AFLATOXIN, ZEARELENONE, DEOXYNIVALENOL AND FUMONISIN CONTAMINATION OF MAIZE FROM THE AUTONOMOUS PROVINCE OF VOJVODINA

KONTAMINACIJA KUKURUZA IZ AUTONOMNE POKRAJINE VOJVODINE SA AFLATOKSINIMA, ZEARELENONOM, DEOKSINIVALENOLOM I FUMONIZINIMA

Jovana KOS*, Radmilo ČOLOVIĆ*, Đuro VUKMIROVIĆ*, Olivera ĐURAGIĆ*,
Vojislava BURSIC**, Ferenc BAGI**, Elizabet JANIĆ HAJNAL*

*Institute of Food Technology, University of Novi Sad, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia

**Faculty of Agriculture, University of Novi Sad, Trg Dositeja Obradovića 8, 21000 Novi Sad,
e-mail: jovana.kos@fins.uns.ac.rs

ABSTRACT

Aflatoxins (AFs), fumonisins (FUMs), zearalenone (ZEA) and deoxynivalenol (DON) have been recognized as major contaminants of maize. Therefore, the presence of AFs, ZEA, DON and FUMs was examined in a total of 100 maize samples from the Autonomous Province of Vojvodina. Sample analyses were performed using the Enzyme Linked Immunosorbent Assay method. The results obtained indicate that 74 % of the maize samples were contaminated with FUMs (540.1-5076 µg/kg), followed by 52 % contaminated with DON (275.2-882.1 µg/kg), 15 % contaminated with ZEA (35.6-183.5 µg/kg) and 5 % contaminated with AFs (2.28-4.31 µg/kg). Although 78 % of the samples were contaminated with at least one mycotoxin, the concentration of FUMs exceed the maximum level (ML) proscribed by the Serbian regulations only in 4 % of the samples. Furthermore, the detected concentrations of AFs, ZEA and DON were in accordance with national regulations. This study is the first research of its kind on the presence of AFs, ZEA, DON and FUMs in maize harvested in the APV in 2016.

Key words: mycotoxins, maize, monitoring

REZIME

Aflatoksini (AF), zearalenon (ZEA), deoksiniivalenol (DON) i fumonizini (FUM) prepoznati su kao jedni od najznačajnijih kontaminanata kukuruza. Prisustvo navedenih mikotoksina u kukuruзу neophodno je kontinuirano pratiti kako bi se zaštitilo zdravlje ljudi i životinja. Iz tog razloga, u 100 uzoraka kukuruza (rod iz 2016. godine) iz Autonomne pokrajine Vojvodine (APV) ispitan je sadržaj AF, ZEA, DON i FUM. Za određivanje koncentracije mikotoksina korišćena je validovana ELISA metoda. Analizama je utvrđeno da je 74%, 52%, 15% i 5% od ispitanih uzoraka kukuruza bilo kontaminirano sa FUM, DON, ZEA i AF, u sledećim koncentracionim opsezima 540,1-5076, 275,2-882,1, 35,6-183,5 i 2,28-4,31 µg/kg, respektivno. Dobijeni rezultati ukazuju da je čak 78% uzoraka kukuruza bilo kontaminirano sa najmanje jednim od četiri ispitivana mikotoksina. Isto tako, sledeće kombinacije mikotoksina su pronađene u ispitivanim uzorcima: DON i FUM u 46%, ZEA i FUM u 15%, DON i ZEA u 13% analiziranih uzoraka kukuruza, dok je kombinacija DON, FUM i ZEA pronađena u 12%, a kombinacija sva četiri ispitivana mikotoksina u 1% analiziranih uzoraka. Iako je analizama utvrđen visok nivo kontaminacije (78%) uzoraka kukuruza, samo 4% uzoraka je sadržalo koncentraciju FUM veću od maksimalno dozvoljene koncentracije (MDK) propisane Pravilnikom Republike Srbije. Koncentracije ostalih detektovanih mikotoksina bile su ispod propisanih MDK vrednosti. Dobijeni rezultati u vezi sa kontaminacijom kukuruza mikotoksini mogu se dovesti u vezu sa vremenskim uslovima zabeleženim tokom proizvodnje 2016. godine. Takođe, potrebno je naglasiti da rezultati dobijeni u ovom radu predstavljaju prve objavljenje podatke u vezi prisustva AF, ZEA, DON i FUM u kukuruзу iz 2016. godine na teritoriji APV.

Cljučne reči: mikotoksini, kukuruz, monitoring.

INTRODUCTION

Maize is very susceptible to substrates for fungal growth and toxinogenesis. Many surveys confirmed that maize can be contaminated with numerous different mycotoxins. However, aflatoxins (AFs), zearalenone (ZEA), deoxynivalenol (DON) and fumonisins (FUMs) are recognized as major potential contaminants of maize (Trung et al., 2008).

The presence of AFs, ZEA, DON and FUMs in maize depends on several factors: agronomic factors (type of hybrid, soil, tillage and previous crop), weather conditions during the maize growing season, storage conditions (temperature, humidity, handling, presence of insects, rodents and birds), as well as storage duration (Hell et al., 2000; Sredanovic et al., 2008). Furthermore, some authors indicate that climate conditions exert the strongest influence on the occurrence of

mycotoxins in maize (Sanchis and Magan, 2004; Medina et al., 2015). The occurrence of AFs in maize is considered a topical agricultural issue in tropical and subtropical regions (Medina et al., 2015), while DON and ZEA generally occur in regions with cooler climates (Sudakin, 2003). Conversely, many studies confirmed the contamination of maize with FUMs in regions characterized by tropical as well as cooler climates (Munkvold and Desjardins, 1997; Kos et al., 2014).

Mycotoxins are undesirable contaminants of maize due to their potentially adverse health effects. AFs are highly toxic, mutagenic, teratogenic and carcinogenic compounds, and the International Agency for Research on Cancer categorizes AFs as primary carcinogenic compounds (IARC, 2012). Conversely, based on the limited data and evidence in humans and experimental animals, DON and ZEA were classified in Group 3 by the IARC (IARC, 1993), whereas FB1 was classified in

Group 2B as potentially carcinogenic to humans (IARC, 2002). In addition to potentially adverse effects on the human and animal health, the presence of mycotoxins in maize could also result in significant economical losses. It should be emphasised that maize represents one of the most important export items of the Republic of Serbia, making the country a leader in maize export both in Europe and the world (Maslac, 2015). Therefore, the presence of mycotoxins in maize should be continuously controlled.

The presence of AFs, ZEA, DON and FUMs in maize causes poisoning, i.e. mycotoxicosis, and exerts teratogenic, carcinogenic, neurotoxic, estrogenic or immune-suppressive effects (IARC, 2002). The maximum levels (ML) for those toxins have been established in the Republic of Serbia (Serbian Regulations, 2011) as well as in numerous countries in the world. According to the Serbian regulations, the MLs for AFs, ZEA, DON and FUMs in unprocessed maize intended for human consumption are 10, 350, 1750 and 4000 µg/kg, respectively. Moreover, if maize is intended for animal feed, the concentration of AFB1 cannot exceed 30 µg/kg, whereas the concentrations of ZEA and DON cannot exceed 4000 µg/kg and 8000 µg/kg respectively. However, the European Union Regulations have set 20, 2000 and 60000 µg/kg as the MLs for AFB1 (European Commission, 2002), ZEA and FUMs (European Commission, 2006b) in maize intended for animal feed, respectively.

Previous European studies on the contamination of maize with mycotoxins indicate that the absence or presence of certain mycotoxins depend on many factors such as weather conditions throughout the maize growing season (Pleadin et al., 2012; Kos et al., 2014; Streit et al., 2013; Kos et al., 2017). Therefore, the purpose of this study is to examine the maize samples (harvested in the Autonomous Province of Vojvodina (APV) in 2016) contaminated with AFs, ZEA, DON and FUMs in relation to weather conditions recorded during the maize growing season.

MATERIALS AND METHODS

A total of 100 maize samples were collected in the major maize-growing regions of the Autonomous Province of Vojvodina. The samples were collected after harvest during October and at the beginning of November in 2016. The sampling was performed according to the requirements set by the European Commission (2006a). The analyses of mycotoxin contamination in the maize samples were done by the Enzyme Linked Immunosorbent Assay (ELISA) method using test kits for AFs (AFB1, AFB2, AFG1 and AFG2), ZEA, DON and FUMs (FB1, FB2 and FB3) determination. All test kits were produced by Neogen Corporation (Neogen Veratox®, Lansing, USA) and the ELISA method was performed strictly according to the manufacturer's instructions. Methanol and ethanol of analytical purity (Merck, Darmstadt, Germany), as well as distilled water (Millipore, Bedford, MA, USA), were used for the ELISA analysis. The analytical quality of the ELISA methods applied was confirmed in numerous international interlaboratory studies (Progetto Trieste, Test Veritas, Padova, Italy; Neogen Corporation, Technical Services Division, Natural Toxins, Lansing, USA; The Grain and Feed Trade Association, GAFTA, London, United Kingdom). Furthermore, the analysis of certified reference materials (CRMs) and the certified AFs, ZEA, DON (A-C-268, Z-C-310, D-W-158, Trilogy Analytical Laboratory, Washington, USA) and FUMs (MI1140-2/CM, Progetto Trieste, Test Veritas, Padova, Italy) contents of 4.5, 59.4, 1400 and 5115 µg/kg respectively were used for determining the validation parameters. The validation parameters obtained (Table 1) were in accordance with the

recommendations stipulated in the Commission Decision (European Commission, 2006a).

Table 1. Validation parameters of ELISA methods for determining the AFs, ZEA, DON and FUMs contents

Validation parameters	Mycotoxins			
	AFs	ZEA	DON	FUMs
LOD (µg/kg)	0.50	10.0	100	200
LOQ (µg/kg)	1.00	25.0	250	250
RSD _r (-)	8.06	7.15	6.09	7.52
RSD _R (-)	9.17	11.2	9.54	10.4
Rec. (%)	103.2	94.5	105.9	96.1

LOD: limit of detection (µg/kg)

LOQ: limit of quantification (µg/kg)

RSD_r: relative standard deviation calculated under repeatability conditions

RSD_R: relative standard deviation calculated under reproducibility conditions

Rec: recovery (%)

RESULTS AND DISCUSSION

Table 2 displays the occurrence of AF, ZEA, DON and FUM contamination in the maize samples harvested in the APV in 2016. The results obtained indicate that 74 % of the maize samples were contaminated with FUMs (540.1-5076 µg/kg), followed by 52 % contaminated with DON (275.2-882.1 µg/kg), 15 % contaminated with ZEA (35.6-183.5 µg/kg) and 5 % contaminated with AFs (2.28-4.31 µg/kg).

Table 2. Contamination of maize samples with AFs, ZEA, DON and FUMs

Mycotoxins	Positive over total	Mean ± SD	Range (µg/kg)
AFs	5/100	3.22±0.79	2.28-4.31
ZEA	15/100	83.3±43.8	35.6-183.5
DON	52/100	541.0±175.0	275.2-882.1
FUMs	74/100	2750±1078	540.1-5076

Mean ± SD: mean concentration (µg/kg) ± standard deviation (µg/kg).

In order to determine the influence of weather conditions on the occurrence of mycotoxins in the maize samples analyzed, the values of air temperature and precipitation in the period of maize planting, growing and harvesting (April-September) in 2016 were examined. Figure 1 shows the average monthly values of air temperature (a) and precipitation (b) in the APV in the maize growing season of 2016 in comparison with the average values of these parameters in the long-term period 1981-2010 (Republic Hydrometeorological Service of Serbia, 2016). Evidently, the average monthly values of air temperature in 2016 were comparable to (May, August) or slightly higher (April, June, July, September) than the values recorded in the same months in the long-term period 1981-2010. Furthermore, during the entire experimental period, with the exception of April, the precipitation amounts recorded were higher than the average precipitation amount in the long-term period. The average amount of precipitation recorded in the APV during the maize growing season of 2016 approximated to 430 mm, which is slightly higher than the average amount recorded in recent years. However, only in July of 2016, the amount of precipitation was significantly higher than the average value recorded in the long-term period 1981-2010.

The contamination frequency obtained, as well as the concentration ranges of four mycotoxins examined in this study, could be explained by weather conditions recorded during the maize growing season of 2016. According to the literature data, the growth of *Aspergillus* species and AFs production are facilitated by hot and dry weather conditions during the maize growing season (Santin, 2005). Therefore, it is evident that the weather conditions recorded in 2016 were not favorable to the AFs synthesis since only 5 % of the maize samples were contaminated with AFs. Moreover, the AFs concentration determined was less than the ML set by the Serbian regulations (Serbian Regulations, 2011). Although the production of AFs occurs primarily in regions with tropical or subtropical climates, the drought conditions recorded in Serbia during the maize growing season of 2012 caused the occurrence of AFs in 68.5 % of the maize samples analyzed, despite the fact that AFs were not detected in maize from Serbia in the previous year (Kos et al., 2013).

On the basis of the results obtained, it could be assumed that the weather conditions in 2016 were much more favorable to certain *Fusarium* species. With regard to FUMs, a contamination frequency of 74% indicate that the weather condition recorded in 2016 were favorable to their synthesis. Furthermore, previous studies confirmed that FUMs were often present as maize contaminants in Serbia regardless of weather conditions. Different authors reported that 52.9%, 50%, 100% and 100% of the maize samples harvested in Serbia in 2008, 2009, 2010 and 2012 were contaminated with FUMs, respectively (Matić et al., 2009; Jakšić et al., 2011; Jakšić et al., 2012; Kos et al., 2014). It is clearly noticeable that a high frequency of the FUM contamination was detected even though the maize growing seasons of 2008 and 2010 were characterized by low amounts of precipitation, compared to that of 2009 which indicated high amounts of precipitation, whereas drought conditions were recorded in 2012. Although a high contamination frequency (74%) of FUMs was observed in the maize samples from 2016, the concentrations of FUMs exceed the ML only in 4% of the samples.

Slightly higher amounts of precipitation, recorded during the maize growing season of 2016, were favorable to the DON synthesis, which was detected in 52 out of 100 maize samples examined. However, all detected concentrations of DON were lower than the ML. In addition, one of the rainiest maize growing seasons (2014) in recent years resulted in contaminating 576 (96.0 %) out of 600 maize samples with a mean DON concentration of 3063.3 ± 1264.4 $\mu\text{g/kg}$. Almost 50 % of the maize samples from 2014 were unsuitable for human consumption (Kos et al., 2017), whereas the obtained concentrations of DON in all the positive samples from 2016 were lower than the ML. The present study confirmed that the appearance of DON in maize is mainly facilitated by abundant precipitation.

A *Fusarium* toxin with the lowest determined contamination frequency of 15 % in maize from 2016 is ZEA. Therefore, weather conditions encompassing high amounts of precipitation and lower air temperatures are favorable to the ZEA synthesis. The

results obtained in 2014 indicate that 85 % of the maize samples were contaminated with ZEA (Kos et al., 2016). However, none of the maize samples from the dry and hot maize growing season of 2012 was contaminated with ZEA (Kos et al., 2014).

The results obtained in this study also indicate that 78% of the maize samples analyzed were contaminated with at least one mycotoxin. Furthermore, the co-occurrence of the mycotoxins examined in the maize samples from 2016 was found to exist, and the combinations of mycotoxins are presented in Table 3. Some authors reported that the co-occurrence of mycotoxins should be observed with special attention because the combination of two or more mycotoxins could provoke synergistic interactions, which may result in additive combinations of toxic effects (Grenier and Oswald, 2011).

Fig. 1. Average monthly temperatures (a) and precipitation amounts (b) in 2016 (April-September) in comparison with the average values in the period 1981-2010

Table 3. Co-occurrence of AFs, ZEA, DON and FUMs in the maize samples analyzed

Mycotoxins combination	Number of samples with the co-occurrence of mycotoxins
DON- FUMs	46
ZEA- FUMs	15
DON- ZEA	13
DON- ZEA- FUMs	12
DON- ZEA- FUMs- AFs	1

CONCLUSION

This study confirmed that the contamination of maize samples with AFs, ZEA, DON and FUMs depends greatly on the weather conditions recorded during the maize growing season. Although extreme weather conditions were recorded during previous maize growing seasons, the weather conditions recorded in the maize growing season of 2016 were characterized by similar and/or slightly higher air temperatures and precipitation amounts in comparison with the average values of these parameters recorded in the long-term period 1981-2010. The concentrations of FUMs exceed the maximum level (ML) in only 4 % of the maize samples analyzed. Furthermore, the determined concentrations of AFs, ZEA and DON were less than the MLs established by the Serbian regulations.

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