Presence of some mycotoxins in feedstuffs in Bulgaria during 2017

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Abstract

Mycotoxins are secondary metabolites produced by fungi which can affect a variety of feedstuffs. These compounds elicit toxicological effects which represent risk for both humans and animals.

This study aimed to investigate the presence of mycotoxins Zearalenone (ZEA), Ochratoxin A (OTA), Deoxinivalenol (DON), Aflatoxin, Fumonisin as natural contaminants of maize, wheat, barley and sunflower.

The samples were analyzed by Enzyme-linked immunosorbent assay – ELISA.

A total number of 59 samples had been investigated.

The content of DON for barley was estimated in the range between 0.544 and 2.29 mg/kg. The contamination with aflatoxin was between 2 and 14.49 μg/kg. The highest concentration was estimated in one sample of sunflower. More favorable substrate for Fumonisins accumulation was maize. The occurrence of ZEA varied from 51.65 μg/kg to 126.54 μg/kg in wheat and 48.67–79.64 μg/kg in barley. OTA contamination was between 4.86–7.32 μg/kg in wheat and 4.46–11.70 μg/kg in barley respectively.

These results suggest a high percentage of ZEA and OTA, especially in wheat and barley.

Key words: barley; ELISA; maize; mycotoxins; sunflower, wheat

Introduction

Mycotoxins are secondary metabolites of fungi. The most common mycotoxins are aflatoxins, OTA, fumonisins, DON, T-2 toxin and ZEA. They are produced by the fungal genera of Aspergillus, Fusarium and Penicillium. Many foods and feeds can become contaminated with mycotoxins since they can form in commodities before harvest, during the time between harvesting and drying, and in storage. Mycotoxins produce a wide range of adverse and toxic effects in animals in addition to being foodborne hazards to humans (Zheng et al., 2006).

The impact of mycotoxins on animal health depends on the amount of the mycotoxin consumed, the toxicity of the compound, acute or chronic exposure, the body weight of the individual, the presence of the other mycotoxins and other dietary effects (Kuiper-Goodman, 1991).

Most cereal grains, oil seeds, tree nuts and dehydrated fruits are susceptible to fungus contamination and mycotoxin formation. They are produced under appropriate environmental conditions by fungi species. The most important species are *Aspergillus niger, Aspergillus parasiticus, Aspergillus flavus, Penicillium, Fusarium, Alternaria* etc. (Zinedine et al., 2007; Oancea and Stoia, 2008; Turner et al., 2009).

As can be seen from Table 1 the fungi species produce mycotoxins which are highly toxic compounds. They can cause chronic toxicity in

humans and animals and also they are associated with mycotoxicosis.

These molds can grow on peanuts, corn, cotton seeds, nuts, copra, cereals, oilseeds such as sunflower and soybeans, unrefined vegetable oils, spices (paprika and chili pepper), dried fruits (dried figs and raisins), coffee, cocoa, and feed. *Fusarium* species produced DON, T-2 toxin and Fumonisin. These toxins occur worldwide and are frequently found in maize. OTA is produced by *Penicillium.* The cereal grains are considered to be the main human dietary source of OTA. However, (Cicoňová et al., 2010) suggested that pork products may also be a significant source of this toxin.

Although over 300 different mycotoxins have been identified so far, those of most concern based on their toxicity and occurrence are, aflatoxins, OTA, ZEA, DON, fumonisins and T-2 toxin, which cause significant health implications, mainly through food and feed contamination (Chhonker et al., 2018).

The aim of the study was to investigate the occurrence of common mycotoxins in the main used feedstuffs as maize, wheat, barley and sunflower.

Materials and Methods

Wheat (26 samples), barley (21 samples), maize (7 samples) and sunflower (6 samples) were supplied by different regions of Bulgaria, namely Northeastern, Northwestern, Northern central, South central, Southeastern and Southwestern. Samples were grounded, thoroughly mixed and processed using 70% methanol as solvent for extraction. The filtered samples were screened for aflatoxins, DON, Fumonizin, ZEA, OTA by ELISA method. For the investigations the samples were prepared according to the instructions of the kit manufacturer R-Biopharm.

Using the optical densities (OD) of the standard, the calibration curve is plotted against the concentrations of other standards, and the amount of mycotoxin in the sample is extrapolated from standard curve. The course of the standard curve is shown in the quality Assurance Certificate enclosed in the test kit.

Results and discussion

Samples of wheat, barley, maize and sunflower from different parts of Bulgaria were investigated for ZEA, OTA, fumonisin, aflatoxins and DON.

From the total number (60) of the investigated samples containing wheat (26), barley (21), maize **(**7**)** and sunflower (6) the most contaminated were wheat and barley. Among of the studied mycotoxins the investigations show contamination of ZEA and OTA in wheat and barley,

Table 1. The important moulds species and effects of ingestion

Mould species	Mycotoxins produced	Commodity	Effects of ingestion
Aspergillus parasiticus	Aflatoxins B_1, B_2, G_1, G_2	Wheat, maize, barley peanuts and other commodities	Identified as potent human carcinogens. Adverse effects in various animals, especially chickens
Fusarium graminearum	Deoxynivalenol, Zearalenone	Maize, wheat	Toxic to animals, especially pigs. Affects reproductive system in female pigs Identified as a possible human carcinogen.
Fusarium moniliforme (F. verticillioides)	Fumonisin B_1	Barley, wheat, and many other commodities	Toxic to pigs and poultry. Cause of equine eucoencephalomalacia, a fatal disease of horses.
Penicillium verrucosum, Aspergillus ochraceus, Aspergillus carbonarius	Ochratoxin A	Maize, peanuts, and many other commodities	Suspected as human carcinogen. Carcinogenic in laboratory animals and pigs.

while with another investigated mycotoxins the results are negative. It should be mentioned that only 2 samples of wheat originated from Northwestern region were contaminated with aflatoxins and one sample with DON. 3 samples for barley was found to be contaminated with this mycotoxin too. We did not find any contamination with another investigated mycotoxins. The samples were analysed by ELISA method.

In Fig. 1 it could be seen the contaminated wheat and barley with the investigated mycotoxins.

Among 21 of the tested samples of barley 15 were investigated for zearalenone. The quantity of zearalenon in barley was in the range 48.67–79.64 µg/kg, i.e. the values are lower than 100 µg/kg according to Regulation (EC) 1881/2006 of maximum allowed amounts for certain contaminants.

The results show that 4 samples were contaminated by zearalenon at concentration in the range 40–70 µg/kg. Two samples had a concentration of zearalenon less than 100 µg/kg. These samples are produced from Northeastern and Southeastern regions and the larger number of the investigated samples of barley (9 samples) was not contaminated. These results are in accordance with the previous ones obtained by (Manova and Mladenova, 2009**)** that on the incidence of ZEA in wheat, barley and maize in Bulgaria all obtained ZEA value are bellow of those ones recommended in European regulations.

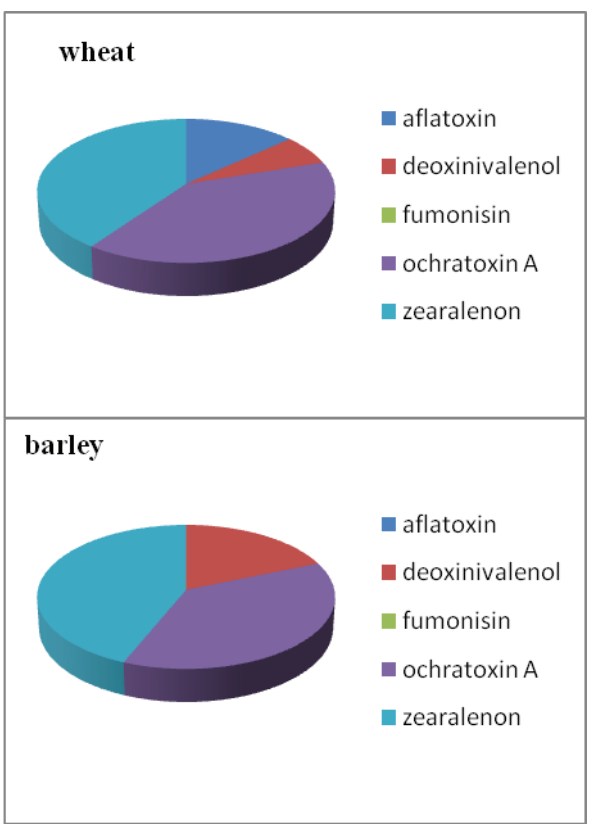

Fig. 1. Shematic depicture of mycotoxins contamination of wheat and barley

Bilal et al. showed that feedstuffs and feeds available in Turkey were contaminated with varied levels of ZEA and that these levels lesser than the tolerable limit.

21 samples of barley were tested for OTA.

Four of the tested samples were contaminated slightly above the norm. It should be mentioned that in one sample we found that the concentration increase by factor of two concerning with maximum determined level of 5 µg/kg and in one sample the concentration is below this value. 15 of the tested samples were negative.

25 samples of wheat were also investigated for ZEA. The situation with detection of ZEA in wheat was a little different. For 6 samples the concentration was varied between 52–92 µg/kg. The detected by us lowest concentration was 51.65 µg/kg and the highest one 126.54 µg/kg. It should be mentioned that this value is up to the recommended limit in the Regulation 1881/2006. It is closed to the value found by (Gutt at al., 2010). They found by ELISA method that 5% (one sample of wheat) of the total number of samples was contaminated with ZEA in an amount greater than the maximum limit permitted by applicable law (100 µg/kg). And it should be note that 17 samples of wheat were not contaminated with zearalenone.

Among of the total 26 wheat samples analyzed for OTA 3 samples were contaminated more than 5 µg/kg according to the Regulation 1881/2006.

Two of wheat samples are contaminated with concentration lower than the limit of 5 µg/kg and it should be noticed that 21 of wheat samples were not contaminated with OTA. (Aydin et al., 2007) in their study concerning the wheat flour

determined that the amount of OTA is bellowing the allowed level of 3 μg/kg in Turkish Food Codex.

From another side (Zinedine et al., 2006) reported that a total of 60 samples consisting of 20 wheat, 20 corn, and 20 barley samples for contamination with OTA in Morocco and found that 40% of 20 wheat samples were contaminated with OTA and the maximum levels of contamination was 1.73 μg/kg.

In Table 2 and Table 3 are shown the mycotoxin's contamination of wheat and barley by regions.

The analyzed samples revealed that the highest contamination with ZEA and OTA in the main cereals – wheat and barley was observed in the Northeastern, Southeastern regions and in South central region. It is noteworthy that in the analyzed samples of wheat produced in the North Central region no zearalenone, DON, ochratoxin A, fumonisin was found.

It should be noted that the wheat produced in the North-Eastearn region is contaminated by factor of 5 than the wheat produced in the South Central Region. It is important to note that the samples supplied from the Southeastern region are "clean" concerning OTA contamination, the results are negative. It has to be mention that the situation with barley contamination is almost the same. Samples supplied from Northeastern are much more contaminated compared with those ones originated from Southeastern and South central regions.

A significant percentage of about 23% of the samples demonstrated the simultaneous presence of both mycotoxins. The amount of both mycotoxins varied in the near range, with ZEA of 48–68 μg/kg and with OTA from 4.46 to 6.43 μg/kg.

These results indicate that the degree of risk in the presence of mycotoxins should be evaluated not only by the number of samples containing several mycotoxins but also by their mean concentrations.

The data on the presence of fungi of the genus Fusarium are similar to those of (Vrabcheva et al., 1996), where they found that 86% of the wheat was contaminated with Fusarium species. These findings confirm the particular importance of Fusarium species as the major grain pollutants in regions with a moderate continental climate where our country also falls. Studies conducted confirm the view that fusarious contamination is high especially for the northern part of the country.

Fumonisin is one of the main contaminants in maize (Munkvold and Desjardins, 1997).

In the mycotoxicological studies carried out on maize, harvest 2017 we found that maize contain mainly fumonisin, fungi of the genus Fusarium. The content was in the range of 1.06 mg/kg to 4.67 mg/kg but it is in the norm. Figure 2 shows contaminated by fumonisin maize and sunflower.

In addition to the presence of fumonisin, the samples were also tested for the other common pollutant in maize aflatoxin.

It can be noted that in 28% of the taken samples, the presence of both mycotoxins-fumonisin and aflatoxin, was found to present at maximum levels of 4.67 mg/kg for fumonisin and 5.7 μg/kg for aflatoxin respectively. These quantities are

Table 2. Zearalenon and ochratoxin A occurrence in wheat and its distributions by regions.

Region	Mycotoxin (%)	
	Zearalenon	Ochratoxin A
Northeastern	42	57
Southeastern	33	0
South central	33	11

Table 3. Zearalenon and ochratoxin A occurrence in barley and its distributions by regions.

Region	Mycotoxin (%)	
	Zearalenon	Ochratoxin A
Northeastern	40	80
Southeastern	42	0
South central	0	1

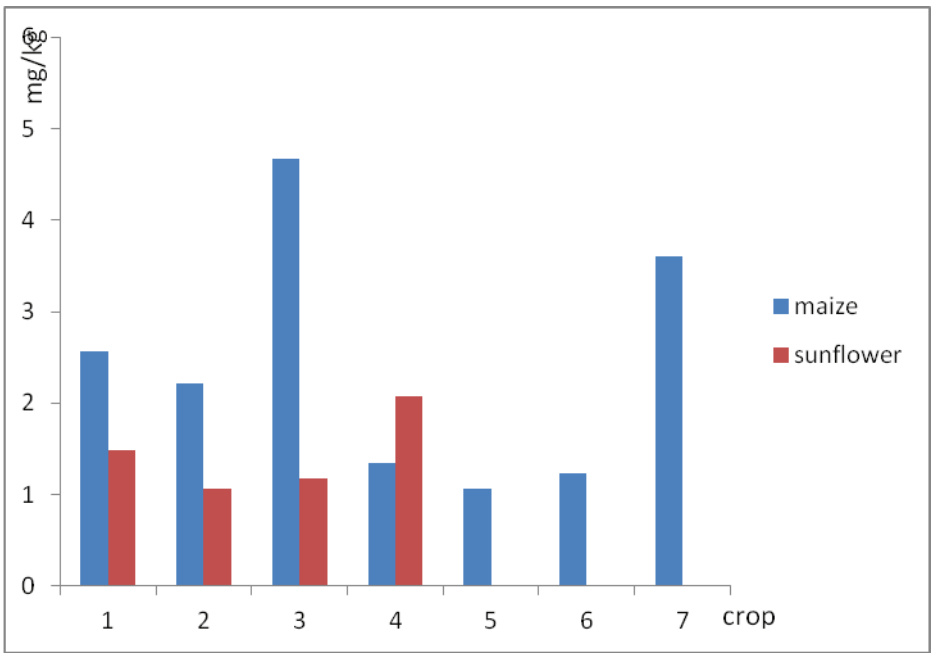

Fig. 2. Maize and sunflower infected by fumonisin

within the normal limits determined in the European regulation where the maximum content of aflatoxin B1 in feed materials is 0,02 mg/kg (i.e., 20 µg/kg).

In the investigated sunflower samples only one sample originated from South central area was contaminated with aflatoxin with concentration of 14.49 µg/kg. This value is closed to the maximum determined value of 20 µg/kg.

Concerning the investigated samples of sunflower we found that 66% of the detected samples were contaminated with fumonisins. 50% of the total contaminated samples are produced in Northwest region and 16 % in Southeastern region.

Conclusion

It can be seen from the obtained results that the studied grains were contaminated with varied levels of ZEA and OTA and that these levels lesser than the tolerable limits. Only in one barley sample the contamination with OTA increases by factor of two and in one wheat sample the concentration of ZEA is a slightly above the limit set in the valid Regulation. The results do not show the presence of DON, Fumonisins and Aflatoxins.

For maize and sunflower Fumonisins pollution predominates compared to those one connected with Aflatoxin contamination where we found that only one sample of maize and one of sunflower were contaminated.

The change in climatic conditions leads to the emergence of mold species with different toxicological characteristics and as a result there is contamination of cereal raw materials with a wide range of mycotoxins.

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