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THE INFLUENCE OF DROUGHT ON THE OCCURENCE OF AFLATOXINS IN MAIZE

ABSTRACT: In this study, a total of 78 maize samples harvested during September and October 2012 in Vojvodina were analyzed. Presence of aflatoxins (AFs) was determined by enzyme-linked immunosorbent assay (ELISA) method. Among the 78 analyzed maize samples, even 44 (56.4%) samples were contaminated with AFs. Concentration interval between 1-10 µg/kg, 10-50 µg/kg and 50-80 µg/kg were found in 23.1%, 17.9% and 15.4% of analyzed maize samples, respectively. It was supposed that prolonged drought during spring and summer of 2012 had a great influence on high contamination frequency and concentration of AFs.

KEY WORDS: Aflatoxins, maize, drought, ELISA

INTRODUCTION

Aflatoxins (AFs) are secondary metabolites produced mainly by three species of *Aspergillus* including *A. flavus*, *A. parasiticus* and *A. nomius*. The most known AFs which can be found as contaminants of food and feed are B_1, B_2, G_1 and G_2 and their two metabolic products M1 and M2 (D e c a s t e l l i et al., 2007). AFs are most common in granular food, including cereals, grains and groundnuts. Milk, eggs, meat and their products can be contaminated if animal consume contaminated feed. However, the commodities with the highest risk of AFs contamination are corn, peanuts, and cottonseed (E l l i s et al., 1991).

The occurrence of AFs is influenced by certain environmental factors and they are especially found in tropical and subtropical regions where temperature and humidity are optimum for growth of molds and for the production of toxins (R u s t o m, 1997). The incidence of AFs and their amount in contaminated products especially depend on the conditions of temperature and humidity during crop growth and storage. Furthermore, the extent of AFs contamination will vary with geographic location, agricultural and agronomic

practices, and the susceptibility of commodities to fungal invasion during pre harvest, storage, and/or processing periods (G a r r i d o et al., 2012).

AFs have been associated with various diseases, such as aflatoxicosis, in livestock and humans throughout the world and they have received great attention because of their potential high toxic, mutagenic, teratogenic and carcinogenic effect on humans and animals. The International Agency for Research on Cancer classified AFs as primary carcinogenic compounds (IARC, 2012).

According to the literature data, *Aspergillus* species and their metabolites AFs are one of the most known contaminants of maize and maize products (B a n k o l e et al., 2003). Maize is one of the most important crops cultivated in Serbia with approximate annual production of six million tons. The weather conditions and drought in spring and summer of 2012 significantly contributed to the reduction in maize production (M a s l a c, 2012). However, it should be noted that there is still a lack of data regarding the occurrence of *Aspegillus* toxins in maize from Serbia.

Many countries, including Serbia, have introduced regulations which stipulate maximum allowed limits of AFs in maize intended for food and feed production. The Serbian regulations (Sl. Glasnik RS 4/2010; Sl. Glasnik RS, 28/2011;) on the control of mycotoxins in food and feed were adopted and complied with the European Union regulation (2002/32/EC, 2010/165/EC) in 2010 and 2011, respectively.

Due to the significant health risks associated with the presence of AFs in food and feed, it is important to establish a data collection on the occurrence of these toxins in Serbian commodities. There are no enough data regarding the occurrence of AFs in maize from Serbia. Hence, the purpose of this work was to screen the presence of AFs in maize harvested in Vojvodina, Northern Province of Serbia, during 2012.

MATERIALS AND METHOD

Samples

A total of 78 maize samples were collected in Vojvodina. Samples were collected after harvest, during September and October 2012. All samples were stored at the temperature of 4 °C in refrigerator before analysis.

Determination of AFs

Content of AFs (B_1, B_2, G_1 i G_2) was determined by the enzyme immunosorbent assay method (ELISA). All samples were analyzed in duplicate with Quantitative Aflatoxin High Sensitivity (HS) test kit (Neogen Veratox®, Lansing, USA). Range of quantitation for AFs HS test kit was between 1 and 8 µg/kg and the analyses were done according to the manufacturer´s description. The analyzed samples which contained AFs more than 8 µg/kg were analyzed

with Aflatoxins Quantitative test kits (standards: 0, 5, 10, 50 μg/kg). Samples with content of AFs more than 50 μg/kg were analyzed again after dilution.

RESULTS AND DISCUSION

In this study, 78 maize samples were analyzed in order to evaluate the AFs contamination. The obtained results (Table 1) showed that 44 (56.4%) of analyzed maize samples were positive. AFs concentration in the positive samples ranged from 1.20 to 80.0 μg/kg. Positive results were classified into three groups. Concentration interval from 1 to 10 μg/kg of AFs were found in 18 (23.1%) of analyzed maize samples, while concentration interval 10-50 μg/kg and 50-80 μg/kg were found in 14 (17.9%) and 12 (15.4%) samples, respectively. Mean level of aflatoxin concentration for all positive samples was 27.0 μg/kg. The obtained results show that negative samples (34) and samples with AFs concentration less than 10 μg/kg (18) can be used for human consumption. Maximum allowed limit for AFs in maize intended for human consumption was stipulated by Serbian (Sl. List 28/2011) and the European Union (2010/165/EC) regulation. Both regulations prescribe 10 μg/kg as the maximum allowed level for sum of AFs (B_1, B_2, G_1 and G_2). Fourteen samples of maize were found to be contaminated with AFs in range of concentration from 10 to 50 μg/kg, and those samples can be used as feed by Serbian (Sl. List 4/2010) regulation. This regulation set maximum level of 50 μg/kg for AFs in maize intended for animal feed. The regulation of the European Union (2002/32/EC) prescribed 20 μg/kg as the maximum allowed limit only for AFB_1 in maize intended for animal feed.

Tab. 1. – Contamination frequency (CF), interval (CI) and mean level (CM±SD) of AFs

	1–10 μg/kg	10–50 μg/kg	50–80 μg/kg
CF	23.1	17.9	15.4
CI	1.20–9.97	10.2–43.2	51.2–80.0
CM	4.87 ± 2.99	18.9 ± 12.0	68.1 ± 12.4

CF(%), CI (μg/kg), CM (μg/kg) and SD (standard deviation)

The results from Table 1 show that AFs were found in 12 (15.4%) samples with concentration higher than 50 μg/kg. Those samples with high AFs contamination cannot be used for human and animal consumption. After mixing with non-contaminated maize, those samples can be used in animal diet, with the use of an appropriate adsorbent.

The obtained results also show that weather conditions in spring and summer of 2012 had a very strong influence on the production of AFs in the examined maize samples. According to the few previously published literature data, *Aspergillus* species and AFs are rarely found in Serbia (B o č a r o v -

- S t a n č i ć, et al., 2001). Furthermore, our previous results showed that none of the analyzed maize sample from Serbia was contaminated with AFs (M a t i ć et al, 2009; M a t i ć et al., 2010).

Maize planting in Serbia usually starts in the middle of April and lasts until middle of May, and farmers harvest the crops during October and November. Due to unusually high temperatures in August and September 2012, harvest was finished earlier, in the second part of September. Considering the fact that high contamination frequency of AFs was found (56.4%) in maize harvested during 2012, weather conditions for that year were analyzed (Figure 1, Figure 2).

Figure 1 shows the average amount of precipitation in Vojvodina for the period from April to September 2012 compared to the average values for the long-term period of 1971–2000 (Republic Hydrometeorogical Service of Serbia, 2012). The figure shows that lack of rainfall characterized the period from April to September. Extremely low amount of rainfall was recorded in the period of four months (June-September), and during that period, the amount of rainfall was lower by 66, 78, 93 and 60% than average rainfall for multiannual period (1971-2000), respectively. For the period of four months, precipitation covered more than 50% of Vojvodina for only 11 days, while during August, there was only 4 mm of rain in the entire region.

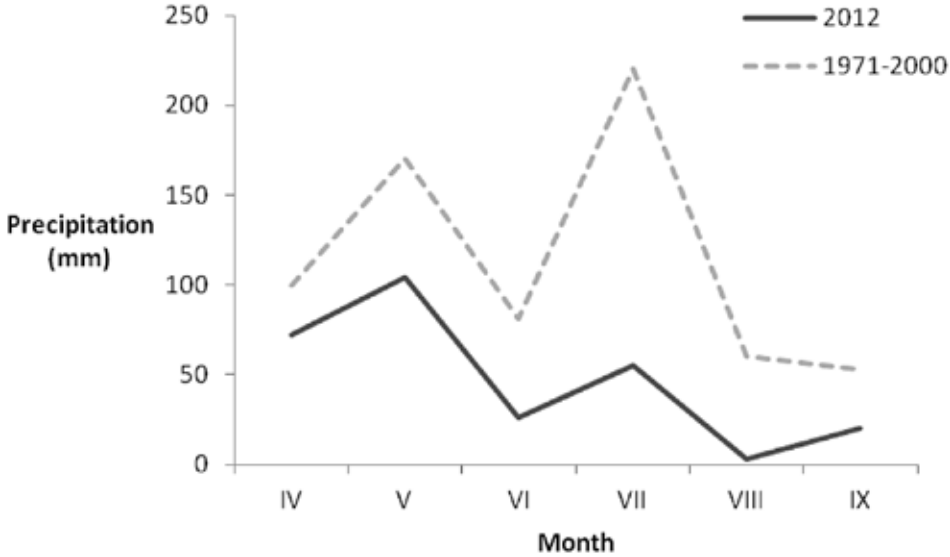

Fig. 1. – Average amount of precipitation in Vojvodina for the period April-September 2012 compared to the average values for the long-term period of 1971–2000

Despite the lack of precipitation, period from June to September was characterized with high temperatures. Monthly average temperature was higher

than the average value, from 1.2 °C (May) to 4.1 °C (July), (Figure 2). The period of four months from June to September was very warm with average monthly temperature in the interval from 20.2 to 25.2 °C and very often daily temperature was near 40 °C. This phenomenon resulted in the favorable conditions for mold growth and emergence of various pests and damage to corn. The lack of moisture and high temperature in these months caused considerable damage to corn.

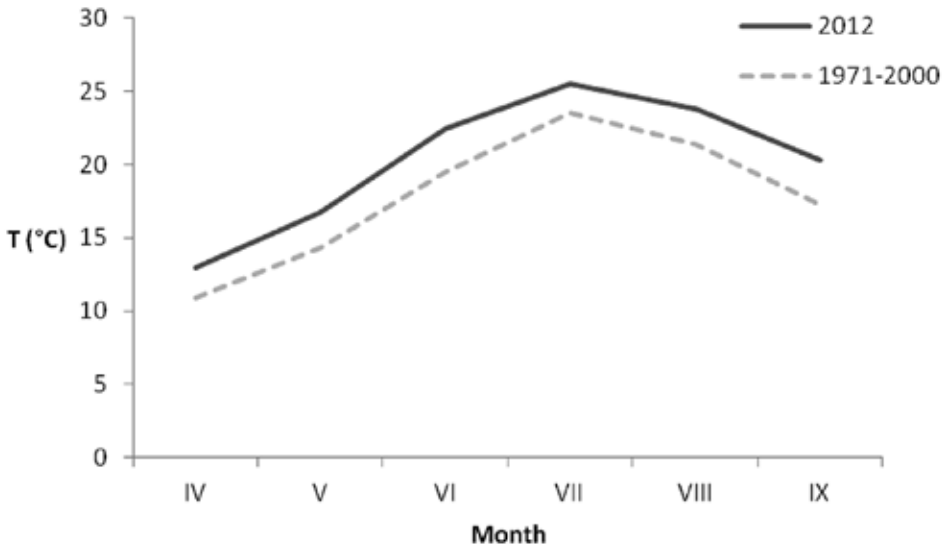

Fig. 2. – Average temperature in Vojvodina for the period April-September 2012 compared to the average values for the long-term period of 1971–2000

According to the figures, it can be concluded that hot and dry weather with prolonged drought characterized spring and summer of 2012 and it was assumed that these weather conditions caused higher presence of AFs. Previous reports have confirmed that if the maize growth and development is followed by hot and dry weather conditions, it can be contaminated with *Aspergillus flavus* with increased probability for AFs synthesis (S t a c k et al., 2003). These authors reported that prolonged drought and high temperatures during the growing season favored the development of *A. flavus*, restricted the development of competitors of *A. flavus*, and inhibited normal pollination in the corn plant. This often leads to an increase in the extent and amount of AFs contamination. Furthermore, K a r a m i-O s b o o et al. (2012) analyzed the presence of AFB_1 in maize harvested for three years and concluded that weather conditions, primarily low rainfall and drought, had a great influence on the presence of AFB_1.

The obtained results for maize harvested in 2012 confirmed that this crop should be continuously controlled in order to protect the population against the unacceptable risk of AFs contamination.

Prevention and control of AFs in food and feed is required, considering the fact that AFs have toxic effect on humans and animals and for the economic reasons as well.

REFERENCES

B a n k o l e, S. A., A d e b a n j o, A. (2003): Mycotoxins in food in West Africa: current situation and possibilities of controlling. African Journal of Biotechnology, 2 (9): 254-263.

B o č a r o v-S t a n č i ć, A., G o j k o v, M., L a c o, D. (2001): Occurrence of mycotoxins in food and food additives sampled in middle Banat region in the period of 1993–1998. In Proceedings of the 4th Yugoslav Symposium Chemistry and Protection of Environment (pp. 193–195). Zrenjanin, Yugoslavia.

Commission regulation 165/2010, Official Journal of the European Union, L 50/8

Commission regulation 32/2002, Official Journal of the European Union, L 140/10

D e c a s t e l l i, L., L a i, J., G r a m a g l i a, M., M o n a c o, A., N a c h t m a n n, C., O l d a n o, F. (2007): Aflatoxins occurrence in milk and feed in Northern Italy during 2004-2005. Food Control, 18: 1263-1266.

E l l i s, W. O., S m i t h, J. P., S i m p s o n, B. K., O l d h a m, J. H. (1991): Aflatoxins in food: occurrence, biosynthesis, effect in organisms, detection and methods of control. Critical Reviews in Food Science and Nutrition, 30: 403-439.

G a r r i d o, C. E., H e r n á n d e z P e z z a n i, C., P a c i n, A. (2012): Mycotoxins occurrence in Argentina's maize (*Zea mays* L.), from 1999 to 2010. Food Control, 25: 650-665.

IARC, International Agency for Research on Cancer (2012). IARC monographs on the evaluation of carcinogenic risks to humans, 1-104.

K a r a m i-O s b o o, R., M i r a b o l f a t h y, M., K a m r a n, R., S h e t a b-B o u s h e h r i, M. (2012): Aflatoxin B1 in maize harvested over 3 year in Iran. Food Control, 23: 271-274.

M a s l a c, T. (2012): US Department of Agriculture (USDA) Grain and Feed, Update on Wheat and Corn for Serbia.

M a t i ć, J., M a s t i l o v i ć, J., Č a b a r k a p a, I., M a n d i ć., A. (2009): Mycotoxins as a risk in the grain food, Matica Srpska, Proceedings for Natural Sciences, 117: 79-86.

M a t i ć, J., M a n d i ć, A., L e v i ć, J., Z d j e l a r, G., V a s i l j e v i ć, I., K o k i ć, B., Č a b a r k a p a, I. (2010): Occurrence of Mycotoxins and genetically modified organisms in feed and food containing corn and soybean, XIV International Symposium Feed Technology, Novi Sad 19-21 October, Serbia, 288-294.

Republic Hydrometeorological Service of Serbia. (2012): Agrometeorološki uslovi u proizvodnoj 2011/2012 godini na teritoriji Republike Srbije. (Sr)

R u s t o m, I. Y. S. (1997): Aflatoxin in food and feed: occurrence, legislation and inactivation by physical methods, Food Chemistry, 59, pp. 57-67.

Službeni glasnik Republike Srbije (2010). Pravilnik o kvalitetu hrane za životinje, 4, 30-61. (Sr)

Službeni glasnik Republike Srbije (2011). Pravilnik 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, 28, 9-18. (Sr)

S t a c k, J., C a r l s o n, M. (2003): *Aspergillus flavus* and Aflatoxins in Corn, Plant Diseases, C-18, Field Crops, Historical Materials from University of Nebraska, Lincoln, 43.

УТИЦАЈ СУШЕ НА ПОЈАВУ АФЛАТОКСИНА У КУКУРУЗУ

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Резиме

У току истраживања анализирани су узорци кукуруза који су сакупљани на територији Војводине непосредно након бербе 2012. године. Имуноафинитетном методом (ELISA) анализирано је присуство афлатоксина (B_1, B_2, G_1 i G_2) у 78 узорака кукуруза. Од укупног броја анализираних узорака кукуруза у 34 (43,6%) није детектовано присуство афлатоксина, док је чак 44 (56,4%) узорка било позитивно на присуство овог токсина. У 18 испитаних (23,1%) узорака концентрација афлатоксина је била између 1 и 10 μg/kg, у 14 (17,9%) између 10 и 50 μg/kg, а чак у 12 (15,4%) узорака концентрација афлатоксина је била изнад 50 μг/кг. Овако висока учесталост и концентрација афлатоксина у узорцима кукуруза највероватније је последица екстремних климатских услова током вегетационог периода 2012. године. Сезону развоја и сазревања кукуруза у периоду од априла до октобра 2012. године обележиле су изузетно високе просечне температуре ваздуха уз ретку појаву кише. Топло време и суша на територији Војводине допринели су и развоју *Aspergillus* врста и синтези афлатоксина. С обзиром на добијене резултате и изузетну токсичност афлатоксина на организам људи и животиња, неопходно је спроводити појачану контролу рода кукуруза из 2012. године.

КЉУЧНЕ РЕЧИ: афлатоксин, кукуруз, суша, ELISA

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