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AFLATOXINS – MAIZE SAFETY ECONOMY CHALLENGE IN 2012.

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SUMMARY: The aim of this study was to investigate the presence of aflatoxins in maize, and their safety and economic impact. As our country is among the largest exporters of corn, the consequences which infected corn leaves certainly are not negligible. Monitoring implementation is the only solution that will stop and prevent further aflatoxins contamination. In this paper, 44 commercial maize samples were analyzed for aflatoxins presence. It was found that 63.6% of samples infected. The level of aflatoxins in infected samples ranged from 5-367 ppb, while the average value of 74.5 ppb, with a standard deviation of 79.5.

Key words: *aflatoxins, maize, Serbia, export.*

INTRODUCTION

Aflatoxin is persistent mycotoxin produced by several Aspergillus fungi, primarily *Aspergillus flavus*, under certain conditions when it grows in ears or on grain. They are commonly found in peanuts, cottonseed, corn and their products. In wheat, rice, soybeans oats, and sorghum aflatoxins only rarely are noted (Sweets and Wrather, 2009).

Infection of corn by *Aspergillus spp.* occurs through the silk. The spores germinate, and the fungus colonizes the silks in hot conditions. The fungus can then grow down the silk channel and around the developing ear. Yellow-brown silks that are still moist are most susceptible to colonization and invasion down the silk channel. Fresh, not pollinated silks are relatively resistant, and brown, dry silks can be colonized, but growth of the fungus down the silk channel is limited (Vincelli, 1995). The fungus appears to grow from the ear tip toward the base by colonizing the silk first, then the glumes and the kernel surface. After the silks die, the growth of the fungus is rapid in hot weather. If conditions are favorable for the production of aflatoxin, it is much more difficult to prevent infection from *Aspergillus spp.* and subsequent aflatoxin production (Anderson, 1983; Gardisser, 1989).

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Another means of infection is kernel injury from insects or birds provides that are easily colonized by the fungus. Even if kernels are uninfected at harvest, the presence of *Aspergillus flavus* spores on kernel surfaces sets the stage for post harvest contamination with aflatoxin. When temperature and moisture conditions permit, *Aspergillus flavus* spores can germinate and infect injured or broken kernels within a day or two of harvest (Vincelli, 1995). According to some studies, *Aspergillus* observed somewhat greater representation during the summer period (Škrinjar et al, 2008). Aflatoxin production by the fungus is most active at 25-32°C, but development of the fungus usually stops when the temperature is below 13°C and grain moisture is 15% or less (Herman and Trigo-Stockli, 2002).

Corn (*Zea mays L.*) is a major crop in Serbia, where it plays an important role in the economy, in animal feed production, alcohol fermentation and direct human consumption. Although aflatoxin contamination in corn is uncommon in Serbia, occasional incidents do occur and can create significant economic losses for individual producers. Our limited survey data and general experience indicate that the incidence of aflatoxin contamination in Serbia is similar to that of other European countries. Furthermore, one or more cases of contamination occur when shelled corn is not promptly dried or properly stored. Rapid detection of contaminated corn is important because aflatoxin normally survives processing and may be concentrated in products or processed fractions (Payne, 1992; Abbas et al., 2002).

The economic impact of aflatoxins derive directly from crop and livestock losses as well as indirectly from the cost of regulatory programs designed to reduce risks to animal and human health. Throughout the world there are many advisory bodies concerned with food safety, including the World Health Organization (WHO), Codex Alimentarius and the European Food Safety Authority (EFSA), and they regularly assess the risk from mycotoxins and advise on controls to reduce consumer exposure. According to the Commission Regulation (EC) No 1881/2006 maximum level for certain contaminants regarding to aflatoxin in maize for human consumption is 10 ppb (μg/kg). In Serbia, maximum permissible level of aflatoxin is 50 ppb (μg/kg) in feed (Službeni glasnik, 4/2010). Although maximum levels for aflatoxins and some other mycotoxins are included in Serbian legislation, there is not enough information about the results of mycotoxicological food and feed control (Jakšić et al., 2011).

Aflatoxins losses to livestock and poultry producers from aflatoxin-contaminated feeds include death and the more subtle effects of immune system suppression, reduced growth rates, and losses in feed efficiency. Other adverse economic effects of aflatoxins include lower yields for food and fiber crops (Anon, 1989). At present, economically disastrous events like the widespread aflatoxins contamination in region are unpredictable even in the short term. However, it will be necessary to study the effect of heat stress on mycotoxin level in corn over a longer time period to establish the significance of maize safety.

The aim of this study was to examine the content of aflatoxin in maize samples, and inquire the effect of infection on maize export from Serbia.

MATERIAL AND METHOD OF THE STUDY

Samples: Samples of maize from the 2012 harvest were collected from different locations in the Republic of Serbia. Samples were taken immediately after the harvest. There were 44 samples of maize. Immediately after sampling, 1000 g of each sample were prepared by grinding in a laboratory mill in such a way that >93% passed through a sieve with pore diameter of 0.8 mm. After that, the sample was homogenized by mixing. Samples thus prepared were packed in plastic bags and stored in a freezer at -20°C until analysis. Prior to each analysis, the samples were allowed to reach room temperature.

Analysis: 20 g of samples were extracted with 100 ml of 70% methanol and filtered through the filter paper. The immunochemical analysis was performed using the Veratox, Aflatoxin (Total), Quantitative Test Kit (Neogen, Lansing, MI, USA) with four calibration standard solutions (0, 5, 15 and 50 ppb). In the mixing well were placed the conjugate (100 µl) and the standard or sample (100 µl). To make a homogeneous mixture, the liquid was drawn up and dispensed three times with the aid of a micropipette. A volume of the mixed solution (100 µl) was transferred to the well with antibodies and incubated at room temperature for 2 minutes. The solution was then removed and wells were rinsed 5 times by distilled water. After that, the substrate (100 µl) was added and three-minute incubation was stopped by adding "stop" reagent (100 µl). Optical densities on the basis of which aflatoxins content was calculated were read using the reader of microtitration plates with a 630 nm filter. According to the manufacturer the limit of determination was 5 µg aflatoxins/kg sample, and range of quantitation 5-50 ppb.

RESULTS

In this paper there were 44 samples of commercial maize processed, from different producers. As it shown in Table 1, for 63.6% samples presences of aflatoxins were detected. Results are represented in ppb (µg/kg).

Table 1. Analyzed samples of corn for aflatoxins content
Tabela 1. Analizirani uzorci kukuruza na sadržaj aflatoxina

Number of analyzed samples / *Broj analiziranih uzoraka*	44
% of infected samples / *% kontaminiranih uzoraka*	63.6
Range AFs (ppb) / *Opseg AFa (ppb)*	5-367
Average for positive samples (ppb) / *Prosečna vrednost pozitivnih uzoraka (ppb)*	74.5
SD / *SD*	79.5
Number of samples that exceeded the ML set by the EU regulative (%) / *Broj uzoraka koji premašuju MDK prema EU (%)*	20 (45.5)
Number of samples that exceeded the ML set by Serbian regulative (%) / *Broj uzoraka koji premašuju MDK prema srpskoj regulativi (%)*	13 (29.6)

Aflatoxins content in positive samples ranged from 5 to 367 ppb. The average value of aflatoxins in contaminated samples was 75.5 and standard deviations of these samples were 79.5. Level of aflatoxins exceeded in 45.5% analyzed samples, according to EU Regulative. In aforementioned samples, 29.6% exceeded level of 50 ppb set by Serbian regulation.

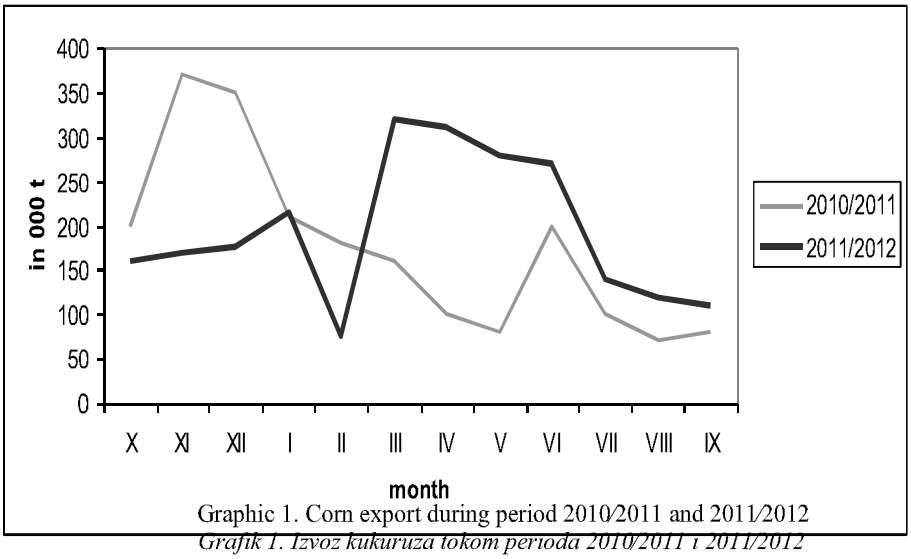
DISCUSSION

In September 2012, harvest area of maize was 1,275,888 ha with expected yield/ha of 2.79 t, which is 45.9% yield/ha less than the year before, and 52.4% less than in 2010, although the harvest area in 2012 was greater than in previous years. Expected production of maize in 2012, was reduced for 45.5% compared to previous year, and 51.0% lower in comparison to 2010. These results are represented in Table 2.

Table 2. Maize production from 2010. to 2012.
Tabela 2. Proizvodnja kukuruza od 2010 do 2012.

REPUBLIC OF SERBIA, Maize/ Year *REPUBLIKA SRBIJA, Kukuruz/ Godina*	2010	2011	2012
Harvest area, ha *Žetvena površina, ha*	1,262,000	1,258,167	1,275,888
Production, t *Proizvodnja, t*	7,207,191	6,479,564	3,532,602
Yield per ha, t *Prinos po ha, t*	5.86	5.15	2.79

In period from March to June, maize grain export was up to 52% higher than the season before (2010/2011). Summary, in 2011/2012 exports of corn was achieved economic record of 2,317,303 t, compared to the year 2010/11 exports increased for 16% (Statistical office of the Republic of Serbia, 2012). Corn export during these two periods has shown in graphic 1.



Graphic 1. Corn export during period 2010/2011 and 2011/2012
Grafik 1. Izvoz kukuruza tokom perioda 2010/2011 i 2011/2012

Increased frequency of controls related to aflatoxins: Most notifications on aflatoxins are related to product/ country of origin combinations for which imposed increased frequencies of controls at import are in force. As such, the number of notifications is enhanced by the increased frequency of control which resulted from the problem identified (RASFF, 2012). Report for feed product, from October 2012, has shown in Table 3. It represents alert for Serbian corn which is released from Italy. Aflatoxins content in corn was exceeded from permitted value, with 126.5, 161.1, 57.1 and 80.8 ppb, thus distribution status for Serbia was restricted to notifying country (RASFF, 2012).

Table 3. Reports feed Week 41 (10/08/2012 - 14/10/2012)
Tabela 3. Izveštaj za hranu za životinje, nedelja 41 (10/08/2012 - 14/10/2012)

Date	Ref.	Notification Type	Notification reason	Notified By	Topic subject	Undertaken actions
10/10 2012	2012 .142 7	Informatio n for Attention	Border control Consignmen t released	Italy	Aflatoxins (B1 = 126.5 / B1 = 161.1 / B1 = 57.1 / B1 = 80.8 µg/kg - ppb) in corn from Serbia, via Croatia	Official detention

CONCLUSION

In addition to high percentage of aflatoxins presence in analyzed samples (63.6%), it is devastating fact that even 45.5% and 29.6% of samples exceeded the maximum tolerable level of aflatoxins, given in the EU, and Serbian regulations, respectively. From the safety point of view, these figures are extremely worrying, also there are crucial for the export of this culture.

For these reasons it is necessary to carry out monitoring in order to prevent occurrence of aflatoxins and other mycotoxins and applied all actions to prevent or decrease the occurrence of these hazardous contaminants to of humans and animals health. Also it is necessary to take care of economic parameters.

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AFLATOKSIN – BEZBEDNOST KUKURUZA, EKONOMSKI IZAZOV U 2012. GODINI

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Izvod

Cilj ovog rada je bio ispitati kukuruz na prisustvo aflatoksina, i na koji način će ova informacija uticati, pre svega na bezbednost ovog hraniva, ali i na ekonomski parametar kada je u pitanju izvoz kukuruza. Naša zemlja spada među najveće izvoznike kukuruza, tako da prisutnost aflatoksina u kukuruzu ima značajne posledice po ekonomske parametre trgovanja ovim hranivom. Ispitana su 44 komercijalna uzorka kukuruza, pri čemu je utvrđeno da je 63,6% ispitanih uzoraka kontaminirano aflatoksinom. Nivo aflatoksina u zaraženim uzorcima se kretao od 5-367 ppb, dok je prosečna vrednost iznosila 74,5 ppb.

Ključne reči: *aflatoksin, kukuruz, Srbija, izvoz.*

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