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Co-occurrence of Fumonisins and Deoxynivalenol in Wheat and Maize Harvested in Serbia

Sandra Jakšić · Biljana Abramović · Igor Jajić · Milica Živkov Baloš · Željko Mihaljev · Vesna Despotović · Daniela Šojić

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Abstract A survey was undertaken to determine total fumonisins (FUMs) and deoxynivalenol (DON) in wheat and maize. Out of 75 wheat samples, 50.7 % contained FUMs in the span from 27 to 614 ng/g, while 65.3 % contained DON in the span from 64 to 1,604 ng/g. Out of 24 maize samples, contents of FUMs in one and of DON in three samples were above the maximal limit. This is the one of rare reports of the natural co-occurrence of FUMs and DON in wheat and maize, and the first report of their correlation in different wheat cultivars.

Keywords Fumonisins · Deoxynivalenol · Co-occurrence · Mycotoxin · Wheat · Maize

Agricultural crops, especially cereals, are commonly colonized by *Fusarium* spp. and often contaminated by their secondary metabolites that have a major impact on livestock health, welfare, and productivity. *Fusarium* spp. are frequently found in the Serbian climatic area which is suitable for cereal production (Jakšić et al. 2011). Among mycotoxins produced by *Fusarium* spp., fumonisins (FUMs) are usually present in maize and maize products, while deoxynivalenol (DON) is a common contaminant of wheat. In the literature, there are different opinions about the possibility of FUMs production on wheat. Marin et al. (1999) demonstrated that, although FUMs producers could colonize barley and wheat more rapidly than maize, they did not produce FUMs in those grains. These isolates were originally from maize, and they were unable to adapt their metabolism to produce FUMs in different substrates, or the nutritional components from wheat could act as inhibitors of fumonisin biosynthesis, or a certain component could have the capacity for initiation of the biosynthesis of fumonisins only in maize. Contrary to these studies, there are data indicating a significant potential for FUM contamination of wheat infected with *F. proliferatum* at similar levels as maize (Desjardins et al. 2007). Shephard et al. (2005) concluded that analytical methods and confirmation are insufficient in some studies of FUMs in wheat, and because of that these results should be validated and confirmed by definitive techniques. However, there are several reports in the literature about natural FUMs in wheat and non-corn based foodstuffs (Kushiro et al. 2009; Cirillo et al. 2003). Preliminary toxicological analysis showed that isolates of *F. verticillioides* and *F. proliferatum* from wheat kernels in Serbia have a high potential to produce fumonisin B_1, and its high incidence and levels in wheat kernels from the 2005 and 2007 harvests were reported (Stanković et al. 2012).

Emerging evidence suggests that mycotoxins may have synergistic effects in vivo (Javed et al. 1993). Therefore, the detection of different toxins simultaneously occurring in infected cereals is useful to better evaluate the risk for human and animal health (Cirillo et al. 2003). Our previous studies were concerned with maize and wheat contamination with FUMs (Jakšić et al. 2011) and DON (Jajić et al.

S. Jakšić · M. Ž. Baloš · Ž. Mihaljev
Scientific Veterinary Institute “Novi Sad”, 21000 Novi Sad, Serbia

B. Abramović (✉) · V. Despotović · D. Šojić
Department of Chemistry, Biochemistry and Environmental Protection, Faculty of Science, University of Novi Sad, Trg D. Obradovića 3, 21000 Novi Sad, Serbia
e-mail: biljana.abramovic@dh.uns.ac.rs

I. Jajić
Department of Animal Science, Faculty of Agriculture, University of Novi Sad, Trg D. Obradovića 8, 21000 Novi Sad, Serbia

2008), but there are very little data about their co-occurrence in Serbian cereals (Stanković et al. 2012).

Owing to the importance of production of cereals in the Vojvodina Province of Serbia, this study evaluated the natural contamination and co-occurrence of FUMs and DON in 25 varieties of wheat from three locations in Vojvodina and in 24 stored maize samples.

Materials and Methods

Twenty-five cultivars of winter wheat, representing most of the cultivars released in Serbia from 1955 to 2006, were used in the study. Banatka-BA is a native population, Bankuty 1205-BK is an old Hungarian, San Pastore-SP and Libellula-LI are Italian, Bezostaja-BZ is worldwide known old Russian, Zlatna Dolina-ZD is Croatian and Skopljanka-SK is FYR Macedonian. The Serbian cultivars were created in two breeding centers Small Grains Research Center, Kragujevac (Kragujevacka-56 KG) and Institute of Field and Vegetable Crops, Novi Sad (Sava-SA, Partizanka-PZ, Novosadska rana 2-N2, Balkan-BL, Yugoslavia-YU, Lasta-LA, Evropa 90-EV, Pobeda-PO, Novosadska rana 5-N5, Renesansa-RE, Pesma-PE, Ljiljana-LJ, Cipovka-CI, Dragana-DR, Simonida-SI, NS 40S-NS and Zvezdana-ZV).

Wheat samples of 25 winter cultivars grown in 2010 were taken at the following locations: Novi Sad (Bačka), Sremska Mitrovica (Srem) and Pančevo (Banat). The selected locations represent a broad range of environmental conditions in the Vojvodina Province (Serbia). They are characterized by semiarid conditions, with dry, hot spring and summer, neutral autumn and moderately cold winter. At each location, the plots were rotated with soybean (*Glycine max* L.). All cultivars were agronomically suitable for production on the given locations.

The wheat cultivars were planted in a randomized complete block design with three replicates at each location. Each plot consisted of 10 rows, 10 cm apart and 5 m long, so that the harvested area was 5 m^2, and seedling density 500 seeds/m^2. All the trails were sown in the middle of October (optimum sowing date) and reached maturity in late June or early July. Weeds were controlled by hand. All wheat samples were asymptomatic (no evident kernel damages).

Maize samples, grown in 2010, were collected during the 2011 from the silos in Bačka. After collecting, 1,000 g of each sample were homogenized by grinding in a laboratory mill in such a way that >93 % passed through a sieve with pore diameter of 0.8 mm, and analyzed immediately for mycotoxins.

Contents of FUMs were determined by the enzymatic immunoaffinity (ELISA) method using Ridascreen® test kit (Art. No. R:3401, R-Biopharm, Germany), with limit of detection 25 ng/g for total FUMs. A recovery experiment was performed in triplicate by spiking 5 g of ground fumonisin-free wheat sample, i.e. maize sample, with FB_1 at a level of 800 ng/g. Spiked samples were left overnight at room temperature to allow solvent evaporation prior to the extraction. Mean total FUMs recovery for wheat was 78 ± 14 % and 110 ± 15 % for maize.

Content of DON in maize samples was determined by ELISA method, using *Ridascreen*® test kit (Art. No. R:5906, R-Biopharm, Germany), with limit of detection 18.5 ng/g. The recovery experiment was performed in triplicate by spiking 5 g of ground maize sample with DON at a level of 300 ng/g. Spiked samples were left overnight at room temperature to allow solvent evaporation prior to extraction. Mean total DON recovery was 86 ± 20 %.

Content of DON in wheat was determined by the HPLC method (Abramović et al. 2005). The accuracy of the method was determined with the recovery of fortified blank grain samples at four levels (80, 160, 400 and 800 ng/g), with three replicates for each level and three injections for each replicate. Recoveries obtained ranged from 93.7 % to 105.8 %. Precision of the method was calculated in terms of standard deviation (SD) and ranged between 4.5 % and 12.6 %. The linearity of the method was estimated in the working range of 0.1–3.0 μg/mL, at eight calibration levels, each injected in duplicate. The correlation coefficient was 0.996. The calculated limit of detection (LOD) and the corresponding limit of quantification (LOQ) (verified by the respective signal-to-noise ratio of 3 and 10) were 18, and 60 ng/g, respectively.

The obtained recoveries comply with the requirements of the European Commission concerning development of analytical methods (EC 2006), and show that the analytical methods used fit the purposes. The results for the samples were not corrected for the recovery of the spike. The results of mycotoxicological analyses were subjected to multiple regression analysis using the software package Statistica, version 9.1 (StatSoft, Inc. 2010, www.statsoft.com).

Results and Discussion

The results of the determination of mycotoxins in wheat are shown in Table 1. The occurrence of total FUMs contamination was the same on all locations (52 %), with similar average levels from 192 ng/g in Srem to 290 ng/g in Banat, whereas the total FUMs levels ranged from 38 to 614 ng/g in the samples from Bačka, from 32 to 586 ng/g in those from Banat, and from 27 to 548 ng/g in the Srem samples. In 37 samples (49.3 %), no FUMs were found, i.e. their content was below the limit of detection. The mean of total FUMs content in the positive samples was 241 ng/g, and the amount of FUMs was in the range from 27 to 614 ng/g.

Table 1 Total FUMs and DON occurrence in wheat samples from different locations, harvest 2010

Cultivar	Location/content (ng/g)					
	Pančevo (Banat)		Novi Sad (Bačka)		Sremska Mitrovica (Srem)	
	FUMs	DON	FUMs	DON	FUMs	DON
Banatka	ND	229	ND	ND	28	392
Bankuty 1205	ND	ND	614	ND	ND	92
San Pastore	ND	ND	ND	ND	ND	352
Bezostaja	385	ND	310	ND	ND	484
Libellula	81	312	ND	144	27	136
Zlatna Dolina	ND	ND	38	ND	ND	312
Sava	427	104	359	ND	414	356
Partizanka	32	512	ND	1,604	ND	456
Novosadska rana 2	ND	132	ND	156	ND	364
Kragujevacka56	ND	226	69	ND	50	692
Balkan	419	ND	ND	ND	ND	316
Yugoslavia	98	136	100	188	38	292
Skopljanka	ND	72	ND	540	ND	628
Lasta	ND	288	ND	384	263	164
Evropa 90	ND	64	102	ND	ND	840
Novosadska rana 5	68	ND	55	ND	37	864
Pobeda	522	304	451	148	548	620
Renesansa	351	196	417	ND	525	660
Pesma	72	292	84	ND	78	984
Ljiljana	ND	280	ND	ND	37	267
Cipovka	401	ND	ND	ND	ND	288
Dragana	36	232	ND	ND	ND	308
Simonida	ND	ND	441	ND	394	292
NS 40S	586	176	ND	80	ND	492
Zvezdana	ND	ND	86	ND	52	692
No. of positive samples (%)	13 (52.0)	16 (64.0)	13 (52.0)	8 (32.0)	13 (52.0)	25 (100.0)
Average	290	222	240	406	192	454
Range	32–586	64–512	38–614	80–1,604	27–548	92–984
Median	351	228	102	172	52	364

ND not detected

The frequency of wheat contamination with DON was somewhat higher: 65.3 % of samples contained DON at an average level of 370 ng/g. The highest level of DON (1,604 ng/g) was found in the sample from Bačka, in spite of the much greater contamination frequency in Srem (100 %). Only in one sample, DON was above the maximum tolerable level (1,250 μg/kg) adopted by EU and Serbian regulations.

It was interesting to examine whether there is a correlation between the content of FUMs and DON, as *Fusarium* toxins. Statistical analysis showed a significant negative correlation ($p < 0.05$) only between the average FUMs and DON contamination (Fig. 1) on three locations (−0.46). The DON median levels for Bačka and Srem samples were higher than FUMs median levels, and the median level of FUMs were higher for Banat. In 36 % of wheat samples, both toxins (DON and FUMs) were detected.

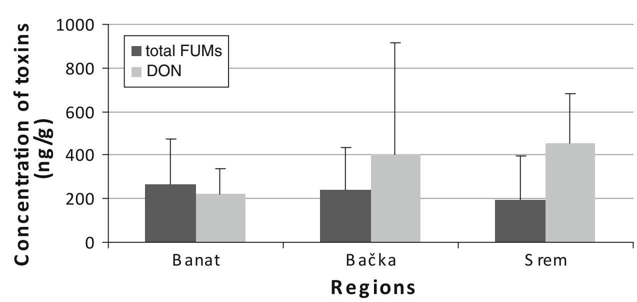

Fig. 1 Concentrations (mean of positive samples ± SD) of total FUMs and DON in wheat samples from different regions in Serbia

Table 2 shows the results of maize contamination with FUMs and DON. Statistical analysis showed no significant

Table 2 Total FUMs and DON occurrence in maize samples harvested in 2010

Toxin	LOD (ng/g)	Min (ng/g)	Max (ng/g)	Average (ng/g)	Positive/total samples	Positive samples (%)
FUMs	25	60	12,800	1,084	24/24	100.0
DON	18.5	154	16,528	1,263	22/24	91.7

Table 3 Distribution of maize samples by total FUMs content and corresponding average content of DON

FUMs content (ng/g)	No. of samples[a]	Average content of DON (ng/g)
<100	5	551
100–1,000	13	427
1,000–4,000	4	1,264

[a] Without outliers (Nalimov test)

correlation ($p < 0.05$) between FUMs and DON contamination, but, as it is evident from Table 3, the samples with higher contents of FUMs had also higher average contents of DON.

Although FUMs were present in maize samples in 100 %, and at higher levels than in our previous investigations (Jakšić et al. 2011), the total FUMs content was below the values set by EU regulations for animals (60,000 ng/g), and in only one sample exceeded the maximum level for humans (4,000 ng/g), by EU and Serbian regulations. Content of DON was above the maximum level for unprocessed maize (1,750 ng/g) in three samples (12.5 %).

The weather in Serbia in 2010 had some deviations from the usual features of climate. Production year was warmer but much wetter. This has significantly influenced the agricultural crop production. The summer of 2010 in Serbia was very warm and extremely warm, rainfalls in the northern regions were significantly above normal and extreme. In May, temperatures were normal, while the rainfall in Vojvodina was extremely above normal. In June, July and August, it was very and extremely warm, with the August rainfall exceeding the maxima (RHSS 2010). Our results showed that these field conditions were favorable for the development of fungi and formation of mycotoxins. In comparison with the results for the years 2004–2007 (Jajić et al. 2008), when the rainfalls and average temperatures were at the level of long-term mean, the incidence and levels of DON contamination in the 2010 harvest were higher. Wet conditions before the harvest influenced probably the higher frequency and higher contents of FUMs in maize in 2010 compared with 2009 (Jakšić et al. 2011). It should be noted that in this study maize was sampled from the silos, while in 2009 this was done immediately after harvest. This means that besides weather conditions, inadequate drying before storage as well as storage conditions could cause a higher level of contamination in samples from the 2010 harvest.

The results of wheat contamination obtained in this study are somewhat different from those of the 2005 and 2007 harvest years (Stanković et al. 2012), although a few of the same locations and the same cultivars were investigated. Our results indicate lower frequency of both *Fusarium* toxins, and the levels of both FUMs and DON are lower. This could be explained by different sample collection. Samples of the cereals from 2005 and 2007 were collected after 4–6 months of storage in family barns. Therefore, storage could have an impact on toxin production, like in the case of maize.

Our results are comparable to the data of Chehri et al. (2010) for stored wheat in Iran, who reported FUMs levels that were lower, ranging from 12 to 155 ng/g, but the frequency of contamination was somewhat higher: 68.2 % for B_1, 42.6 % for B_2 and 31.7 % for B_3. Kushiro et al. (2009) detected FUMs in Japanese wheat, and although the amount was much lower than generally found in maize, they pointed out that the combination of several mycotoxins co-occurring in wheat could have synergistic effects. They also emphasized the importance of identification of fungal species in wheat plant. In a review concerning FUMs and DON in Turkey, these toxins were reported to be present in maize products, but not in wheat (Omurtag et al. 2007). In an investigation of different mycotoxins in 22 wheat varieties in Pakistan FUM was not detected, whereas DON was found at 100 ng/g in 12 samples of 4 varieties (Sahar et al. 2009). The DON frequency and levels in wheat and corn-based food from Italy appear much higher than that of FUMs B_1 and B_2 (Cirillo et al. 2003). However, when comparing results of different surveys it is necessary to take into account different analytical methods with different limits of detection.

Our study indicated significant presence of FUMs and DON in Serbian cereals. Results of the 2010 harvest are interesting because of the unusual weather conditions for this climate that had affected agricultural production. Although mycotoxins are present, it can be concluded that wheat cultivars in Serbia are well adapted to the environment and even to the major changes in climate, including the drought stress and extremely humid conditions. High percentage of positive samples of maize obliges to more frequent control of stored samples during the whole period of storage. This study confirms the importance of continued surveillance of *Fusarium* mycotoxins in wheat and maize cultivars grown in Serbia, as well as of forming database of their occurrence in Serbian weather conditions.

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