Contamination with Aflatoxins and Fumonisins of some raw feed materials in Bulgaria

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Abstract

Mycotoxins are naturally occurred contaminants. They can enter the human and animal dietary system by direct or indirect contamination.

Aflatoxins and fumonisins are hepatotoxic and carcinogenic metabolites produced by *Aspergillus flavus* and *Fusarium moniliforme*, respectively. *Aspergillus flavus* is common and widespread in nature and is most often found when certain grains are grown under stressful conditions such as draught. Several *Fusarium* species are important pathogens of cereals and corn, causing severe crop yield reduction. To determine whether these mycotoxins exist on maize, sunflower and beans under natural conditions, 30 samples originated from different regions of Bulgaria, harvest 2017 were investigated by using Enzyme-linked immunosorbent assay. Among of the investigated maize samples 20% were contaminated with aflatoxins in the range 2.73–5.74 µg kg^{-1}, in 20% of sunflower samples the contamination was in the range 8.23–14.49 µg kg^{-1} and aflatoxins contamination of beans samples was 40% with concentration 3–15.99 µg kg^{-1}. Fumonisins pollution was registered in 60% of maize samples in the range 1.06–4.67 mg kg^{-1}, in half of examined sunflower samples it was in the range 1.06–2.07 mg $kg^{-1}$and in beans samples we did not detect any contamination of this mycotoxin. The obtained results are lower than the limit set in the EC Regulation 1126/2007. An exception was found in one maize sample where the value for fumonisins was a little up of the referred one.

Key words: mycotoxins, maize, sunflower, beans

Introduction

Aflatoxins and fumonisins are toxic secondary metabolites produced by moulds belonging to various genera such as *Aspergillus* and *Fusarium* (Azizi et al., 2012; Bosco & Mollea, 2012).

They are low molecular weight toxic metabolites, that when ingested, inhaled or absorbed through the skin have the potential to seriously affect human and animal health by acute and chronic effects such as the induction of hepatocellular carcinoma, liver cancer or sudden death due to acute toxicity in the case of aflatoxins (Lewis et al., 2005).

Aflatoxins are acute and chronic toxicity genotoxic and carcinogenic compounds produced by two major *Aspergillus* species; *A. flavus* which produces aflatoxin B1 and B2, and *Aspergillus parasiticus* which produce aflatoxin G1, G2, B1 and B2 (Filazi & Sireli, 2013; Omar, 2013).

The source of aflatoxins are from agricultural commodities like oil seeds such as sunflower groundnut, soybean, and cotton; cereals like maize, tree nuts such as almonds, pistachio, walnuts and coconut; sorghum, pearl millet, rice and wheat; spices like cumin, cinnamon, clove, black pepper, cardamom, ginger, and coriander;

vegetables, milk, meat and dried fruits (Marasas et al., 2008; Wild & Gong, 2010).

Fumonisins produced by *Fusarium* spp. cause oesophageal cancer and neural tube defect leading to abortion (Marasas et al., 2008). Fumonisins occur in maize and infrequently in foodstuffs such as sorghum, asparagus, rice, beans and beer (Creppy, 2002; Zain, 2011), and animal feed from maize sources (Morgensen et al., 2010). The effect of fumonisin on humans has not been fully established, but much evidence suggests a role in human oesophageal cancer from consumption of fumonisin-contaminated maize and maize based products in South Africa, specifically to regions where maize was their staple food (Marasas et al., 2008).

The aim of the present work was to survey fumonisins and aflatoxins concentrations in maize, sunflower and beans collected from four producing regions in Bulgaria during 2017 crop year.

Materials and Methods

The raw samples of maize (10 samples), sunflower (10 samples) and beans (10 samples) were supplied by some regions of Bulgaria, i.e. Northwestern, Northeastern, Southwestern and South central. They were investigated for aflatoxins and fumonisins by Enzyme-linked immunosorbent assay (ELISA) method.

For the investigations, the samples were prepared according to the instructions of the kit manufacturer R-Biopharm.

Reagents

All reagents including standard solutions, conjugate solution, antibody, substrate/chromogen and stop solution must be bringing at room temperature before use. Microtitre plate with 48 wells. Wells are coated with antibodies. 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 measurement was made photometrically at 450 nm. The absorbance was inversely proportional to the mycotoxin concentration in the sample. The values calculated for the standards were entered the Ridawin program, Computer Systems (ELx800 Universal Microplate Reader, BIOTEK® Instruments, Inc., USA).

Results and Discussion

Results obtained after harvest indicated that 20% and 60% of maize samples were contaminated with aflatoxins and fumonisins respectively, 20% and 50% of sunflower samples were contaminated with aflatoxins and fumonisins while for beans the incidence was 40% for aflatoxins and no sample was infected with fumonisins. The range of aflatoxins and fumonisins concentrations for maize, sunflower and beans is reported in Table 1 and as well as the obtained positive values (%) of the investigated mycotoxins.

As can be seen in all positive samples aflatoxins were the major contaminant. The overall lev-

Table 1. Incidence, minimum and maximum values of aflatoxins and fumonisins in maize, sunflower and beans across four regions

Maize	N	Positive samples (%)	Minimum	Maximum
Aflatoxins (µg/kg)	10	20	2.73	5.74
Fumonisin (mg/kg)	10	60	1.06	4.67
Sunflower				
Aflatoxins (µg/kg)	10	20	8.23	14.49
Fumonisin (mg/kg)	10	50	1.06	2.07
Beans				
Aflatoxins (µg/kg)	10	40	3	15.99
Fumonisin (mg/kg)	10	0	0	0

els of aflatoxins ranged from 2.73 µg kg^{-1} to 15. 99 µg kg^{-1} and the overall incidence was 26.7%.

The most contaminated samples with aflatoxins are beans, followed by maize and sunflower (Fig. 1).

Aflatoxins and fumonisins occur in many animal feed concentrates including cereal grains, soybeans products (soybeans meal), oil cakes (from groundnuts, cottonseed, sunflower, palm and copra. McDonald et al. (1995) defined animal feeds as any material provided to animals as part of its daily ration and when ingested is capable of being digested, absorbed and utilised by the body of the animal to meet its nutritional need.

Aflatoxins and fumonisins levels are important for animal health as maize is an important feed component used as an energy-yielding material for animal feeding.

In the previous studies the levels of aflatoxins and fumonisins in maize and raw feed samples are different because of different climatic conditions which are on the base for mycotoxins formation.

The mean aflatoxins concentration of the analyzed maize samples (4.2 µg kg^{-1}) in the current study was quite lower than the results reported by Peker et al. (1994) (36.83 µg kg^{-1}) and Oruc et al. (2006) (10.94 µg kg^{-1}) in Turkey, a little bit higher than the results reported by Solovey et al. (1999)

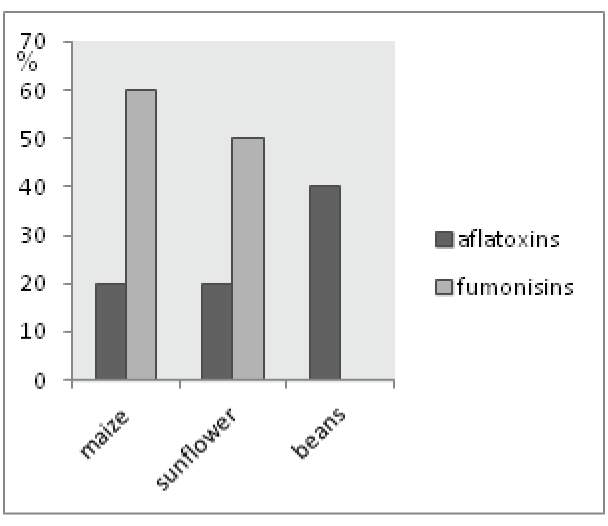

Fig. 1. Aflatoxins and fumonisins occurrence in the investigated feedstuff materials (%)

in Argentina (< 2 µg kg^{-1}) and closed to the results found by Warth et al. (2012) for maize from Mozambique with a mean value of 2.4 µg kg^{-1}.

In this connection is interesting to note that Chauhan et al. (2016) detected in maize samples massive presence of *Aspergillus* species (75%) followed by *Fusarium* (11%). The samples are produced in Gedeo zone in Ethiopia. They concluded that fungal contaminant are potential threat to the agricultural industry and require urgent intervention.

It has to be mentioned that the concentration of aflatoxins (mean 4.2 µg kg^{-1}) in our survey was much lower than the levels set by Ordinance № 10 of 3.04.2009 on the maximum admissible concentrations of undesirable substances and products in animal feed. This means that this concentration will not have any influence on the toxic effect in the animal species and these raw feed materials can be use for animal nutrition.

The mean fumonisin level in maize samples was 2.87 mg kg^{-1}. The level being higher than the levels reported by Solovey et al. (1999) (0.55 mg kg^{-1}) and Scudamore and Patel (2000) (0.01 mg kg^{-1}).

If we follow the incidence (%) our results are closed to these one by Vrabcheva (2004) where 50% of corn samples in Bulgaria were positive for fumonisins contamination in the range 0.03–5.65 mg kg^{-1}.

In another study performed by Manova and Mladenova (2009) for fumonisins occurrence in cereal production (wheat, barley and maize) in Bulgaria they found in the tested samples contamination with fumonisins (94.7%) and only one of them exceeded the maximum limit in EU legislation of 4 mg kg^{-1}.

Comparing to our results we also found in one maize sample fumonisins content of 4.67 mg/kg, which is a little up to this one set in EC Regulation 1126/2007 of 4 mg kg^{-1} and the incidence value (60%) in our survey is lower than this one detected by them.

As it is known the molds produced by *Fusarium spp.* are called "field" mycotoxins that is formed during growth and harvest. The presence of fumonisins in most of studied maize samples can be attributed to the raining period during the harvest in 2017. In Bulgaria, mycotoxins produced by *Fussarium spp.* are the major fungus in maize and infections by this fungus usually occurs when there is cold, wet weather before harvest (Vrabcheva, 2004).

It should be mentioned that such samples might have more hazardous influence on animal health, particularly non-ruminants, due to the cumulative effect of these mycotoxins.

The most susceptible animals to aflatoxin contamination are rabbits, turkeys, chickens, pigs, cows and goats (Lizárraga-Paulín et al., 2011), and for fumonisins the most susceptible animals are horses, pigs and rats (Voss et al., 1995; Smith et al., 1996; Segvic and Pepeljnjak, 2001).

Another lot of tested samples were beans samples. They were also analyzed for aflatoxins and fumonisins. It has to be noted that in the collected samples we have detected only the presence of aflatoxins. All of the contaminated samples were originated from Northeastern region. The level of aflatoxins ranged from 3–15.99 μg kg^{-1} with a mean value of 9.8 μg kg^{-1}. These values are lower than the recommended limit of 20 μg kg^{-1} in Ordinance № 10 of 3.04.2009. Moreover, from another side they are much lower than those one reported by Aiat (2006) in Egypt where he has found the levels of total aflatoxins to be 1463 μg kg^{-1}. Another study was conducted in Rwanda (Nyinawabali, 2013) and reported levels of aflatoxins ranged between 0.2–154.9 μg kg^{-1} with a mean value of 28.1 μg kg^{-1}.

The low aflatoxin levels and the absence of fumonisins in our batch of samples could be attributed to environmental characteristics of the different climatic zones and different agricultural practices which have shown to have an impact on aflatoxins development.

For instance Stössel (1986) reported that soybean seed coat, integrity acts as a barrier against fungal attack, and hence mycotoxins contamination, other factors being constant, this might be the reason for low levels of aflatoxin and fumonisin's absence.

The set of sunflower samples was also analyzed in a way to see the presence of the tested mycotoxins. In the studied samples, we found that the contamination is caused by the both mycotoxins.

Half of the samples were contaminated with fumonisins in the range 1.06–2.07 mg kg^{-1}. It should be noted that the values are below those ones in the EC Regulation 1126/2007 – 4 mg kg^{-1}.

Among of the infected 50% with fumonisins samples 30% were originated from the Northwestern region.

20% of the analyzed samples were positive for aflatoxins. The maximum aflatoxins level (14.49 μg kg^{-1}) was below 20 μg kg^{-1} as referred in the Ordinance № 10 of 3.04.2009. The minimum level of 8.23 μg kg^{-1} found by us was for sample originated from South central part. In Fig. 2 can be seen the contamination by regions.

As can be seen from the figures the aflatoxins and fumonisins contamination of the investigated feed materials is mainly in the Northwestern and Northeastern regions. This could be con-

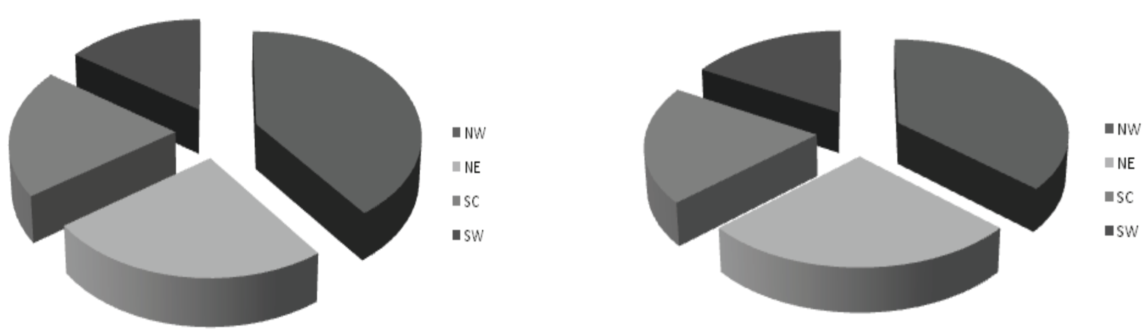

Fig. 2. Distribution of mycotoxins pollution by regions; a) with aflatoxins; b) with fumonisins

nected with atmospheric conditions during the harvest period.

Within the scope of this survey, the low incidence of aflatoxins indicates that the possibility of its contamination with maize and sunflower seeds is remote.

Conclusion

The results of this study demonstrated that the studied raw feed materials maize and sunflower were contaminated with the both mycotoxins while in beans samples fumonisins contamination is absent. The contaminated samples were originated mainly from Northwestern and Northeastern and particularly from Southwestern and South central parts. The detected results were below the maximum permitted levels by European Commmunity standards. Only in one maize sample the fumonisins concentration was a little up than the recommended one of 4 mg kg^{-1}.

These results confirm that the farmers have to make control on feed materials by routine analyses in order to protect the risk of mycotoxins infections and in this will help to decrease the risk of mycotoxicosis.

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