

Biotechnology in Animal Husbandry 31 (2), p 291-302 , 2015
Publisher: Institute for Animal Husbandry, Belgrade-Zemun

ISSN 1450-9156
UDC 632.4'633.15
DOI: 10.2298/BAH1502291K

MYCOBIOTA AND MYCOTOXINS IN FRESHLY HARVESTED AND STORED MAIZE

V. Krnjaja, M. Lukić, N. Delić, Z. Tomić, V. Mandić, Z. Bijelić, M. Gogić

Institute for Animal Husbandry, Autoput 16, 11080, Belgrade-Zemun, Serbia
Corresponding author: vesnkrnjaja.izs@gmail.com
Original scientific paper

Abstract: The incidence of mycobiota and mycotoxin levels were investigated in the freshly harvested maize kernel samples from October 2014 and in the samples of stored maize kernels from February 2015. Toxigenic fungal species (moulds) were isolated, cultivated and identified on agar plates according to standard mycological methods, while mycotoxins were detected by enzyme-linked immuno-sorbent assay (ELISA).

Mycological analyses of kernels showed the presence of toxigenic species from genera *Aspergillus*, *Fusarium* and *Penicillium*. Among the *Aspergillus* species, *Aspergillus flavus* was identified with higher incidence in the stored kernels (10.25%), than in freshly harvested kernels (3.67%) whereas *A. parasiticus* was the predominant species in the freshly harvested kernels (4.17%) compared to the stored kernels (0%). From the genus *Fusarium* three species were identified: *F. graminearum*, *F. subglutinans* and *F. verticillioides*, with the incidence of 1.08%, 8% and 25.75%, respectively in freshly harvested kernels and the incidence of 2.50%, 7.10% and 29.75%, respectively in the stored kernels. Species from genus *Penicillium* had higher incidence in freshly harvested kernels (14.25%) than in the stored kernels (9%).

In addition, tested samples of harvested and stored maize kernels were 100% positive with aflatoxin B₁ (AFB₁), deoxynivalenol (DON) and total fumonisins B₁, B₂ and B₃ (FBs). The mean levels of AFB₁, DON and FBs were 2.77 µg kg⁻¹, 117.83 µg kg⁻¹, and 3700.84 µg kg⁻¹, respectively in the freshly harvested kernels and a mean levels of 2.16 µg kg⁻¹, 2034.40 µg kg⁻¹, and 5976.50 µg kg⁻¹, respectively in the stored maize kernels.

In the freshly harvested maize kernel samples, statistically significant ($P \leq 0.05$) positive correlations of kernel moisture content with the incidence of *Penicillium* spp. ($r = 0.47$), and levels of AFB₁ ($r = 0.46$) and FBs ($r = 0.47$), and between the incidence of *Penicillium* spp. and level of AFB₁ ($r = 0.53$) were established. In the stored maize kernel samples, statistically significant ($P \leq 0.05$) positive correlations were found between the incidence of *F. subglutinans* and level of FBs ($r = 0.50$) and between levels AFB₁ and FBs ($r = 0.52$). A highly significant

($P \leq 0.01$) positive correlation was established between the incidence of *F. verticillioides* and level of FBs ($r = 0.64$) in freshly harvested maize kernel samples.

These results indicate that the incidence of toxigenic fungi and levels of mycotoxins, in particular DON and FBs, were higher in the stored maize kernel samples than in freshly harvested maize kernels. Therefore, to prevent the development of toxigenic fungi and mycotoxins accumulation in post-harvest period it is necessary to thoroughly dry maize and keep it in hygienic food storages.

Key words: mycobiota, mycotoxins, harvest, storage, maize

Introduction

Maize is cereal crop used for human and animal nutrition. According to data of *Statistical Yearbook of Serbia (2012)*, maize has been grown on about 1.2 million hectares with an average yield of 5.4 t ha^{-1} and with a production of 6.5 million tons in Serbia in 2011.

The contamination of maize with fungi (moulds) and their secondary metabolites (mycotoxins) represents a serious hazard to humans and animals. The most important mycotoxins in maize kernels are aflatoxins produced by *Aspergillus flavus* and *A. parasiticus*. Equally important mycotoxins in maize kernels are fumonisins produced mostly by *Fusarium verticillioides* and *F. proliferatum*, as well as deoxynivalenol produced by *F. graminearum* (Chulze, 2010; Pereyra et al., 2011).

Climatic conditions and maize growing on large areas in Serbia are suitable for development of numerous toxigenic species, resulting with frequent animal feed contamination by toxic products of fungi – mycotoxins. Development of toxigenic fungi and bio-synthesis of mycotoxins most often depend on ample precipitation and low temperatures at the end of summer or beginning of autumn during sensitive phenophase of maize growing (Krnjaja et al., 2009). Mould growth and mycotoxin contamination can occur in the field pre-harvest and later during storage condition in the post-harvest period. The higher moisture and temperature are abiotic factors which positively influence on fungal growth and mycotoxin accumulation (Niaz et al., 2011; Kocasari et al., 2013). Production of farm animals, poultry and swine, in particular, requires a large amount of cereal grain. For this reason, most of the grain is stored until utilized. Storage conditions are determined by abiotic and biotic factors including microorganisms, insects, mites, rodents and birds (Santin et al., 2005).

Consumption of mycotoxin contaminated diet may cause acute and chronic toxicity in humans and animals. Among farm animals, swine are considered to be

the most sensitive species to mycotoxins. Aflatoxin B₁ (AFB₁) is main hepatotoxin that causes various pathological effects on organs and tissues. Deoxynivalenol (DON) causes feed refusal, emesis, anaemia, haemorrhage, immunosuppression and neurotoxic effects. Fumonisin B₁, B₂ and B₃ (FBs) cause leukoencephalomalacia and porcine pulmonary edema (Biagi, 2009; Berardo, 2011; Pereyra et al., 2011).

Unfortunately, there are no direct measures for prevention of fungal infection and mycotoxin contamination. However, unfavourable conditions for development of toxigenic fungi could be provided by implementation of appropriate agricultural practices as preventive measures in the field. Pre-harvesting control strategies primarily consist of crop practices designed to reduce the fungal development and mycotoxin accumulation and the utilization of genetically resistant hybrids (Blandino et al., 2008). Post-harvest control strategy includes the practices directed at reducing mycotoxin levels, mycotoxin risk assessment in crop products and controlling its use through regulation. Monitoring of fungal and mycotoxin contamination in crops and products can be implemented in pre-harvest and post-harvest period (Dohlman, 2003). Reduction moisture in grains to moisture level of 13% or below is also very important post-harvest control measure (Lutfy et al., 2008).

The aim of this research was to determine the incidence of toxigenic fungal species, levels of mycotoxins (AFB₁, DON and FBs) and to establish the signification of correlation coefficients between investigated variables in freshly harvested and stored maize kernel samples, used for animal feed.

Material and Methods

During the harvest time in October of 2014 and storage time in February of 2015, 20 and 20 maize kernel samples intended for farm animals feeding (pigs, sheep and poultry) were randomly collected from parcels for agricultural production and warehouse with natural-air-drying conditions in the Institute for Animal Husbandry, Belgrade. The samples were collected according to the Commission Regulation (EC) No 401/2006 (European Commission, 2006). All samples were kept at 4°C in the refrigerator before further analysis. Moisture content of milled maize kernels was determined using a moisture analyzer (OHAUS MB35, USA).

For the mycological analysis, maize kernels were disinfected in 1% sodium hypochlorite (NaOCl) for 2-3 minutes, and rinsed twice in distilled water. A total of 50 maize kernels per each sample were distributed in Petri dishes (5 kernels per Petri dish) with water-1,8% salt agar (18g NaCl per 1 litre of agar medium) (Krnjaja et al., 2015). Plates were incubated for 14 days at 20°C with alternating light and darkness. Potential toxigenic fungi were identified according to the fungal

key of Singh *et al.* (1991). The incidence of potential toxigenic fungal species was calculated according to Lević *et al.* (2012).

For the mycotoxicological analysis, the maize kernel samples were ground to a fine powder with an analytical mill (IKA A11, Germany). The levels of AFB₁, DON and FBs were detected using the competitive ELISA method according to the manufacturer's instructions Celer Tecna® ELISA kits. Absorbance was determined at a wavelength of 450 nm on an ELISA plate reader spectrophotometer (Biotek EL x 800TM, USA). The limit of detection for AFB₁, DON and FBs were 1 µg kg⁻¹, 40 µg kg⁻¹ and 750 µg kg⁻¹, respectively.

The correlation between individual values for moisture content of maize kernels, the incidence of toxigenic fungal species and the levels of AFB₁, DON and FBs was determined using the Pearson correlation coefficient.

Results

The average moisture content for freshly harvested maize kernel samples was 15.20% (range 14.28 – 16.05%) and for stored maize kernel samples was 14.04% (range 13.41 – 14.92%).

In mycological analyses, toxigenic fungi from genera *Aspergillus*, *Fusarium* and *Penicillium* were identified. Fungal species identified in both, freshly harvested and stored maize kernels, were *A. flavus*, *F. graminearum*, *F. subglutinans*, *F. verticillioides* and *Penicillium* spp., with the exception of the species *A. parasiticus* which was identified only in the freshly harvested maize kernels. The most frequent fungal species was *F. verticillioides*, with an average incidence of 25.75% (range 10 - 43%) in freshly harvested and 29.75% (range 17 – 50%) in stored maize kernels. Considering the average values, the incidence of *A. flavus* was higher in stored (10.25%) than in freshly harvested maize kernels (3.67%). The species *A. parasiticus* was identified only in freshly harvested kernels with presence of 4.17% on average. The presence of *F. graminearum* was higher in stored (on average 2.50%) than in freshly harvested kernels (on average 1.08%), while the incidence of *F. subglutinans* and *Penicillium* spp. was higher in freshly harvested (on average 8% and 14.25% respectively) than in stored maize kernels (on average 7.10% and 9% respectively) (Table 1).

In the mycotoxicological analyses, freshly harvested and stored maize kernels samples were 100% positive for the presence of all tested mycotoxins. In the freshly harvested samples the mean levels of AFB₁, DON and FBs were 2.77 µg kg⁻¹ (range 2.31 – 3.34 µg kg⁻¹), 117.83 µg kg⁻¹ (range 42 – 238 µg kg⁻¹) and 3700.84 µg kg⁻¹ (range 1519 – 9780 µg kg⁻¹), respectively (Table 2). In the stored maize samples the mean levels of AFB₁, DON and FBs were 2.16 µg kg⁻¹ (range 1.03 – 4.11 µg kg⁻¹), 2034.40 µg kg⁻¹ (range 380 – 10684 µg kg⁻¹) and 5976.50 µg kg⁻¹ (range 760 – 35760 µg kg⁻¹), respectively (Table 3).

Table 1. Incidence of fungal species in freshly harvested and stored maize kernel samples

Fungal species	Freshly harvested kernel		Stored kernel	
	Incidence (%)			
	Average	Range	Average	Range
<i>Aspergillus flavus</i>	3.67	0 - 10	10.25	0 - 28
<i>Aspergillus parasiticus</i>	4.17	0 - 17	0	0
<i>Fusarium graminearum</i>	1.08	0 - 7	2.50	0 - 10
<i>F. subglutinans</i>	8	0 - 20	7.10	0 - 20
<i>F. verticillioides</i>	25.75	10 - 43	29.75	17 - 50
<i>Penicillium</i> spp.	14.25	0 - 27	9	0 - 27

Table 2. Levels of aflatoxin B₁ (AFB₁), deoxynivalenol (DON) and total fumonisins (FBs) in freshly harvested maize kernel samples

Item	Freshly harvested kernel		
	AFB ₁	DON	FBs
Sample size ^a	20/20	20/20	20/20
Incidence (%)	100	100	100
Range (µg kg ⁻¹)	2.31 – 3.34	42 - 238	1519 - 9780
Mean ^b (µg kg ⁻¹)	2.77	117.83	3700.84

^a Number of positive samples/Number of total samples

^b Mean level in positive samples

Table 3. Levels of aflatoxin B₁ (AFB₁), deoxynivalenol (DON) and total fumonisins (FBs) in stored maize kernel samples

Item	Stored kernel		
	AFB ₁	DON	FBs
Sample size ^a	20/20	20/20	20/20
Incidence (%)	100	100	100
Range (µg kg ⁻¹)	1.03 – 4.11	380 - 10684	760 - 35760
Mean ^b (µg kg ⁻¹)	2.16	2034.40	5976.50

^a Number of positive samples/Number of total samples

^b Mean level in positive samples

Statistical analyses and Pearson's correlation coefficient, established highly significant positive correlation ($P \leq 0.01$) between the incidence of *F. verticillioides* and level of FBs ($r = 0.64$) in the freshly harvested kernel maize samples. There was also significant positive correlation ($P \leq 0.05$) of kernel moisture content with levels of AFB₁ ($r = 0.46$) and FBs ($r = 0.47$) and between the incidence of *Penicillium* spp. and level of AFB₁ ($r = 0.53$). Likewise, the positive correlation, but not statistically significant, was found between kernel moisture content and level of DON ($r = 0.27$), then between levels of AFB₁ and FBs ($r = 0.31$), between the incidence of *F. subglutinans* and level of FBs ($r = 0.30$), and between the incidence of *A. flavus* and AFB₁ ($r = 0.20$). It was only established the

statistically insignificant negative correlation between the incidence of *A. parasiticus* and level of AFB₁ ($r = -0.06$) in the freshly harvested maize kernels. Considering correlation coefficients in the stored maize kernel samples, statistically significant positive correlation ($P \leq 0.05$) was also found between levels of AFB₁ and FBs ($r = 0.52$), then between the incidence of *F. subglutinans* and level of FBs ($r = 0.50$) and between the incidence of *A. flavus* and AFB₁ ($r = 0.34$). There were also positive correlations but not significant between the incidence of *F. verticillioides* and level of FBs ($r = 0.31$), then positive correlation of kernel moisture content with levels of FBs ($r = 0.26$), AFB₁ ($r = 0.17$) and DON ($r = 0.03$). The negative, but not significant, correlation was found only between the incidence of *F. graminearum* and level of DON ($r = -0.30$) in the stored maize kernel samples (data not presented).

Discussion

In this study the incidence of mycobiota and levels of mycotoxins in the samples of freshly harvested and stored maize kernels were researched. The contamination of cereals with toxigenic fungi under favourable climatic and storage conditions may lead to mycotoxin accumulation to injurious levels for farm animals and human health. The production of mycotoxins is consequence of increased presence of toxigenic fungi. Therefore, determination of fungal species at the right time is very important step to reduce the detrimental effects of mycotoxin problems.

All tested samples of freshly harvested and stored maize kernels were positive for the presence of mycotoxins AFB₁, DON and FBs. In samples of freshly harvested maize kernels, mean levels of mycotoxins did not exceed the maximum permitted limits for unprocessed maize prescribed by the regulations of the Republic of Serbia (*Sl. glasnik*, 2014), but in some samples FBs level was above the maximum permitted limit ($4000 \mu\text{g kg}^{-1}$), with a maximum level of $9780 \mu\text{g kg}^{-1}$ (Table 2). In samples of stored maize kernels, mean level of DON was above the maximum permitted limit ($1750 \mu\text{g kg}^{-1}$) with a maximum level of $10684 \mu\text{g kg}^{-1}$, as well as certain samples with FBs levels above the maximum permitted limit – maximum level of $35760 \mu\text{g kg}^{-1}$ (Table 3). The incidence of DON producer, *F. graminearum*, and FBs producers, *F. subglutinans* and *F. verticillioides*, was higher in the stored than in the freshly harvested maize kernels (Table 1). These results can be explained by the favourable climate conditions during the maize harvest in 2014 and during the winter period in 2015. Namely, according to data of the Republic Hydro-meteorological Service of Serbia for 2014, heavy total rainfall was recorded in September (126 mm) with a mean daily temperature of 18.3°C before maize harvest. For this reason the average moisture content of harvested maize kernels was high ($> 15\%$). After harvest, the maize was stored in a warehouse and naturally dried without conditions control (temperature and

humidity). Mild winter during 2015 and uncontrolled conditions of temperature and relative humidity in the warehouse caused the intensive development of moulds and increased mycotoxins content, especially of DON in samples of stored kernels.

Similarly to this, in earlier mycotoxicological studies in Serbia, *Krnjaja et al. (2013a)* have found in maize kernels harvested in 2012 the mean level of AFB₁ in 100% of tested samples exceeding the maximum permitted limit (5 µg kg⁻¹; *Sl. glasnik, 2014*) in unprocessed maize, with incidence of *A. flavus* of 36.69%. These authors also concluded that the mean levels of DON and FBs have not exceeded the maximum permitted limits. It has been assumed that the drought in 2012 had also been the reason for the occurrence of aflatoxigenic fungi and high level of AFB₁ up to >40 µg kg⁻¹. In addition, in the stored maize samples collected from October 2011 to October 2012 with an average kernel moisture content of 11.02% *Krnjaja et al. (2013b)* have detected AFB₁, DON and FBs with the mean levels of 1.39 µg kg⁻¹, 128.17 µg kg⁻¹ and 1610.83 µg kg⁻¹, respectively. Compared to year 2014, in 2011 less rainfall was recorded before the maize harvest in September (47.7 mm) with a mean daily temperature of 17.2°C. Such climatic conditions before harvesting and especially kernel moisture content during the period of maize storage were not suitable for intensive development of toxigenic fungi and thus for the increased production of mycotoxins.

In total 26 maize samples from Turkey (n=19) and USA (n=7) collected between April 2002 and 2003, the mean aflatoxin and fumonisin levels were higher in samples from Turkey (10.94 µg kg⁻¹ and 88240 µg kg⁻¹, respectively) than in samples from USA (0.78 µg kg⁻¹ and 74150 µg kg⁻¹, respectively) (*Oruc et al., 2006*). By examining a total of 82 consignments of French and Argentinean maize as raw, imported in the United Kingdom between 2004 and 2007, *Scudamore and Patel (2009)* have detected the maximum level of 444 µg kg⁻¹ for DON and 5002 µg kg⁻¹ for FBs. These authors have found clear differences in the levels of mycotoxins between harvests and geographic regions. Maize from Argentina contained lower levels of DON and higher levels of FBs than maize from France, although the level of FBs up to 2000 µg kg⁻¹ or more were present in samples taken from both regions. Likewise, levels of fumonisins were higher in 2004 in Argentina and in 2006 in France due to exceptionally hot and dry summer and dry period before harvest. In the USA, *Dowd and Johnson (2010)* over a 4-year period (2005-2008) conducted mycotoxicological studies of popcorn samples and have rarely detected AFB₁, but FBs and DON were present in all years, with mean levels in fields up to 1700 µg kg⁻¹ (sample max. 2770 µg kg⁻¹) and 1900 µg kg⁻¹ (sample max. 2660 µg kg⁻¹). These authors have concluded that the damage from insects is the main cause of higher levels of FBs in relation to the DON levels, while higher levels of DON are caused by higher rainfall and lower temperatures during the maize ripening. In mycotoxicological analysis of 2258 maize samples collected over a 3-year period (2006-2008) from 93 storage centres in Italy, *Berardo et al.*

(2011) have found a high level of FBs, with the highest mean level in 2006 (10900 $\mu\text{g kg}^{-1}$) and lowest in 2008 (4800 $\mu\text{g kg}^{-1}$). These authors have assumed that climatic factors in conjunction with the specific growing area, played an important role in the accumulation of FBs in maize. In maize samples collected from July through August 2011, from 15 swine farms in the Beijing region in China, *Li et al.* (2014) have reported the natural occurrence of AFB₁ and DON with the highest levels of 58.9 $\mu\text{g kg}^{-1}$ (on average 6 $\mu\text{g kg}^{-1}$) and 2130 $\mu\text{g kg}^{-1}$ (on average 1091 $\mu\text{g kg}^{-1}$), respectively. Similar to the above mentioned data our results confirm high maximum levels of FBs in both harvested and stored maize kernels and high maximum level of DON in stored maize kernels.

After examining the correlation coefficients, in most variables from both tested groups in this research, harvested and stored maize samples, positive correlations were found, particularly emphasizing the positive correlation between the incidence of *F. verticillioides* and *F. subglutinans* with FBs level, and the incidence of *A. flavus* and AFB₁ level, then positive correlation between AFB₁ and FBs levels and between kernel moisture content and DON level. These results are similar to studies of *Kimanya et al.* (2008), *Sun et al.* (2011), *Berardo et al.* (2011) and *Krnjaja et al.* (2013a).

In this study, in tested harvested and stored maize kernel samples, the presence of potentially toxigenic fungi and mycotoxins AFB₁, DON and FBs was recorded. Because of favourable conditions for the growth and development of toxigenic fungi and mycotoxin contamination of maize kernels, it is of outmost importance to implement the preventive measures to reduce the risk of these contaminants in Serbia, especially in years when weather conditions are suitable for their development. Preventive measures, such as fast drying of maize for the medium- and long-term storage in hygiene maintained warehouses, without the presence of insects and microorganisms, and proper regulation of the moisture content of kernels, could significantly reduce the mycotoxins contamination of maize grains (*Bruns, 2003*). Good ventilation of warehouse has also been one of the most important preventive measures for the reduction of mycotoxins production (*Jakic-Dimic et al., 2011*).

Conclusion

Fungal and mycotoxin contamination of maize has been increasing worldwide, as a result of climate change. Some mycotoxins could be synthesized in maize before harvest but their level may increase after harvest during the storage period and further in the food chain.

Based on the obtained results it can be concluded that the potentially toxigenic species of fungi from the genera *Aspergillus*, *Fusarium* and *Penicillium* were present in the harvested and stored maize kernel samples. Species of the genera *Aspergillus* and *Fusarium* had higher incidence in stored samples compared

to freshly harvested maize. Also, in both groups of maize samples, AFB₁, DON and FBs were detected, but in the samples of stored maize kernels, the mean levels of DON and FBs exceeded the maximum permitted levels for unprocessed maize as stipulated by Serbian Regulation. For this reason, constant supervision and monitoring of mycotoxins occurrence in maize pre- and post-harvest and application of preventive measures are very important to reduce risks to human and animal health.

Acknowledgment

This research was funded by the Ministry of Education, Science and Technological Development, Republic of Serbia within projects TR-31023, TR-31033, TR-31053 and TR-31081.

Mikrobiota i mikotoksini u sveže požnjevenom i uskladištenom zrnu kukuruza

V. Krnjaja, M. Lukić, N. Delić, Z. Tomić, V. Mandić, Z. Bijelić, M. Gogić

Rezime

U radu je proučavana učestalost mikrobiota i sadržaj mikotoksina u uzorcima zrna kukuruza sakupljenih tokom berbe u oktobru 2014. godine i u uzorcima uskladištenog zrna sakupljenih u februaru 2015. godine. Toksigene vrste gljiva (plesni) su izolovane, odgajene i identifikovane na hranljivoj podlozi prema standardnim mikološkim metodama, dok je sadržaj mikotoksina detektovan primenom imunoadsorpcione enzimske metode (ELISA).

Mikološkim analizama zrna kukuruza ustanovljeno je prisustvo toksigenih vrsta iz rodova *Aspergillus*, *Fusarium* and *Penicillium*. Među *Aspergillus* vrstama, *Aspergillus flavus* je identifikovana u većem procentu u uzorcima uskladištenog zrna (10,25%) nego u uzorcima sveže požnjevenog zrna (3,67%), a *A. parasiticus* bila je dominantna vrsta (4,17%) u uzorcima sveže požnjevenog u odnosu na uskladišteno zrno kukuruza (0%). Tri vrste roda *Fusarium* su identifikovane sa učestalošću od 1,08% (*F. graminearum*), 8% (*F. subglutinans*) i 25,75% (*F. verticillioides*) u požnjevenom zrnu, i sa učestalošću od 2,50% (*F. graminearum*), 7,10% (*F. subglutinans*) i 29,75% (*F. verticillioides*) u uskladištenom zrnu kukuruza. Vrste iz roda *Penicillium* imale su veću učestalost u uzorcima požnjevenog (14,25%) nego u uzorcima uskladištenog zrna (9%).

Ispitivani uzorci sveže požnjevenog i uskladištenog zrna bili su 100% pozitivni sa aflatoksinom B₁ (AFB₁), deoksinivalenolom (DON) i ukupnim

fumonizinima FB₁, FB₂ i FB₃ (FBs). Prosečne koncentracije ovih toksina su iznosile 2,77 µg kg⁻¹ (AFB₁), 117,83 µg kg⁻¹ (DON) i 3700,84 µg kg⁻¹ (FBs) u uzorcima sveže požnjevenog zrna i 2,16 µg kg⁻¹ (AFB₁), 2034,40 µg kg⁻¹ (DON), i 5976,50 µg kg⁻¹(FBs) u uzorcima uskladištenog zrna.

Statistički značajne ($P \leq 0.05$) pozitivne korelacije ustanovljene su između sadržaja vlage zrna sa učestalošću *Penicillium* spp. ($r = 0,47$) i koncentracijama AFB₁ ($r = 0,46$) i FBs ($r = 0,47$), kao i između učestalosti *Penicillium* spp. i koncentracije AFB₁ ($r = 0,53$). U uzorcima uskladištenog zrna, statistički značajne ($P \leq 0.05$) pozitivne korelacije ustanovljene su između učestalosti *F. subglutinans* i koncentracije FBs ($r = 0,50$) i između koncentracija AFB₁ i FBs ($r = 0,52$). Statistički veoma značajna ($P \leq 0.01$) pozitivna korelacija ustanovljena je između učestalosti *F. verticillioides* i koncentracije FBs ($r = 0,64$) u uzorcima sveže požnjevenog zrna kukuruza.

Rezultati ovih istraživanja ukazuju da su učestalosti toksigenih vrsta gljiva i koncentracije mikotoksina, posebno DON i FBs, bile više u uzorcima uskladištenog zrna nego u uzorcima sveže požnjevenog zrna. Zbog toga, da bi se sprečio razvoj toksigenih gljiva i akumulacija mikotoksina u postžetvenom periodu neophodno je kukuruz dobro osušiti i čuvati u higijensko ispravnim skladištima.

References

- BERARDO N., LANZANOVA C., LOCATELLI S., LAGANÁ P., VERDERIO A., MOTTO M. (2011): Levels of total fumonisins in maize samples from Italy during 2006-2008. *Food Additives and Contaminants: Part B*, 4, 2, 116-124.
- BIAGI G. (2009): Dietary supplements for the reduction of mycotoxin intestinal absorption in pigs. *Biotechnology in Animal Husbandry*, 25, 5-6, 539-546.
- BRUNS H.A. (2003): Controlling aflatoxin and fumonisin in maize by crop management. *Journal of Toxicology Toxin Reviews*, 22, 2-3, 153-173.
- CHULZE S.N. (2010): Strategies to reduce mycotoxin levels in maize during storage: a review. *Food Additives and Contaminants*, 27, 5, 651-657.
- DOHLMAN E. (2003): "Mycotoxin Hazards and Regulations: Impacts on Food and Animal Feed Crop Trade," chapter 6 in *International Trade and Food Safety: Economic Theory and Case Studies*. J. Buzby (ed.) US Department of Agriculture, Economic Research Service, AER-828, Nov. 2003.
- DOWD P.F., JOHNSON E.T. (2010): Field incidence of mycotoxins in commercial popcorn and potential environmental influences. *Mycotoxin Research*, 26, 15-22.
- JAKIĆ-DIMIĆ D., NEŠIĆ K., ŠEFER D. (2011): Mycotoxicoses of poultry caused by trichothecenes. *Biotechnology in Animal Husbandry*, 27, 3, 713-719.
- KIMANYA M.E., MEULENAER B.De., TIISEKWA B., NDOMONDO-SIGONDA M., DEVLIEGHERE F., VAN CAMP J., KOLSTEREN P. (2008): Co-

occurrence of fumonisins with aflatoxins in home-stored maize for human consumption in rural villages of Tanzania. *Food Additives and Contaminants*, 25, 11, 1353-1364.

KRNJAJA V., LEVIĆ J., STANKOVIĆ S. (2009): Ubiquity of toxigenic fungi and mycotoxins in animal feeds in Republic of Serbia. *Biotechnology in Animal Husbandry*, 25, 5-6, Book 1, 477-491.

KRNJAJA V.S., LEVIĆ J.T., STANKOVIĆ S.Ž., PETROVIĆ T.S., LUKIĆ M.D. (2013a): Molds and mycotoxins in freshly harvested maize. *Proc. Nat. Sci. Matica srpska Novi Sad*, 124, 111-119.

KRNJAJA V.S., LEVIĆ J.T., STANKOVIĆ S.Ž., PETROVIĆ T.S., TOMIĆ Z., MANDIĆ V., BIJELIĆ Z. (2013b): Moulds and mycotoxins in stored maize grains. *Biotechnology in Animal Husbandry*, 29, 3, 527-536.

KRNJAJA V., TOMIĆ Z., STANKOVIĆ S., PETROVIĆ T., BIJELIĆ Z., MANDIĆ V., OBRADOVIĆ A. (2015): *Fusarium* infection and deoxynivalenol contamination in winter wheat. *Biotechnol. Anim. Husbandry*, 31, 1, 123-131.

KOCASARI F.S., MOR F., OGUZ M.N., OGUZ F.K. (2013): Occurrence of mycotoxins in feed samples in Burdur Province, Turkey. *Environ. Monit. Assess.*, 185, 4943-4949.

LEVIĆ J., STANKOVIĆ S., KRNJAJA V., BOČAROV-STANČIĆ A., IVANOVIĆ D. (2012): Distribution frequency and incidence of seed-borne pathogens of some cereals and industrial crops in Serbia. *Pesticide and Phytomedicine*, 27, 1, 33-40.

LI X., ZHAO L., FAN Y., JIA Y., SUN L., MA S., JI C., MA Q., ZHANG J. (2014): Occurrence of mycotoxins in feed ingredients and complete feeds obtained from the Beijing region of China. *Journal of Animal Science and Biotechnology*, 5, 37, 3-8.

LUTFY O.F., MOHD-NOOR S.B., ABBAS K.A., MARHABAN M.H. (2008): Some control strategies in agricultural grain driers: A review. *Journal of Food, Agriculture and Environment*, 6, 74-85.

NIAZ I., DAWAR S., SITARA U. (2011): Effect of different moisture and storage temperature on seed borne mycoflora of maize. *Pak. J. Bot.*, 43, 5, 2639-2643.

PEREYRA C.M., CAVAGLIERI L.R., CHIACCHIERA S.M., DALCERO A.M. (2011): Mycobiota and mycotoxins contamination in raw materials and finished feed intended for fattening pigs production in eastern Argentina. *Vet. Res. Commun.*, 35, 367-379.

ORUC H.H., CENGİZ M., KALKANLI O. (2006): Comparison of aflatoxin and fumonisin levels in maize grown in Turkey and imported from the USA. *Animal Feed Science and Technology*, 128, 337-341.

SANTIN E. (2005): Mold growth and mycotoxin production. Pp. 225-234. *In: Diaz, D.E. (eds), The mycotoxin blue book. Nottingham University Press, Nottingham, United Kingdom.*

-
- SCUDAMORE K.A., PATEL S. (2009): Occurrence of *Fusarium* mycotoxins in maize imported into the UK, 2004-2007. *Food Additives and Contaminants*, 26, 3, 363-371.
- SINGH K., FRISVAD J.C., THRANE U., MATHUR S.B. (1991): An Illustrated Manual on Identification of some Seed-borne *Aspergilli*, *Fusaria*, *Penicillia* and their Mycotoxins. 1st Ed., Danish Government Institute of Seed Pathology for Developing Countries, Ryvangs Alle 78 DK 2900 Hellerup, Denmark, pp. 133.
- SLUŽBENI GLASNIK RS (2014): Pravilnik o izmeni Pravilnika o maksimalno dozvoljenim količinama ostataka sredstava za zaštitu bilja u hrani i hrani za životinje i o hrani i hrani za životinje za koju se utvrđuju maksimalno dozvoljene količine ostataka sredstava za zaštitu bilja, br. 37/2014.
- STATISTICAL YEARBOOK OF SERBIA (2012): Statistical Office of the Republic of Serbia, Belgrade, pp. 410.
- SUN G., WANG S., HU X., SU J., ZHANG Y., XIE Y., ZHANG H., TANG L., WANG J.-S. (2011): Co-contamination of aflatoxin B₁ and fumonisin B₁ in food and human dietary exposure in three areas of China. *Food Additives and Contaminants*, 28, 4, 461-470.

Received 7 April 2015; accepted for publication 21 May 2015