Зборник Матице српске за природне науке / Proc. Nat. Sci, Matica Srpska Novi Sad, № 120, 49—59, 2011

UDC 615.918:582.281/.282
DOI: 10.2298/ZMSPN1120049J

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FUMONISINS AND CO-OCCURRING MYCOTOXINS IN NORTH SERBIAN CORN

ABSTRACT: The presence of fumonisin has not been regulated in the legislation of the Republic of Serbia. Therefore, the data on contamination of cereals, especially corn, which is highly susceptible to contamination by this toxin, are not sufficient. This paper presents the results of testing the corn samples collected in the autumn 2009 on the territory of Bačka. Samples were analyzed for the contents of fumonisins and it was determined whether there is a correlation between the moisture content, total number and class of fungi, as well as the content of aflatoxin, ochratoxin and zearalenone. Using enzymatic immunoaffinity method it was discovered that the highest percentage of samples were contaminated with fumonisins, which was probably due to the presence of *Fusarium* molds as the most abundant ones. The positive samples contained fumonisin in the concentrations from 0.030 to 1.52 mg kg^{-1}. The influence of the climate and moisture content of grain on fungal contamination and mycotoxin production was analyzed in order to investigate the predictability of the presence of mycotoxins.

KEY WORDS: corn, ELISA, fumonisins, fungi, mycotoxins

INTRODUCTION

Fumonisins, secondary metabolites of fungi from the genus *Fusarium,* are mycotoxins that are most frequently found worldwide as natural contaminants in corn and corn-based products (WHO, 2000). They are important for both human and animal health. Experiments confirmed fumonisins to be causative agents of equine leukoencephalomalacia, porcine pulmonary oedema syndrome and producers of liver cancer in rats (WHO, 2000). In addition, oesophageal cancer in humans has been observed in distinct areas of the world, i.e. Southern Africa (Sydenham et al., 1990), Northern China (Chu and Li, 1994) and Northern Italy (Franceschi et al., 1990), where extremely high levels of fumonisins occur in corn and corn-based products. International

Agency for Research on Cancer (IARC) has classified *Fusarium moniliforme* toxins as possibly carcinogenic to humans (Group 2B carcinogens), similar to ochratoxin A (IARC, 2002).

Due to especially favorable climatic conditions and fertile soil in Serbia, especially in Vojvodina, its northern part, the most extensively grown crop is corn, which is mainly used as livestock feed. According to the data from the years 2005–2009, corn was planted on about 665.000 ha of arable land, with a total yield of about 3.7 million metric tons, indicating that corn growers are mainly individual agricultural producers (Statistical Office of the Republic of Serbia, 2010). Apart from somewhat lower yields, these producers face the problems concerning appropriate drying and storage, i.e. preservation of harvested corn quality, which raises questions about microbiological and mycotoxycological food and feed integrity. Namely, the data on mycologycal contamination of corn (Djilas et al., 2001; Lević et al., 2009) indicate a realistic possibility for mycotoxin production.

By studying the influence of abiotic factors (temperature, moisture content, water activity, and relative humidity) on the microflora and content of fumonisins in freshly harvested and stored corn, Orsi et al. (2000) concluded that there is a negative correlation between the presence of the genus *Fusarium* and mean temperature and air humidity, and a positive correlation between moisture content. Campa et al. (2005), by studying the effects of different factors, found that the production of fumonisins is most dependent on the location (i.e. weather conditions), insects, and finally on the hybrid type. Based on this, they developed a model that can predict fumonisin concentration using the variables such as weather conditions (daily precipitation, minimum and maximum daily temperature, relative humidity) and damages done by the insects. The weather conditions two weeks before and three weeks after the corn silking were found to be critical for the production of fumonisins.

Humidity level during the harvest period and prior to drying is important for the control of the mold growth and fumonisin production, and the data on the grain microflora may point out to the danger of mycotoxin presence. If the corn is not dried to contain less than 14% of moisture, there is a possibility of toxin production during the storage, too (Ono et al., 2002).

Out of more than 300 currently known mycotoxins, the highest effect on the health of humans and animals, apart from fumonisin, have aflatoxins, ochratoxin A, zearalenone and trichothecenes (Binder, 2007). Studies have shown that various mycotoxins present in combination have more severe effects than individual mycotoxins (Kubena et al., 1997).

The objective of this work was to investigate the contamination of corn from the area of northern Serbia with fumonisins, other mycotoxins and molds, as well as the possible influence of climatic factors on the degree of corn contamination.

MATERIALS AND METHODS

Corn samples were collected in the autumn of 2009 from different locations in Bačka. Sampling was conducted in the fields after the harvest, and immediately after, the samples were stored in the silos. After collecting, the samples were analyzed for total count of molds and their prevailing genera were determined. To this end, 1000 g of each sample were homogenized and prepared by grinding in a laboratory mill in such a way that > 93% passed through a sieve with pores 0.8 mm in diameter. After the analysis of the moisture content, samples were stored in a freezer at −20 °C for the analysis on mycotoxins. Prior to each analysis, the samples were allowed to reach room temperature.

Total counts of molds and moisture contents were determined by standard methods (The Official Gazette of SFRY, 25/80, The Official Gazette of SFRY, 15/87).

Contents of total aflatoxins, ochratoxin A, zearalenone and total fumonisins were determined by the enzymatic immunoaffinity (ELISA) method, using *Ridascreen*® test kits (Art. No. R:4701; R:1311; R:1401; R:3401, R-Biopharm, Germany), with limits of detection of 1 μg kg^{-1} for ochratoxin A, 1.75 μg kg^{-1} for aflatoxins and zearalenone, and 0.025 mg kg^{-1} for fumonisins.

The results of mycological and mycotoxicological analyses were subjected to multiple regression analysis using the software package Statistica, version 9.1 (StatSoft, Inc., 2010, www.statsoft.com).

RESULTS AND DISSCUSION

Bearing in mind the literature data on the effect of grain moisture on the mold growth and toxin production, the investigated samples were divided into two groups: in one group moisture content of the samples was ≤ 14%, and in the other it was > 14%; the results of mycological and mycotoxicological analyses were presented accordingly (Tables 1 and 2). It was found that the samples with higher moisture content were significantly more contaminated with molds (85.7%), when compared to the group of samples with lower humidity (65%), and a higher percentage of samples from the former group was contaminated with *Fusarium* spp. They were also characterized by higher frequency of positive samples and higher mean values of the content of total aflatoxins and ochratoxin A. This group of samples had also a somewhat higher mean value of the fumonisin content, although the percentage of positive samples and concentration range were the same or similar.

If all the samples are analyzed together (Table 3), although the contamination with molds (> 1000) was observed for a high percentage of samples (73.5%), only three samples had a total count of molds that exceeded the maximum allowed count predicted by the current regulations (The Official Gazette, 2010). The presence of molds of the genus *Fusarium* was confirmed in 64.7% of the samples. It can be concluded that *Fusarium* molds and

Tab. 1 – Results of mycological and mycotoxicological analyses of corn samples with moisture content ≤ 14%

LOCATION	Moisture content (%)	CFU (x1000/g)	Genus of fungi	Total Aflatoxins (ppb)	Zea-ralenone (ppb)	Total fumoni-sins (ppm)
Veternik	11.19	30	Ab	2.25	–	0.036
Čonoplja	11.77	20	Fu	–	–	0.057
Silo 3	11.98	10	Fu	–	–	0.098
Silo 1	12.03	10	Mu	–	2.43	–
Silo 4	12.27	100	Fu	–	3.02	–
Silo 6	12.34	–	–	–	3.32	0.262
Feketić	12.35	160	Fu	4.33	–	–
Silo 7	12.56	–	–	–	1.81	0.465
Silo 5	12.60	230	Fu	–	2.92	0.496
Lalić	12.81	80	Fu	–	2.24	–
Ruski Krstur	12.86	20	Fu	1.98	–	–
Silo 2	13.02	300	Ab, Fu	–	2.94	1.52
Mali Beograd	13.33	–	–	3.04	–	0.04
Savino Selo	13.40	–	–	–	–	–
Zmajevo	13.49	–	–	2.98	–	–
Lipar	13.47	–	–	–	–	–
Stepanovićevo	13.65	8	Fu, Ab	2.08	–	0.044
Temerin	13.71	220	Ab, Fu	–	–	0.143
Ravno Selo	13.71	–	–	–	–	–
Bačka Topola	13.91	10	Mu	2.58	–	–
Average	12.82	59.9		0.96	0.93	0.158
RSD	0.76	92.8		1.42	1.34	0.354
Average of positive samples		92.2		2.75	2.67	0.316
RSD of positive samples		102.0		0.81	0.53	0.457
% of positive samples		65.0	50 (on Fu)	35.0	35.0	50.0
Range of con-tamination	11.19–13.91	8–300		1.98–4.33	1.81–3.32	0.036–1.52

– not detected, Pe – *Penicillium* spp., Mu – *Mucor* spp., Fu – *Fusarium* spp., Ab – *Absidia* spp., CFU – total fungal colony count

fumonisin are present the most, but there is no significant correlation between the count of molds and contents of their toxins (Table 4), which is in agreement with the findings of M n g a d i et al. (2008). Contamination with toxins was also found in some samples with less than 1000 mold colonies. This can be explained by the fact that mycotoxins are stable compounds which can persist under the conditions that eliminate the molds that produce them (A l d r e d et al., 2004). The survival of molds depends also on the microbiological interaction and competition, that is, it is possible that *Fusarium* spp.

Tab. 2 – Results of mycological and mycotoxicological analyses of corn samples with moisture content > 14%

LOCATION	Moisture content (%)	CFU (x1000/g)	Genus of fungi	Total Aflatoxins (ppb)	Ochratoxin A (ppb)	Zearalenone (ppb)	Total fumonisins (ppm)
Sombor	14.29	15	Fu, Ab	2.34	–	–	0.229
Rumenka	14.40	50	Fu, Ab	4.23	1.07	–	0.43
Svetozar Miletić	14.55	–		–	–	–	–
Sivac	14.23	8	Fu	–	–	–	0.257
Odžaci	15.09	25	Fu, Ab	3.93	–	–	–
Kisač	15.25	625	Ab, Pe, Fu	3.09		1.79	–
Vrbas	15.28	3	Fu	–	–	3.39	0.030
Bački Brestovac	15.68	70	Ab, Fu	–	–	–	–
Futog 1	16.54	10	Fu, Ab	–	–	–	0.036
Crvenka	16.61	–		2.34	–	–	–
Kucura	16.99	15	Fu	7.01	1.26	–	–
Kula	17.29	10	Fu	2.91	1.07	–	–
Despotovo	18.03	20	Fu, Pe, Ab	–	–	–	1.44
Futog 2	19.21	10	Fu, Ab, Pe	–	–	–	0.396
Average	15.96	61.5		1.85	0.24	0.37	0.201
RSD	1.53	163.4		2.21	0.48	0.99	0.389
Average of positive samples		71.7		3.69	1.13	2.59	0.403
RSD of positive samples		178.9		1.65	0.11	1.13	0.502
% of positive samples		85.7	78.6 (on Fu)	50.0	21.4	14.3	50.0
Range of contamination		3–625		2.34–7.01	1.07–1.26	1.79–3.39	0.030–1.44

– not detected, Pe – *Penicillium* spp., Mu – *Mucor* spp., Fu – *Fusarium* spp., Ab – *Absidia* spp. CFU – total fungal colony count.

under the conditions of lower humidity inhibit the growth of *Aspergillus* spp. (O n o et al., 2002), which have not been isolated.

Statistical analysis showed a significant correlation ($p<0.05$) between the moisture content and contamination with *Penicillium* spp. (0.55), *Absidia* sp (0.35), as well as between the moisture content and content of ochratoxin (0.35). Also, a significant coefficient of correlation (0.61) was found between the contamination with ochratoxin and aflatoxins.

In 50% of all analyzed samples, the content of fumonisins was above the limit of detection of the applied method. The mean value of fumonisin content in them was 0.352 ppm (range 0.030–1.52 ppm). Although fumonisins are present in the corn from Serbia, the total fumonisins content is below the values set by the EU regulations for the nutrition of humans (4 ppm, E C , 2007)

Tab. 3 – Average results of mycological and mycotoxicological analyses of corn samples

	Moisture content (%)	CFU (x1000/g)	Genus of fungi	Total Aflatoxins (ppb)	Ochratoxin A (ppb)	Zearalenone (ppb)	Total fumonisins (ppm)
Average	14.11	60.6		1.33	0.10	0.70	0.176
RSD	1.93	124.4		1.81	0.33	1.22	0.364
Average of positive samples		82.4		3.22	1.13	2.65	0.35
RSD of positive samples		139.3		1.33	0.11	0.61	0.46
% of positive samples		73.5	64.7 (on Fu)	41.2	8.8	26.5	50
Range of contamination		3–625		1.98–7.01	1.07–1.26	1.79–3.39	0.030–1.52

Fu – *Fusarium* spp., CFU–total fungal colony count

Tab. 4 – Distribution of total fungal content and the range of corn contamination with fumonisins

Total fungal colony count (in 1 g)	No of samples	No of positive samples on fumonisin	Range of fumonisin (ppm)
<1000	9	3	0.040–0.465
1000-200,000	21	11	0.030–1.44
>200,000	4	3	0.143–1.52

and animals (60 ppm, EC, 2006a). The contaminated samples also contained aflatoxins (41.1%), ochratoxin A (8.8%) and zearalenone (26.5%). None of the samples contained significant concentration of the investigated toxins, but chronic effect of their low concentrations should be taken into consideration.

Since fungal formation and toxin production are influenced by environmental factors during pre-harvest and harvest periods, prior to and during the harvest, the obtained results were discussed with regard to these conditions. The conditions of the vegetation season 2009 were typical of the climate in Serbia, which deviated from the average ones mostly on the territory of Vojvodina. Namely, the humidity conditions, apart from the northeast part, have the characteristics of a drought. In the middle of July, the measured temperatures (above 35°C) exceeded the optimum ones, which could have affected the course of the silking stage. The last month of the vegetation season was characterized by hot dry weather (RHZ, 2009). Such dry conditions caused less intensive production of toxins, which favor the conditions with water activity of 0.93 and humidity of even 25% (Sanchis and Magan, 2004). Lower production of fumonisins in drier ripening conditions is in agreement with our findings for the samples collected in 2001 and 2002 (Jakšić, 2004). Low fumonisin content, however, can be explained by the lack of favorable

conditions for the development of fungi due to warm and dry weather during the 2009 harvest, consequently lowering the kernel moisture.

The obtained results were compared with those obtained in Serbia in the previous year by other authors (Kokić et al., 2009; Matić et al., 2009). The results for fumonisin are similar, whereas our study gave higher frequency of samples positive on aflatoxins, as well as on zearalenone and ochratoxin, but at lower concentrations.

The obtained results were compared to those obtained in the neighboring countries – Croatia, Hungary, Romania and Bulgaria. Data from 1992 are similar to those presented in this work, regarding the frequency of positive samples, i.e. 58% for fumonisin B_1 (FB_1) and 21% for fumonisin B_2 (FB_2) in Croatia, along with 50% for FB_1 and 17% for FB_2 in Romania (Doko et al., 1995). The fumonisin content, however, was lower. Namely, the mean values of FB_1 content in the positive samples were 20 ng g^- (range 10–60 ng g^-) in Croatia, and 10 ng g^- (range 10–20 ng g^-) in Romania, while for FB_2 the value was 10 ng g^- in both countries. Conversely, according to the data obtained by Jurjević et al. (1999) and Domijan et al. (2005), the frequency of corn contamination by fumonisins (FB_1+FB_2) in Croatia, from 1996, 1997 and 2002, was significantly higher with values of 99%, 93% and 100%, respectively. The mean values for fumonisin content in the positive samples were 645 ppb of FB_1+FB_2 in the samples from 1996, 134 ppb of FB_1+FB_2 in the samples from 1997, and 459.8 ppb of FB_1, in the samples from 2002 with the concentrations of FB_2 in three positive samples (out of 49) being 68.4, 109.2 and 3084.0 ppb, respectively. The analysis of corn from 2007 (Šegvić Klarić et al., 2008) detected zearalenone in 91.9%, and ochratoxin A in 16.2 % of the samples, with mean concentrations of 318.3 ppb and 9.8 ppb, respectively.

The fumonisin content of corn flour and corn coarse meal samples has been examined in 1997 in Hungary (Fazekas, 2001). Fumonisins were detected in 67% of the samples, although the contamination levels were very low (16−58 ppb).

In Bulgaria, the analysis of corn samples on fumonisins and zearalenone under the conditions favorable for *Fusarium* molds (Manova and Mladenova, 2009) showed that only 21% of the samples were positive on zearalenone and 94.7% on fumonisin, in the range of 249−4050 ppb, with mean value being 1150 ppb, whereas according to the data from 2001 even 50% of samples contained fumonisin at a level of 0.03−6.56 ppm.

The importance of moisture content for the mold growth and fumonisin production between the harvest and drying phase was also emphasized by Ono et al. (2002). Drying immediately after the harvest and appropriate storage conditions can minimize toxin production. The results obtained by examining the corn from silos indicated the possibility of toxin production in Silo 2 because of the significant contamination, probably due to toxigenic molds, considering the already produced amount of fumonisins.

Contents of the investigated mycotoxins in 5 out of 34 samples were below the detection limit of the applied method, and 13 out of 29 positive samples contained more than one toxin. Content of aflatoxin did not exceed the

maximum tolerable level (The Official Gazette of RS, 2010; EC 2003, EC 2006b), whereas contents of other toxins were below the values prescribed/recommended by the EU legislation (EC, 2006a, 2007).

CONCLUSION

Based on all the above, it can be concluded that fumonisins are present in corn from Serbia, although in relatively low concentrations. Also, it can be concluded that their presence in corn has been confirmed in recent years and that the concentrations are similar to those found in the neighboring countries, with some deviations due to the differences in climatic conditions. It should be pointed out that such investigations indicate the indispensability of introducing the recommended limits for fumonisin in Serbia. Dry conditions, prior to harvest, and low kernel humidity lead to lower mycological and mycotoxicological contamination of corn. Further investigation should examine the effect of traditional corn storing, practiced by individual growers, on the degree of mycological and mycotoxicological contamination, encompassing trichothecene as well, due to their confirmed presence (Jajić et al., 2008).

ACKNOWLEDGMENTS

This work was financially supported by SEE-ERA.NET PLUS No. 139 "CROSSMICOTOX".

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ПРИСУСТВО ФУМОНИЗИНА И ДРУГИХ МИКОТОКСИНА У КУКУРУЗУ СА ПОДРУЧЈА СЕВЕРНЕ СРБИЈЕ

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Резиме

Фумонизини нису обухваћени законским регулативама Републике Србије, па последично и нема довољно података о контаминацији житарица, а посебно кукуруза овим микотоксинима. У раду су приказани резултати испитивања узорака кукуруза прикупљених у јесен 2009. године са територије Бачке. Анализиран је садржај фумонизина и испитано да ли постоји корелација са садржајем влаге, укупним бројем и родом плесни, као и садржајем афлатоксина, охратоксина и зеараленона. Ензимском имуноафинитетном методом је утврђено да је, у односу на остале одређиване микотоксине, највећи проценат узорака био контаминиран фумонизинима, што је вероватно последица присуства плесни рода *Fusarium* као најзаступљенијих. У контаминираним узорцима је утврђена концентрација фумонизина у интервалу од 0,030 – 1,52 mg/kg. Анализиран је утицај климе и влажности зрна на последичну контаминацију плеснима и концентрацију микотоксина у циљу процене предвидљивости присуства микотоксина.