Biotechnology in Animal Husbandry 34 (2), p 239-249, 2018 ISSN 1450-9156
Publisher: Institute for Animal Husbandry, Belgrade-Zemun UDC 632.4:633.15
https://doi.org/10.2298/BAH1802239K

TOXIGENIC FUNGAL AND MYCOTOXIN CONTAMINATION OF MAIZE SAMPLES FROM DIFFERENT DISTRICTS IN SERBIA

Vesna Krnjaja[1], Slavica Stanković[2], Miloš Lukić[1], Nenad Mićić[1], Tanja Petrović[3], Zorica Bijelić[1], Violeta Mandić[1]

[1]Institute for Animal Husbandry, Autoput 16, 11080, Belgrade-Zemun, Serbia
[2]Maize Research Institute "Zemun Polje", Slobodana Bajića 1, 11185, Belgrade-Zemun, Serbia
[3]Institute of Food Technology and Biochemistry, Faculty of Agriculture, University of Belgrade, Nemanjina 6, 11080 Belgrade, Serbia
Corresponding author: vesnakrnjaja.izs@gmail.com
Original scientific paper

Abstract: This study was carried out in order to investigate the natural occurrence of toxigenic fungi and levels of zearalenone (ZEA), deoxynivalenol (DON) and aflatoxin B_1 (AFB_1) in the maize stored immediately after harvesting in 2016 and used for animal feed in Serbia. A total of 22 maize samples were collected from four different districts across the country: City of Belgrade (nine samples), Šumadija (eight samples), Podunavlje (four samples) and Kolubara (one sample). Toxigenic fungi were identified according to the morphological characteristics whereas the mycotoxins contamination were detected using biochemistry enzyme-linked immuno-sorbent (ELISA) assay. The tested samples were mostly infected with *Aspergillus, Fusarium* and *Penicillium* spp., except that one sample originated from Kolubara was not contaminated with *Aspergillus* species. *Fusarium graminearum* was the most common species in the maize sample from Kolubara district (60%), *F. verticillioides* in the maize samples from Podunavlje (43.75%) and City of Belgrade (22.4%) districts, and *Penicillium* spp. in the maize samples from Šumadija district (26.38%). In the analysed maize samples the presence of *Aspergillus* species was low (0-1.78%). Mycotoxicological analysis revealed the presence of zearalenone (ZEA), deoxynivalenol (DON) and aflatoxin B_1 (AFB_1) in all the investigated samples, except that DON and AFB_1 were not recorded in the samples from Podunavlje and Kolubara districts, respectively. The investigated samples were highly contaminated with ZEA, with incidence of 100% for the samples from Šumadija, Podunavlje and Kolubara districts and 88.89% for the samples from City of Belgrade district. In addition, the samples contamination with DON was 100% and 22.2% for the samples from Šumadija, Kolubara and City of Belgrade, districts, respectively. The highest number of AFB_1 positive samples was found in Šumadija district (87.5%), while in the City of Belgrade and Podunavlje districts, 55.56% and 50% AFB_1 positive samples were established, respectively. Generally, remarkable infection of all the tested samples with toxigenic fungal species from *Aspergillus*, *Fusarium* and *Penicillium* genera were recorded. In addition, high contamination with mycotoxins ZEA, DON and AFB_1 were also recorded; nevertheless, only in one sample the level of DON exceeded the allowed legal limit (1750 μg kg^{-1}) according to Regulation for unprocessed maize. Therefore, permanent mycological and mycotoxicological analyses of maize grain are necessary for risk assessment of fungal and mycotoxin contamination throughout the food chain.

Key words: maize, toxigenic fungi, mycotoxins

Introduction

Maize as the most important crop in diet for humans and animals is grown on an area of about 1.03 million hectares in Serbia (*Statistical Yearbook of Serbia, 2017*). Maize grain as a rich source of nutrients represents a very good substrate for the development of toxic fungi (moulds) from the genera *Aspergillus*, *Fusarium* and *Penicillium*. These moulds are producers of secondary metabolites (mycotoxins). The most commonly detected mycotoxins in maize grain are fumonisins, deoxynivalenol (DON), zearalenone (ZEA), and aflatoxins (*Covarelli et al., 2011*). Among aflatoxins, aflatoxin B_1 (AFB_1) is a potent hepatotoxin and carcinogen that is a common contaminant of cereals and feeds. Aflatoxin M_1 (AFM_1) is a 4-hydroxylated metabolite of AFB_1, which is excreted into milk through diet for dairy cows and represents a potential human carcinogen (*Britzi et al., 2013*). Zearalenone (ZEA) and deoxynivalenol (DON) are produced mainly by *F. graminearum*, and aflatoxin B_1 (AFB_1) produced by *Aspergillus flavus* and *A. parasiticus (Nuryono et al., 2005; Zain, 2011*). The harmful effects of aflatoxins, ZEA and trichothecenes on human and animal health are globally known (*Khatoon et al., 2012*).

Temperature, humidity and light are the key factors for *Fusarium* infection (*Doohan et al., 2003*), while the main factor for *Aspergillus* infection is the presence of primary inoculum at the time of maize ripening (*Tédihou et al., 2012*). In addition to field grain infestation, the development of these toxic species can be continued even during the storage period. Frequent adverse abiotic (high humidity and temperature) and biotic factors, including microorganisms, insects, mites, rodents and birds, can greatly contribute to increase contamination of maize grain with moulds and mycotoxins during storage conditions (*Santin et al., 2005).*

The occurrence of moulds and their mycotoxins in food is unpredictable and therefore can sometimes lead to adverse effects (mycotoxicoses) when consuming mouldy food (*Nugmanov et al., 2018*). Mycotoxicoses can be with acute and chronic symptoms. Farm animals, such as cattle, sheep, pigs and poultry are very sensitive to increased mycotoxin concentrations in food. Intoxication with ZEA, DON and AFB_1 leads to disorders of reproductive functions and functions in the gastrointestinal tract in animals (*Biagi, 2009; Liew and Mohd-Redzwan, 2018*).

Due to the inevitable fungal and mycotoxin contamination of maize grain, it is necessary to propose preventive measures in the field in order to increase food safety. The application of maize hybrids less sensitive to fungal infection is one of the ways to reduce mycotoxin level in grain (*Iglesias et al., 2010*).

The aim of this research was to establish the presence of toxigenic fungal species and the level of some mycotoxins (ZEA, DON and AFB_1) in maize grain samples which were used for animal feed and to assess the risk of possible harmful effects of these contaminants in four districts of Serbia.

Materials and Methods

A total of 22 maize samples were collected from the maize stored immediately after harvesting, during November and December in 2016, from four different districts of Serbia, City of Belgrade (nine samples), Šumadija (eight samples), Podunavlje (four samples) and Kolubara (one sample). Most maize samples (eight samples from the City of Belgrade, four samples from Šumadija and Podunavlje districts, each, and one maize sample from Kolubara district) were collected from ventilated maize cribs, while fewer samples (one sample from City of Belgrade and four samples from Šumadija district) were collected from closed concrete warehouses in which the temperature and relative humidity conditions are not controlled. In both types of maize warehouses, maize was dried naturally. Maize grains were harvested manually from cob samples which were collected from maize cribs. The samples of maize grains of about 1 kg were stored in the paper bags in a refrigerator at 4°C prior to fungal and mycotoxin analyses.

Mycological analyses of maize grain samples were conducted according to the previously described methods by *Krnjaja et al. (2015*). Based on morphological properties (colony and spore appearance), toxigenic species have been identified according to fungal keys of *Burgess et al. (1994)* and *Singh et al. (1991).* The incidence of toxigenic species was calculated per sample according to *Lević et al. (2012).*

In order to determine the moisture content and the level of mycotoxins, the tested maize samples were first ground in an analytical mill (IKA A11, Germany). The moisture content was determined in laboratory conditions using a moisture analyser (OHAUS MB35, USA). Prior to the mycotoxicological analysis, maize samples were dried at 60°C for 72h and then the mycotoxin level was determined by a competitive ELISA method. The ELISA assay was done according to the manufacturer's instructions Celer Tecna® ELISA kits, at a wavelength of 450 nm.

The limit of detection for ZEA, DON and AFB_1 were 10 μg kg^{-1}, 40 μg kg^{-1} and 1 μg kg^{-1}, respectively.

The Pearson correlation coefficients between investigated variables (moisture content, incidence of toxigenic fungal species and the level of mycotoxins) were done in Excel 2010.

Results

The average moisture content of tested maize samples from City of Belgrade, Šumadija, Podunavlje and Kolubara districts were 13.57%, 15.45%, 14.35% and 15.38%, respectively.

Mycological analyses confirmed the presence of *Aspergillus, Fusarium* and *Penicillium* spp. in the maize grain samples from all investigated districts, except in the maize grain sample from Kolubara district, in which *Aspergillus* species were not identified. Considering the average values, the most frequent species were *F. graminearum* in the maize grain sample from Kolubara district (60%) and *F. verticillioides* (43.75%) in the samples of maize grain from the Podunavlje district. *Penicillium* species were most prevalent in maize grain samples from Šumadija district (26.38%). *Aspergillus* species were present from 0 to 1.78% in the tested maize grain samples. Among the identified *Aspergillus* species, the species *A. flavus* was the most prevalent in maize samples from the City of Belgrade district (1.78%), while *A. niger* was most prevalent in samples of maize grain from the Podunavlje district (1%) and *A. parasiticus* was equally represented in maize samples from all districts (0.22-0.25%) except for Kolubara district (0%) (Table 1).

Table 1. Average incidence (%) of potentially toxigenic fungal species in tested maize samples from four Serbian districts

Fungal species	Districts			
	City of Belgrade	Šumadija	Podunavlje	Kolubara
A. flavus	1.78	1.50	0.5	0
A. niger	0.44	0	1	0
A. parasiticus	0.22	0.25	0.25	0
F. graminearum	12.33	10.13	8.50	60
F. proliferatum	0.33	0.38	0	0
F. verticillioides	22.4	12.38	43.75	5
F. subglutinans	1.11	1.75	0	2
Penicillium spp.	9	26.38	1.50	1

In mycotoxicological analyses, a high percentage of ZEA positive samples of maize grain originating from all districts was established (88.89-100%). DON was detected in 100% of the samples of maize grain from Šumadija and Kolubara districts and in 22.2% of the samples of maize grain from the City of Belgrade district. AFB_1 was detected in 50, 55.6, and 87.5% of maize grain samples from Podunavlje, City of Belgrade and Šumadija districts, respectively (Table 2).

In the tested samples of maize grain, the mean level of ZEA was from 16.82 (Podunavlje district) to 26.97 μg kg^{-1} (Šumadija district), DON from 445 (City of Belgrade district) to 1977 μg kg^{-1} (Kolubara district) and AFB_1 of 1.3 (Podunavlje district) to 1.39 μg kg^{-1} (City of Belgrade district). DON and AFB_1 were not detected in maize grain samples from Podunavlje and Kolubara districts. In all tested samples, the levels of ZEA, DON, and AFB_1 were not above the allowed limits of 350, 1750, and 5 μg kg^{-1}, respectively, adopted by the European Commission (EC, 2007, 2010), except for DON level in maize grain sample from Kolubara district (1977 μg kg^{-1}) (Table 3).

Table 2. Incidence (%) of mycotoxin positive maize samples from four Serbian districts

	Districts			
Mycotoxin	City of Belgrade	Šumadija	Podunavlje	Kolubara
ZEA	88.89	100	100	100
DON	22.2	100	0	100
AFB_1	55.56	87.5	50	0

Table 3. Mean mycotoxin levels (μg kg^{-1}) in positive maize samples from four Serbian districts

	Districts			
Mycotoxin	City of Belgrade	Šumadija	Podunavlje	Kolubara
ZEA	20.46	26.97	16.82	17.56
DON	445	998.38	ND*	1977
AFB_1	1.39	1.36	1.3	ND*

*ND – non detectable

Examination of the correlation ratios considered for a total of 22 tested maize samples showed positive correlations between moisture content and incidence of *F. graminearum* (r=0.28), *F. proliferatum* (r=0.12) and *Penicillium* spp. (r=0.58) and levels of ZEA (r=0.56), DON (r=0.49) and AFB_1 (r=0.16). Also, a positive correlation was established between the incidence of *F. graminearum* and DON (r=0.13) and between the incidence of *A. parasiticus* and AFB_1 (r=0.52). Likewise, positive correlations were established between the levels of ZEA and DON (r=0.52) and the level of DON and AFB_1 (r=0.42). Negative correlations were found between moisture content and incidence of *A. flavus* (r=-0.01), *A. parasiticus* (r=-0.16), *A. niger* (r=-0.01), *F. verticillioides* (r=-0.11) and *F subglutinans* (r=-0.09) and between the incidence of *A. flavus* and AFB_1 (r=-0.20) and incidence of *F. graminearum* and ZEA (r=-0.09).

Discussion

In the present study, the presence of toxigenic fungal species from *Aspergillus*, *Fusarium* and *Penicillium* genera and mycotoxins such as ZEA, DON and AFB_1 was confirmed in most of the maize samples originated from four Serbian districts. In all investigated districts, among *Fusarium* species, *F. verticillioides* was the most prevalent, except in maize sample from Kolubara district where the most dominant species was *F. graminearum* (Table 1). Similarly, in Argentina, in mycological studies including 52 maize samples, *Pacin et al. (2001)* have found that in all examined departments *F. verticillioides* was the prevalent toxigenic species, whereas the incidence of *F. graminearum* was low. Furthermore, in the biennial (2006-2007) mycological studies of maize grain samples originating from different locations in central Italy *Covarelli et al. (2011)* have established the dominance of the species *F. verticillioides* in relation to other *Fusarium* species. Likewise, among *Fusarium* spp., *Czembor et al. (2015)* isolated *F. verticillioides* as commonly presented, with a mean incidence of 16.19% in 30 maize grains samples from three locations in 2011 and in seven locations in 2012, in Poland, while *F. graminearum* was isolated in maize grain samples in only one location (3.57%).

In the present study, *F. graminearum* highly infected the maize sample from Kolubara district with incidence of 60%, while relatively high incidence were recorded (8.5-12.33%) in the samples from other investigated districts. These results may be explained with high average values of grain moisture content (13.57-15.45%). Moisture content is consider as one of the most important physiological factors for successful and safe storage of maize. The recommended moisture content for the safe maize storage is around 13% and below (*Alptekin et al., 2009*). The higher values of moisture content promote the favourable condition for development and proliferation of fungi and the appearance of insects leading to the storage problems (*Weinberg et al., 2007*). Also, *Logrieco et al. (2002)* reported that in some geographic regions the incidence of *F. graminearum* varies considerably from the investigated years and locality, which is highly connected with abiotic and biotic conditions.

Among toxigenic fungal genera, *Covarelli et al. (2011)* found out the dominance of *Fusarium* species in the tested maize samples, followed by *Aspergillus* species of *Flavi* and *Nigri* sections and *Penicillium* spp., which is similar to our findings. In contrast, *Alptekin et al. (2009)* demonstrated a significantly higher incidence of *Penicillium* spp. in the maize samples collected from various counties in Turkey during the 2005-2006 growing season, relative to the species from *Fusarium* and *Aspergillus* genera.

Incidence of positive maize samples for all investigated mycotoxins was relatively high (50-100%) in all examined districts, except for DON in maize samples from City of Belgrade district (22.2%) and Podunavlje district (0%) and for AFB_1 in maize samples from Kolubara district (0%). Mean levels of ZEA, DON and AFB_1 were not above the allowed limits of 350, 1750 and 5 μg kg^{-1}, respectively, prescribed by the European Commission (EC, 2007, 2010) for unprocessed maize, except for DON level in maize sample from Kolubara district (1977 μg kg^{-1}). Similar results were reported by *Covarelli et al. (2011)* in Italy, with DON and AFB_1 levels in some samples of maize grain being very high, 14,000 μg kg^{-1} and 820 μg kg^{-1}, respectively. *Czembor et al. (2015)* detected the incidence of DON and ZEA of 66.67% and 43.33%, respectively in the 30 tested maize samples originated from Poland, with the mean levels for positive samples of 50.77 μg kg^{-1} and 18.39 μg kg^{-1}, for DON and ZEA, respectively. In Turkey, *Alptekin et al. (2009)* detected AFB_1 in 72.4% of maize samples, with a concentration in the range of 0.63-108.86 μg kg^{-1}, with 43% of maize samples having an AFB_1 concentration above the permitted limit (5 μg kg^{-1}). In Argentina, *Pacin et al. (2001)* have not detected ZEA and DON, while AFB_1 was detected in only one maize sample at a concentration of 16.8 μg kg^{-1}. In North Korea, *Kim et al. (1993)* have established 30% DON and 8% ZEA positive maize samples with an average concentration of 310 and 151 μg kg^{-1}, respectively. In Indonesia, *Nuryono et al. (2005)* have established a low percentage (3%) of ZEA positive maize samples for feed with an average concentration of 25.5 μg kg^{-1}.

Considering the correlation values for the total number of 22 samples examined, medium positive correlations between moisture content and incidence of *Penicillium* spp. were found (r=0.58) and levels of ZEA (r=0.56) and DON (r=0.49), also between the incidence of *A. parasiticus* and AFB_1 (r=0.52), ZEA and DON levels (r=0.52). Slight positive correlations were found between moisture content and incidence of *F. graminearum* (r=0.28) and *F. proliferatum* (r=0.12) and levels of AFB_1 (r=0.16), and between incidence of *F. graminearum* and DON levels (r=0.13). Slight negative correlations were found between moisture content and incidence of *A. flavus* (r=-0.01), *A. parasiticus* (r=-0.16), *A. niger* (r=-0.01), *F. verticillioides* (-0.11) and *F. subglutinans* (-0.09) and between incidence of *F. graminearum* and ZEA (r = -0.09) and incidence of *A. flavus* and AFB_1 levels (r = -0.20). In similar studies, *Alptekin et al. (2009)* have established slight positive correlations between relative humidity (RH) and fungal count (r=0.378) and AFB_1 level (r=0.258) and slight negative correlations between fungal count and AFB_1 level (r=-0.249). In contrast, *Gourama and Bullerman (1995)* have established a positive correlation between fungal growth and AFB_1 production.

Conclusion

Based on the obtained results, it can be concluded that the considerable presence of certain potentially toxic fungi species, especially from the *Fusarium* genus, was determined in the maize samples, depending on the district tested. *F. graminearum* was the most common species (60%) in the maize sample from Kolubara district, followed by *F. verticillioides* in the maize samples from Podunavlje (43.75%) and City of Belgrade districts (22.4%) and *Penicillium* spp. in the maize samples from Šumadija district (26.38%). In regard to the tested mycotoxins, ZEA, DON and AFB_1, only DON exceeded the allowed limit (1750 $\mu g\ kg^{-1}$; EC, 2007) in the maize sample from Kolubara district. This was expected due to the high incidence of *F. graminearum* in the maize sample from this district and a positive correlation between incidence of *F. graminearum* and DON level.

These studies have confirmed the potential danger and risk from toxigenic species, primarily *Fusarium* species and their mycotoxins in the production of maize in agro-ecological conditions in Serbia. The obtained results justify the need for constant fungal and mycotoxin analyses of maize grain and other types of feeds, in order to find preventive measures for reducing these contaminants in the food chain. Future research should focus on the examination of the incidence and analysis the greater number of the samples from a number of localities, as well as a more detailed examination of the dependence of fungal and mycotoxin contamination from climatic factors in order to more accurately assess the effect of the locality (district) on the natural occurrence of these contaminants in the production of maize.

Kontaminacija toksigenim vrstama gljiva i njihovim mikotoksinima uzoraka kukuruza iz različitih regiona u Srbiji

Vesna Krnjaja, Slavica Stanković, Miloš Lukić, Nenad Mićić, Tanja Petrović, Zorica Bijelić, Violeta Mandić

Rezime

Ispitivanja u ovom radu izvedena su s ciljem da se odredi prirodna pojava potencijalno toksigenih gljiva iz rodova *Aspergillus*, *Fusarium* i *Penicillium* i sadržaj mikotoksina zearalenona (ZEA), deoksinivalenola (DON) i aflatoksina B_1 (AFB_1) u kukuruzu uskladištenom neposredno posle berbe u 2016. godini i korišćenom za ishranu životinja. Ukupno 22 uzoraka zrna kukuruza sakupljeni su iz četiri regiona u Srbiji: Beogradski (devet uzoraka), Šumadijski (osam uzoraka), Podunavski (četiri uzorka) i Kolubarski (jedan uzorak). Toksigene vrste gljiva su identifikovane na osnovu morfoloških osobina, a sadržaj mikotoksina određen je pomoću biohemijske, imunoadsorpcione enzimske metode (ELISA).

Ispitivani uzorci kukuruza većinom su bili inficirani sa *Aspergillus*, *Fusarium* i *Penicillium* spp., izuzev što u uzorku iz Kolubarskog regiona nisu bile identifikovane *Aspergillus* vrste. *Fusarium graminearum* bila je najučestalija vrsta

u uzorku kukuruza iz Kolubarskog regiona (60%), *F. verticillioides* u uzorcima iz Podunavskog (43,75%) i Beogradskog regiona (22,4%) i *Penicillium* spp. u uzorcima iz Šumadijskog regiona (26,38%). U ispitivanim uzorcima kukuruza zastupljenost *Aspergillus* vrsta bila je niska (0-1,78%).
Mikotoksikološkim analizama ustanovljeno je prisustvo zearalenona (ZEA), deoksinivalenola (DON) i aflatoksina B_1 (AFB_1) u svim ispitivanim uzorcima kukuruza, izuzev što DON nije detektovan u uzorcima iz Podunavskog a AFB_1 u uzorku iz Kolubarskog regiona. Ispitivani uzorci su visoko kontaminirani sa ZEA, 100% uzoraka iz Šumadijskog, Podunavskog i Kolubarskog regiona i 88,89% uzoraka iz Beogradskog regiona. Isto tako, sa DON bilo je kontaminirano 100% uzoraka iz Šumadijskog i Kolubarskog regiona i 22,2% iz Beogradskog regiona. Najveći broj AFB_1 pozitivnih uzoraka ustanovljen je u Šumadijskom regionu (87,5%), dok je u Beogradskom i Podunavskom regionu ustanovljeno 55,56% i 50% AFB_1 pozitivnih uzoraka, respektivno.
Uopšteno razmatrajući, u ovim analizama ustanovljena je visoka zastupljenost toksigenih vrsta u svim ispitivanim uzorcima kukuruza. Isto tako, ustanovljena je visoka kontaminiranost uzoraka sa mikotoksinima ZEA, DON i AFB_1, iako je samo u jednom uzorku sadržaj DON premašio dozvoljeni limit (1750 $\mu g\ kg^{-1}$) prema zakonskoj regulativi za neprerađeni kukuruz. Zbog toga, stalne mikološke i mikotoksikološke analize zrna kukuruza neophodne su radi ocene rizika od gljivične i mikotoksin kontaminacije u lancu ishrane.

Ključne reči: kukuruz, toksigene gljive, mikotoksini

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-46010.

References

ALPTEKIN Y., DUMAN A.D., AKKAYA M.R. (2009): Identification of fungal genus and detection of aflatoxin level in second crop corn grain. Journal of Animal and Veterinary Advances, 8, 9, 1777-1779.

BIAGI G. (2009): Dietary supplements for the reduction of mycotoxin intestinal absorption in pigs. Biotechnology in Animal Husbandry, 25, 5-6, 539-546.

BRITZI M., FRIEDMAN S., MIRON J., SOLOMON R., CUNEAH O., SHIMSHONI J.A., SOBACK S., ASHKENAZI R., ARMER S., SHLOSBERG A. (2013): Carry-over of aflatoxin B1 to aflatoxin M1 in high yielding Israeli cows in mid- and late- lactation. Toxins (Basel), 5, 1, 173-183.

BURGESS L.W., SUMMERELL B.A., BULLOCK S., GOTT K.P., BACKHOUSE D. (1994): Laboratory Manual for *Fusarium* Research. Third edition. Fusarium Research Laboratory, Department of Crop Sciences, University of Sydney and Royal Botanic Gardens, Sydney, pp. 133.

COVARELLI L., BECCARI G., SALVI S. (2011): Infection by mycotoxigenic fungal species and mycotoxin contamination of maize grain in Umbria, central Italy. Food and Chemical Toxicology, 49, 2365-2369.

DOOHAN F.M., BRENNAN J., COOKE B.M. (2003): Influence of climatic factors on *Fusarium* species pathogenic to cereals. European Journal of Plant Pathology, 109, 755-768.

EUROPEAN COMMISSION (2007): Commission regulation (EC) No 1126/2007 of 28 September 2007 Amending Regulation (EC) N0 1881/2006 Setting maximum levels for certain contaminants in foodstuffs as regards *Fusarium* toxins in maize and maize products. Official Journal of the European Union L 255/14.

EUROPEAN COMMISSION (2010): Commission regulation (EC) No 165/2010 of 26 February 2010 amending Regulation (EC) No 1881/2006 Setting maximum levels for certain contaminants in foodstuffs as regards aflatoxins. Official Journal of the European Union L 50/8.

GOURAMA H., BULLERMAN L.B. (1995): Relationship between aflatoxin production and mold growth as measured by ergosterol and plate count. LWT Food Science and Technology, 28, 185-189.

KHATOON S., HANIF N.Q., TAHIRA I., SULTANA N., SULTANA K., AYUB N. (2012): Natural occurrence of aflatoxins, zearalenone and trichothecenes in maize grown in Pakistan. Pak. J. Bot., 44, 1, 231-236.

KIM J.C., KANG H.J., LEE D.H., LEE Y.W., YOSHIZAWA T. (1993): Natural occurrence of *Fusarium* mycotoxins (trichothecenes and zearalenone) in barley and corn in Korea. Applied and Environmental Microbiology, 3798-3802.

KRNJAJA V., LUKIĆ M., DELIĆ N., TOMIĆ Z., MANDIĆ V., BIJELIĆ Z., GOGIĆ M. (2015): Mycobiota and mycotoxins in freshly harvested and stored maize. Biotechnology in Animal Husbandry, 31, 2, 291-302.

LIEW W.P.P., MOHD-REDZWAN S. (2018): Mycotoxin: its impact on gut health and microbiota. Frontiers in Cellular and Infection Microbiology, 8, 1-17, doi: 10.3389/fcimb.2018.00060

NURYONO N., NOVIANDI C.T., BÖHM J., RAZZAZI-FAZELI E (2005): A limited survey of zearalenone in Indonesian maize-based food and feed by ELISA and high performance liquid chromatography. Food Control, 16, 85-71.

NUGMANOV A., BESHOVA I., KOKANOV S., LOZOWICKA B., KACZYNSKI P., KONECKI R., SNARSKA K., WOŁEJKO E., SARSEMBAYEVA N., ABDIGALIYEVA T. (2018): Systems to reduce mycotoxin contamination of cereals in the agricultural region of Poland and Kazakhstan. Crop Protection, 106, 64-71.

PACIN A.M., BROGGI L.E., RESNIK S.L., GONZÁLEZ H.H.L. (2001): Mycoflora and mycotoxins natural occurrence in corn from entre Rios Province, Argentina. Mycotoxin Research, 17, 31-38.
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.
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.
STATISTICAL YEARBOOK OF SERBIA (2017): Statistical Office of the Republic of Serbia, Belgrade, pp. 481.
TÉDIHOU E., OLATINWO R., HELL K., HAU B., HOOGENBOOM G. (2012): Effects of variety, cropping system and soil inoculation with *Aspergillus flavus* on aflatoxin levels during storage of maize. Tropical Plant Pathology, 37, 1, 25-36.
WEINBERG Z.G., YAN Y., CHEN Y., FINKELMAN S., ASHBELL G., NAVARRO S. (2007): The effect of humidity level on high-humidity maize (*Zea mays* L.) under hermetic storage conditions – *in vitro* studies. Journal of Stored Products Research, 44, 136-144.
ZAIN M.E. (2011): Impact of mycotoxins on humans and animals. Journal of Saudi Chemical Society, 15, 2, 129-144.

Received 24 May 2018; accepted for publication 22 June 2018