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Fusariotoxins in Wheat Grain in Serbia

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SUMMARY

Samples of wheat grain (41), collected during the 2010 harvest from seven localities in Serbia, were analysed for the presence of zearalenone (ZEA), T-2 toxin, deoxynivalenol (DON) and fumonisine B_1 (FB_1). Results of Enzyme-Linked Immunosorbent Assay (ELISA) showed that all analysed samples were positive for the presence of at least one of four observed fusariotoxins. The most distributed mycotoxins were ZEA (90.2%, with the average concentration of 442.6µg kg^{-1}) and T-2 (90.2%, with the average concentration of 24.2 µg kg^{-1}). DON (73.2%) and FB_1 (84.4%) were detected in a somewhat smaller number of samples, but their average concentrations were higher (1988.1 µg DON kg^{-1} and 882.7 µg FB_1 kg^{-1}). The established correlations between concentrations of DON and FB_1 ($r = 0.32$) or DON and ZEA ($r = 0.22$) were not statistically significant. A negative correlation was established between concentrations of T-2 and FB_1 ($r= -0.24$), as well as, between T-2 and DON ($r = -0.36$). Detected concentrations of ZEA and T-2 were bellow the level prescribed by the World Health Organisation (WHO), while concentrations of FB_1 and DON detected in five that is, 17 samples, respectively, were above the permissible limit for human consumption.

Keywords: Wheat; Zearalenone; T-2 toxin; Deoxynivalenol; Fumonisin B_1

INTRODUCTION

Seed diseases caused by pathogenic fungi, insect damages and injuries caused by inappropriate storage after harvest, can significantly deteriorate technological quality of wheat grain and can cause economic damages important for any country (Lević et al., 2008a, 2008b).

In many countries world-wide, *Fusarium* species are considered to be the most common pathogens in cereals, while among them *F. avenaceum*, *F. culmorum*, *F. graminearum*, *F. equseti*, *F. poae* and *F. tricinctum* are the most important (Burgess et al., 1994; Nirenberg et al., 1994).

The greatest importance of representatives of the genus *Fusarium*, beside their role in decreasing the yield and grain quality (Nelson et al., 1983), is their ability to biosynthesise mycotoxins in infected plants (Marasas et al., 1984).

Species of the genus *Fusarium* biosynthesise a wide spectrum of mycotoxins, but their concentrations are not always proportional to the intensity of fusariosis, which they cause on cereal grains. Climatic factors, first of all, temperature and relative humidity during the growing season, affect the development of certain species of the genus *Fusarium*, and therefore have an effect on mycotoxins which will be synthesised and in which concentration. Beside climatic factors, a number of other factors, such as genotype susceptibility, storage types, affect the development of fungi and the synthesis of mycotoxins (Lević et al., 2008a).

Out of 300 identified toxic fungal metabolites, 139 are fusariotoxins. Among them, the most distributed are trichothecenes, then zearalenone (ZEA), fumonisins (FB_1) and other mycotoxins (moniliformin, beauvericin, fusaproliferin, fusarin, etc.), which differ in their chemical structure, mode of action and symptoms that cause diseases in humans and animals (Marasas et al., 1984).

As fusariotoxins enter the nutrition chain by consuming cereals and cereal-based products, risk to human and animal health varies depending on entered concentrations and types of mycotoxins.

Different species of the genus *Fusarium* produce different mycotoxins, and also one species can produce several different mycotoxins, such as *F. graminearum* that biosynthesises more than 17 types of mycotoxins: zearalenone (ZEA), trichothecenes of the type B (deoxynivalenol – DON, nivalenol – NIV), rarely trichothecenes of the type A, culmorin, fusarin C, fusarochromanone, steroids, etc. (Lević et al., 2004a; Lević, 2008).

Agroecological conditions in Serbia favour the development of numerous pathogenic and toxigenic species of the genus *Fusarium* (Lević et al., 2004b) out of which, *F. graminearum* is the most important pathogen of wheat grain (Lević et al., 2008b). Isolates of this fungus, originating from grain of wheat grown in Serbia, are important producers of fusariotoxins (Stanković et al., 2008a). However, species of the section *Liseola,* especially *F. proliferatum*, have been very frequently occurring during the past few years (Stanković et al., 2006). Mycotoxins produced by these species differ from those produced by *F. graminearum* (Lević, 2008).

Zearalenone (ZEA) is a prevalent contaminant of stored products, such as maize, wheat, barley, oat, as well as, other substrates on which fungi belonging to the genus *Fusarium* develop. *F. graminearum* and *F. culmorum* are the best known producers of zearalenone, though some other species of this genus can biosynthesise it under certain conditions (Marasas et al., 1984). Under agroecological conditions of Serbia species and strains of the genus *Fusarium*, particularly *F. graminearum*, have relatively high potential for the synthesis of ZEA (Bočarov-Stančić, 1996). ZEA and its metabolites are known for estrogenic and anabolic properties, as well as, for the growth improvement, and therefore they can cause: estrogenism in animals, cancer of reproductive organs and early occurrence of puberty in humans (Hagler et al., 2001).

Trichothecenes are natural contaminants of wheat, maize and other cereals, as well as, cereal-based products. There are the greatest group of toxins synthesised by species of the genus *Fusarium,* with over 170 different chemical structures (Jurić et al., 2007). The natural occurrence of trichothecenes of the type A is important for the territory of Serbia, while there are hardly any data on trichothecenes of the type B. In Serbia, more intensive studies on DON were initiated in the second half of the first decade of the 21st century, although this group of trichothecenes have been thoroughly studied in the world (Prelusky et al., 1994). On the other hand, producers of this mycotoxin are widely distributed in cereals in Serbia (Lević et al., 2009) and therefore there is no justification for insufficient studying of DON in Serbia. DON can cause an emetic syndrome and feed refusal, as well as, a disease called akakabi-byo, whose incidence has been recorded in Japan and was associated with consumption of wheat and barely 100% contaminated with the species *F. graminearum* and *F. sporotrichioides*, which synthesise DON, NIV, T-2 and ZEA (Marasas et al., 1984; Desjardinis and Hohn, 1997). T-2 toxin has been used as a very effective poison weapon in Vietnam and Laos (Watson et al., 1984).

Fumonisins are broadly distributed in maize grain, as well as, in maize-based feed and food (Lević and Stojkov, 2002). This group consists of 15 compounds grouped into four series designated with A, B, C and P. Under natural conditions, fumonisins of the series B prevail, with 70.0% of FB_1, while FB_2 and FB_3 amount to 10.0-20.0% of the total level of fumonisins (Nelson et al., 1993). *F. verticillioides, F. proliferatum* and *F. subglutinans* are significant producers of fumonisins. It has been determined that these fungi synthesise FB_1 in maize grain more than in wheat and barely grain (Visconti and Doko, 1994). In Serbia, there are hardly any data on the presence of fumonisins in cereals, particularly in wheat and barely grain, than on other toxins, such as DON. Fumonisins can cause hepatotoxicity and hepatocarcinogenicity in the majority of animals, oesophagal cancer in humans, equine leukoencephalomalacia and pulmonary oedema in pigs (Nelson et al., 1993).

The objective of this study was to determine the presence and concentrations of mycotoxins ZEA, T-2, DON and FB_1 in grain of wheat collected from Serbian locations under different agroecological conditions, as well as, to establish interrelationships in their incidence.

MATERIAL AND METHODS

Forty one wheat samples, collected at harvest from the following seven localities in Serbia, were analysed: Zemun Polje (26), Bela Crkva (5), Loznica (3), Morović

(3), Kraljevci (2), Samoš (1) and Padina (1). Each sample contained 1 kg of wheat grain with the average moisture of 14.0%. The basic sample was divided into 100-g sub-samples that were dried at the temperature of 50°C for 3 d, and then were powdered. The mycotoxicological analysis for the presence of ZEA, T-2, DON and FB_1 was performed by the Enzyme-Linked Immunosorbent Assay (ELISA).

Samples for a quantitative and qualitative analysis of mycotoxins were prepared in the following manner: 25 ml of the appropriate solvent were added to the 5-g powdered sample, while 1 g NaCl was added for the analysis of FB_1. The sample homogenisation was done in an Osterizer blender at 1300 rpm for 3 min. ZEA, T-2 and FB_1 were extracted with 70.0% methanol solution in distilled water, while DON was extracted with distilled water. A homogenised mixture was filtered through Whatman No. 1 filter paper. If a concentration of mycotoxins was very high in the samples, filtrates of the samples were diluted with appropriate solvents according to the manufacturer's instructions.

The mycotoxin analyses were performed according to the manufacturer's instructions (Tecna S.r.l., Italy, Celer Fumo Test Kit, Celer DON Gold Test Kit, Celer Zon Test Kit and Celer T-2 Toxin Test Kit). Concentrations of mycotoxins were quantitatively determined at wave lengths of 450 nm by ELISA reader (BioTek EL x 800TM).

The Pearson's Product-Moment Correlation test was used to assess relationships among concentrations of particular mycotoxins.

RESULTS

The presence of mycotoxins was established in all observed samples of wheat grain (Table 1). ZEA, type A (T-2) and type B (DON) trichothecenes and FB_1 were detected in different concentrations. Mycotoxins ZEA (90.2%) and T-2 (90.2%) were predominant in observed samples; FB_1 (85.4%) ranked third by its presence, while the presence of DON was the least (73.2%).

Zearalenone. This mycotoxin was detected in concentrations ranging from 10 to 1000 $\mu g\ kg^{-1}$ (average 442.6 $\mu g\ kg^{-1}$) (Table 1). It was not detected in four samples. Its concentrations ranged from 500 to 1000 $\mu g\ kg^{-1}$ in the majority of samples (41.5%), while concentrations greater than 1000 $\mu g\ kg^{-1}$ were recorded in 4.8% of observed samples (Table 2).

Trichothecenes. Concentrations of T-2 in samples were not higher than 500 $\mu g\ kg^{-1}$. Also, concentrations below 100 $\mu g\ kg^{-1}$ were detected in the highest number of samples (92.7%) (Table 2). Detected concentrations of T-2 varied from 25 to 135.6 $\mu g\ kg^{-1}$ (average 24.2 $\mu g\ kg^{-1}$). This mycotoxin was not detected in four samples.

More than a half of observed samples (51.2%) had concentration of DON above 1000 $\mu g\ kg^{-1}$. The average concentration of DON in wheat samples was 1988.1 $\mu g\ kg^{-1}$. This toxin was not detected in 11 samples, while eight samples had the concentration above 5000 $\mu g\ kg^{-1}$.

Fumonisin B_1. The concentration of this mycotoxin ranged from 750 to 2465 $\mu g\ kg^{-1}$ (average 882.7 $\mu g\ kg^{-1}$) (Table 1). FB_1 was not detected in six samples, while four samples were contaminated with this toxin in the concentration above 2000 $\mu g\ kg^{-1}$ (Table 2).

Table 1. Frequency and concentration ($\mu g\ kg^{-1}$) of fumonisin B_1 (FB_1), zearalenone (ZEA), deoxynivalenol (DON) and T-2 toxin in wheat grain in Serbia in 2010

Parameters	Mycotoxin			
	ZEA	T-2	DON	FB_1
Number of positive samples	37/41	37/41	30/41	35/41
Frequency of positive samples (%)	90.2	90.2	73.2	85.4
Range ($\mu g\ kg^{-1}$)	10-1000	25-135.6	50-5000	750-2465
Average ($\mu g\ kg^{-1}$)	442.6	24.2	1988.1	882.7

Table 2. Distribution of mycotoxins in wheat samples collected from different localities in Serbia in 2010

Concentration of mycotoxins ($\mu g\ kg^{-1}$)	Samples contaminated with mycotoxins (%)			
	ZEA	T-2	DON	FB_1
< 100	26.8	92.7	34.1	17.1
100-500	26.8	7.3	7.3	17.1
500-1000	41.5	0	7.3	31.7
> 1000	4.8	0	51.2	34.1

Table 3. Co-occurrence of mycotoxins in 2010 and coefficient of correlation (r)

Mycotoxin	Co-occurrence (%)			Coefficient of correlation (r)		
	DON	T-2	ZEA	DON	T- 2	ZEA
FB_1	63.4	75.6	75.6	0.32	–0.24	0.09
DON		63.4	65.8		–0.36	0.22
T- 2			80.5			–0.09

Interrelationships of two mycotoxins. Co-occurrence of mycotoxins ZEA and T-2 was detected in 80.5% of samples, while a somewhat lower number of samples (63.4%) were co-contaminated with DON and T-2 toxin, as well as, with DON and FB_1 (Table 3)

The coefficients of correlation between two mycotoxins varied from r = –0.36 (between T-2 and DON) to r = 0.32 (between DON and FB_1) and were not statistically significant (Table 3).

DISCUSSION

All samples of wheat grain were positive for the presence of mycotoxins ZEA, T-2, DON and FB_1. This points out to the fact that agroecological conditions during the growing season in 2010 favoured the development of *Fusarium* species producing mycotoxins. DON was detected in the highest concentration (1988.1 μg kg^{-1}), followed by FB_1 (882.7 μg kg^{-1}), ZEA (442.6 μg kg^{-1}) and T-2 (24.2 μg kg^{-1}). These results have been expected, considering that results obtained in previous studies showed that samples originating from wheat grain contained high concentrations of mycotoxins (Stojanović et al., 2005; Stanković et al., 2007). High concentrations of fusariotoxins in wheat grain can be explained by a high frequency of producers of these mycotoxins in Serbia (Lević et al., 2009). At the same time, these producers are characterised by high potential of production of these mycotoxins (Stanković et al., 2008a)

In Serbia, ZEA is one of the most common contaminants of feed and its components. It is also the most prevalent pathogen of mycotoxicoses in domestic animals (Bočarov-Stančić et al., 1995; Lević et al., 2004b). The concentrations of ZEA (average 442.6 μg kg^{-1}) in samples of wheat grain established in our study were higher than concentrations (299.9 μg kg^{-1}) detected in studies conducted by Krnjaja et al. (2011). Samples used in both studies were collected in 2010, but wheat varieties were different and they were grown in different localities. Results on wheat grain contamination by ZEA over years and localities in Serbia point to effects of climatic factors on the production of mycotoxins. According to results obtained by Stanković et al. (2007), ZEA was detected in samples of wheat grain in the range of 37 to 331 μg kg^{-1}, in 64.5% samples. The presence of ZEA was mainly detected in the three wheat varieties most often grown in Serbia (Evropa-90, Pobeda and Renesansa) (Stojanović et al., 2002). According to these authors, the concentration of ZEA in the majority of samples of wheat grain (78.0%) was 160-500 μg kg^{-1}.

In the present study, T-2 toxin was detected in the lowest concentration (25-135.6 μg kg^{-1}), although its frequency was 90.2%. Similar results were obtained by Muthomi et al. (2008), when they studied wheat grain collected during 2004. These authors found that the concentration of this toxin ranged from 20.3 to 65.7 μg kg^{-1}. Stanković et al. (2009) in their two-year studies found out significantly higher concentrations of T-2 toxin (60 to 495 μg kg^{-1} in 2005 and 86 to 200 μg kg^{-1} in 2007). However, the frequency of this toxin was lower than the frequency established in the present study (75.0% in 2005 and 60.0% in 2007).

Although, DON is generally, the most distributed fusariotoxin in the world (Miller et al., 2001; Tomczak et al., 2002), there are little data on the presence of this mycotoxin in wheat grain in Serbia. Our studies show that DON was detected in the highest concentration (average 1988.1 μg kg^{-1}) in comparison with other observed fusariotoxins. Jajić et al. (2008) studied grain samples of wheat (16), maize (76), soybean (24), sunflower (19) and barley (4), collected in Serbia in 2004 and 2005. The average frequency of DON in wheat grain amounted to 37.5% for both years. The concentration of DON ranged from 630 do 1840 μg kg^{-1} (average 1235 μg kg^{-1}) in 2004, and from 57 to 423 μg kg^{-1} (average 124 μg kg^{-1}) in 2005. The authors concluded that differences in the mycotoxin concentrations between two years occurred due to climatic conditions in 2004 that favoured the development of *Fusarium* species on wheat. In this research, samples were taken directly from fields immediately after harvest and this could be the reason why the contamination level with DON was much lower than the contamination level with DON in our research. The analyses performed under conditions of Flanders (Belgium) during the 2002-2005 period showed that infection with the species of the genus *Fusarium* and contamination with DON were caused by climatic conditions, especially during wheat flowering and by applied cropping practices. The value of this mycotoxin ranged from 0 to 15000 μg kg^{-1} (Isebaert, 2009). According to data obtained by Jurić et al. (2007) in Vojvodina during 2004 and 2005, 41.6% of wheat samples were contaminated with DON. The concentration of this toxin varied from 57 to 1840 μg kg^{-1}.

The concentration of FB_1 in wheat grain in our studies ranged from 750 to 2465 μg kg^{-1}. According to Stanković et al. (2009) the concentration of FB_1 determined in wheat grain in 2007 amounted to 918.7 μg kg^{-1}. Stanković et al. (2008b) tested 24 samples of wheat grain collected from 14 localities in Serbia and found out that the concentrations of FB_1 in stored wheat grain were high and ranged from 2000 to 20000 μg kg^{-1}. Such high concentrations were explained by inadequate storage conditions.

There are little data in the world on contamination of wheat grain by fumonisins. Some researchers explain the absence of fumonisins in wheat and barley grain by the competition of wheat grain mycobiotes, which can inhibit the synthesis of fumonisins, or the participation of mycobiotes in biodegradation of mycotoxins immediately after its biosynthesis (Marin et al., 1999). On the other hand, the presence of fungi of other genera can affect the development of producers of fumonosins. For instance, *F. verticillioides* and *F. proliferatum* under particular environmental conditions in the presence of *A. niger*, *A. flavus* and *A. ochraceus* have tendency to biosynthesise greater amounts of fumonisins. Similarly, one or several nutritive substances in wheat could act as inhibitors of biosynthesis of fumonisns. However, Šegvić Klarić et al. (2009) found FB_1 in 27.0% of samples of wheat grain in the concentration of 3690 μg kg^{-1} in Croatia.

Contamination of cereal grain by several mycotoxins is of a great importance in the production of feed and food. Namely, some mycotoxins can have a synergic effect that is much stronger than effects of individual mycotoxins. According to some studies, the presence of DON is an indicator of a possible presence of other fusariotoxins (Lombaert, 2002). Based on our study, DON and FB_1 co-occurred in 63.4% of samples, which was lower by 8.1% than the percentage determined by Stanković et al. (2009). In contrast, T-2 and ZEA co-occurred in the same number of samples (75.6%). The co-occurrence of FB_1 and DON, T-2 and ZEA was, according to Stanković et al. (2012), found in 78.6, 60.7 and 67.9% of samples collected in 2005 and in 86.5, 52.0 and 88.0% of samples collected in 2007.

We detected the co-occurrence of ZEA, DON and T-2 in 56.1% of samples, or higher by 21.1% than those found in the sample analysis performed by Muthomi et al. (2008).

Generally, concentrations of mycotoxins detected in the present study were below values prescribed by the World Health Organisation. A small number of samples contained concentrations slightly above prescribed, which can cause disease with typical clinical symptoms if they occur in the nutrition chain. Concentrations of FB_1 above the permissible limit for human consumption (2000 μg kg^{-1}) were detected in 9.8% wheat samples.

The accumulation of mycotoxins in cereal crops in fields and later during their storage presents risk to human and animal health. Hamilton (1984) pointed to the fact that any level of mycotoxins increased risk of economic losses and that it was not possible to determine safe levels of mycotoxins under field conditions.

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Fuzariotoksini u zrnu pšenice u Srbiji

REZIME

Uzorci zrna pšenice (41), sakupljeni tokom žetve 2010. godine iz 7 različitih lokaliteta na teritoriji Srbije, analizirani su na prisustvo ZEA, T-2 toksina, deoksinivalenola (DON) i FB_1. Primenom ELISA metode utvrđeno je da su svi analizirani uzorci bili pozitivni na prisustvo bar jednog od četiri ispitivana fuzariotoksina. Najzastupljeniji mikotoksini su bili ZEA (90,2%, prosečne koncentracije 442,6 μg kg^{-1}) i T-2 (90,2%, prosečna koncentracija 24,2 kg^{-1}). U nešto manjem broju uzoraka utvrđeni su DON (73,2%) i FB_1 (84,4%), ali u većoj prosečnoj koncentraciji (1988,1 μg DON kg^{-1} i 882,7 μg FB_1 kg^{-1}). Nije utvrđena statistički značajna korelacija između koncentracije DON i FB_1 (r = 0,32) ili DON i ZEA (r = 0,22). Negativna korelacija je utvrđena između koncentracije T-2 i FB_1 (r = -0,24), kao i između T-2 i DON (r = -0,36). Detektovane koncentracije ZEA i T-2 bile su niže od nivoa koji propisuje Svetska zdravstvena organizacija, dok su FB_1 i DON detektovani kod 5, odnosno 17 uzoraka u koncentraciji većoj od dozvoljenih u ljudskoj hrani.

Ključne reči: Pšenica; zearalenon; T-2 toksin; deoksinivalenol; fumonizin B_1