with low concentrations. By analyzing the concentration of FUMs in the samples before and after storage, we concluded that storage had no significant effect on the production of toxins. Comparative analysis of FUMs content and agro-meteorological conditions throughout the research period suggests the necessity of further examination of interconnection of climatic conditions and mycotoxicological safety of maize in the territory of Serbia. The obtained results could be the starting point in developing prognostic model for predicting the risks of mycotoxin contamination in Serbia. These results strongly emphasize the mandatory continuous monitoring of FUMs and establishment of relevant database on their presence under climatic conditions in Serbia with an aim of obtaining relevant data for predicting contamination of FUMs. Further research and analysis of the effects of particular hybrids and applied preventive agrotechnical and agrochemical measures under production conditions in Serbia are required. The obtained results strongly indicate the necessity of preparing the draft on harmonization of legislation on FUMs contents in feed in Serbia with the relevant EU regulations.

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