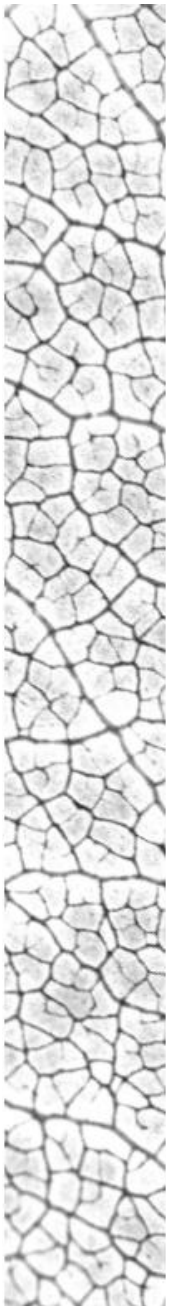

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Determination of mycotoxins in the seeds of sunflower, soybean and corn by enzyme immunoassay

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Abstract
The spread of secondary metabolites of molds in plant products that have adverse effects on humans and animals is an urgent and priority problem from the point of view of food safety. HPLC methods are currently arbitrage in determining mycotoxins and primary metabolites of molds. However, the HPLC method requires a rather large consumption of expensive eluents and solvents, which not only complicates the process, but also significantly increases the cost of analysis. The implementation of the enzyme-linked immunosorbent assay is based on a highly specific reaction “antigen-antibody”, the detection of which occurs due to a change in the color of the corresponding substrate with the introduction of the enzymatic component. To study the contamination of mycotoxins of various varieties of crops (soybean, sunflower, corn) cultivated in the Central Chernozemregion region of Russia. The study was conducted by a direct competitive enzyme immunoassay using the Multiskan FC microbiological analyzer. As standards used ready-made sets “TESTSIP”. As a result of analysis, detectable mycotoxins were found in 9 out of 10 samples of sunflower seed varieties. Exceeding the permissible norm of aflatoxin B1 was recorded in the sample of sunflower seeds of the Mechta variety by 28%. As a result of the analysis, detectable mycotoxins were found in 5 of 6 samples of soybean seeds. The threshold concentration of the acceptable norm of aflatoxin B1 is recorded in samples of soybean varieties Zara and Slavia. As a result of the analysis, detectable mycotoxins were found in all 6 samples of corn seeds. The creation of appropriate conditions for production and storage, as well as the improvement of control methods for agricultural raw materials and products produced from it, is an essential part of the system for ensuring safety and reducing the risk of food mycotoxicosis.

Keywords: mycotoxins, molds, enzyme immunoassay, food products, soybeans, sunflower, corn, aflatoxin B1, fumonisins B1, B2, Zearalenone, T-2 toxin

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INTRODUCTION

Secondary metabolites of microscopic fungi (molds) - mycotoxins, have toxic properties for both humans and animals. There is every reason to believe that mycotoxins perform numerous functions aimed at ensuring the survival of microscopic fungi and their competitiveness in the struggle for a place in various ecological niches (Lessner and Golden 2014, Garibova 2016, Gusev and Mineeva 2003, Shiberu and Tamiru 2016).

The spread of secondary metabolites of molds in plant products that have adverse effects on humans and animals is an urgent and priority problem from the point of view of food safety.

More than 300 mycotoxins are known. More than 10 000 strains belonging to 350 species produce mycotoxins (Efimochkina et al. 2019), mainly the following genera of fungi: Aspergillus, Fusarium, Penicillium, Claviceps and Neotyphodium. The most favorable conditions for mold damage in storages and warehouses are a 60% concentration of CO2, temperature + 20 C and relative humidity above 95%. Some species can breed at lower temperatures.

Mycotoxins accumulate in such organs of fungi as conidia and sclerotia, and can also be in the substrate (Kravchenko and Tutelyan 2005, Tutelyan and Kravchenko 1985). These metabolites are formed from the products of the main metabolism in a chain of sequential enzymatic reactions such as amino acids, malic, citric and other acids of the Krebs cycle. The most important reactions of mycotoxin biosynthesis are condensation, oxidation, reduction, and alkylation, which

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lead to the formation of various mycotoxin precursors (Bhatnagar and Ehrlich 2002, Berthiller et al. 2005, Koteswara et al. 2016). In the Central Chernozem region of Russia, fumazines B1, T-2 toxin, aflotoxin B1, and zearalenone are of greatest importance.

Currently, the common mycotoxins present in products have been studied, their number is strictly regulated.

To date, various methods for the determination of mycotoxins in foods are known:

- thin-layer chromatography (TLC) (Soleas et al. 2001);
- gaschromatography (GC) (Malinkin and Sedova 2016);
- high-performance liquid chromatography (HPLC) (Selimov et al. 2017, Slepchenko et al. 2014);
- voltammetric analysis (Medyantseva et al. 2014);
- determination using amperometric biosensors (Evtyugin and Porfireva 2018, Medvedevsky et al. 2018);
- Enzyme Immunoassay (EIA) (Dubovskaya et al. 2018, Startseva 2019);
- Immunochromatographic assay (ICA) (Chasov et al. 2014);
- Capillary electrophoresis (Tsao et al. 2008, Maragos et al. 2008, GOST standard 34140-2017 2018).

Currently, the following methods are regulated in the Russian Federation: HPLC with mass spectrometric detection and direct solid state competitive EIA (GOST standard 34140-2017 2018, GOST standard P 55569-2013 2015).

It should be noted that HPLC methods are currently arbitrage in determining mycotoxins and primary metabolites of molds. However, the HPLC method requires a rather large consumption of expensive eluents and solvents, which not only complicates the process, but also significantly increases the cost of analysis.

The implementation of the enzyme-linked immunosorbent assay is based on a highly specific reaction “antigen-antibody”, the detection of which occurs due to a change in the color of the corresponding substrate with the introduction of the enzymatic component. This method is widely used for routine studies and has several advantages over chromatographic analyzes: efficiency, high productivity (up to several dozen analyzes at the same time), ease of sample preparation and measurement, low cost of analysis, small sample volume.

Capillary electrophoresis is an intensively developing method for the separation of complex mixtures, which allows the analysis of ionic and neutral components of various nature with high expressivity and unique efficiency. It can be used to study the primary metabolites of molds:

- for the quantitative determination of amino acids (the procedure is regulated as GOSTstandart P) (GOST standardP 56373-2015 2016);
- for the quantification of organic acids (Piven 2007).

The method of capillary electrophoresis has several advantages compared with other methods of separation: high separation efficiency, speed of analysis, low consumption of reagents and solvents, the minimum amount of sample to be analyzed, low cost of a single analysis.

Also, according to publications, the CE method is successfully used for the quantitative determination of mycotoxins.

The aim of this work was to study the contamination of mycotoxins of various varieties of crops (soybean, sunflower, corn) cultivated in the Central Chernozem region of Russia.

MATERIAL AND METHODS

The study was conducted by a direct competitive enzyme immunoassay using the Multiskan FC microbiological analyzer. As standards used ready-made sets “TESTSIP”:

1. EIA TESTSIP® kit Afla B1 (Aflatoxin B1),
2. Set for EIA TESTSIP® Fum (Fumonisins B1, B2),
3. Set for EIA TESTSIP® ZON (Zearalenone),
4. Set for EIA TESTSIP® T-2 (T-2 toxin).

Sunflower varieties were selected for the study: Voronezhkiy 638, Efko14, Hybrid NIKA, Factor, Commandor, Metschta, Rodnik (R-453), Umnik, Buzluk, Photon; soy: Vestochka, Zara, Olimpia, Slavia, Duniza, Chara; maize: Hybrid Mashuk 171, Belogorye S, Belozerny 1 MV, Belkha 234 MV, Belmondo, GS 210, Dniprovskiy 195 SV.

EXPERIMENTAL TECHNIQUE

Cereal samples are crushed and thoroughly homogenized, parallel weighed samples weighing 20.0 g are taken, transferred to a conical flask with a volume of 250 cm3, 100 cm3 of methanol are added with a volume fraction of 70%. Stirred for 40 minutes, filtered. If necessary, dilute the extracts. The extract and the conjugate are mixed, the mixture is introduced into the wells of a plate with immobilized monoclonal antibodies. There is a competition between the mycotoxins of the sample and free mycotoxins for a strictly defined amount of antibodies. Then the remaining mycotoxins are removed from the wells by washing, a chromogenic substrate and a stop solution are added. Measure the optical density of the wells at a wavelength of 450 nm using a tablet photometer. The content of mycotoxins in the samples is determined by the calibration characteristics of the solutions of mycotoxins from the «TESTSIP» kits.

Table 1. The results of studies of samples of oilseed sunflower seeds

№	The variety ofsunflower	The concentration of mycotoxins C, mg/kg			
		Aflatoxin B1	Zearalenone	Fumonisins B1	T-2 toxin
		Norm: no more than 0.005		Notregulated	
1.	Voronezhkiy 638	0.0033±0,0006	-	1.0002±0.0007	-
2.	Efko14	0.0002±0,0001	-	0.2541±0,0021	0.2845±0.0011
3.	Hybrid NIKA	-	0.0525±0,0005	0.2575±0.0005	-
4.	Factor	-	-	-	-
5.	Commandor	0.0013±0.0004	0.1039±0.0009	1.2502±0.0004	-
6.	Metschta	0.0066±0.0005	-	-	-
7.	Rodnik (R-453)	0.0002±0.0001	0.5563±0.0011	-	0.1225±0.0005
8.	Umnik	0.0027±0.0005	0.0474±0.0009	-	-
9.	Buzluk	0.0007±0.0001	-	-	0.0258±0.0008
10.	Photon	-	-	0.5678±0.0010	-

* p≤0.001

Table 2. Soybean seed test results

№	The variety of soy	The concentration of mycotoxins C, mg/kg			
		Aflatoxin B1	Zearalenone	Fumonisins B1	T-2 toxin
		Norm: no more than 0.005		Notregulated	
1.	Vestochka	0.0011±0.0002	-	0.2581±0.0005	-
2.	Zara	0.0050±0.0002	-	-	0.2513±0.0005
3.	Olimpia	-	-	-	-
4.	Slavia	0.0055±0.0004	-	-	-
5.	Duniza	-	0.1522±0.0004	-	-
6.	Chara	0.0025±0.0004	-	-	-

* p≤0.001

Table 3. Corn Seed Test Results

№	The variety of corn	The concentration of mycotoxins C, mg/kg			
		Aflatoxin B1	Zearalenone	Fumonisins B1	T-2 toxin
		Norm: no more than 0.0050	Norm: no more than 1.0000±0.069	Notregulated	Norm: 0.1000
1.	Hybrid Mashuk 171	-	1.1003±0.0008	0.3820±0.0007	-
2.	Belogorye S	-	-	-	0.1548±0.0009
3.	Belozerny 1 MV	0.0021±0.0004	-	-	-
4.	Belkha 234 MV	0.0018±0.0001	-	0.2551±0.0005	-
5.	Belmondo	-	0.3545±0.0009	-	-
6.	Gs 210	-	-	1.5314±0.0010	0.1005±0.0006
7.	Dniprovskiy 195 Sv	0.0076±0.0005	-	0.2515±0.0008	0.1849±0.0008

* p≤0.001

RESULTS AND DISCUSSION

Sunflower samples were examined for the presence of microtoxinsfumazin B1, aflotoxin B1, zaaraleon. The results of the study are presented in **Table 1**.

As a result of analysis, detectable mycotoxins were found in 9 out of 10 samples of sunflower seed varieties. The regulated indicator of mycotoxins in sunflower culture, according to the Technical Regulations of the Customs Union, is the presence of aflatoxin B1 with a concentration of not more than 0.005 μ / kg. Exceeding the permissible norm of aflatoxin B1 was recorded in the sample of sunflower seeds of the Mechta variety by 28%.

Also, during the analysis, 6 soybean varieties of the 2019 harvest were selected (**Table 2**).

As a result of the analysis, detectable mycotoxins were found in 5 of 6 samples of soybean seeds. The regulated indicator of mycotoxins in soybean culture, according to the Technical Regulations of the Customs Union, is the presence of aflatoxin B1 with a concentration of not more than 0.005 μg / kg. The threshold concentration of the acceptable norm of aflatoxin B1 is recorded in samples of soybean varieties Zara and Slavia.

Similar studies were carried out for seed samples of 7 cultivars of the corn crop of 2019 harvest (**Table 3**).

As a result of the analysis, detectable mycotoxins were found in all 6 samples of corn seeds. The regulated indicator of mycotoxins in maize culture, according to the Technical Regulations of the Customs Union, is the presence of aflatoxin B1, with a concentration of not more than 0.005 microns / kg; zearalenone - not more than 1.0000 mg / kg; T-2 toxin - not more than 0.1000 mg / kg. Exceeding the aflatoxin B1 standards was observed in seeds of the Dneprovsky 195 St. variety by 52%. In seeds of Hybrid Mashuk 171, an excess of the permissible concentration of zearalenone by 10% was found. T-2 toxin was detected in three samples of corn seeds - varieties Belogorye C (concentration exceeded by 55%), varieties Gs 210 (threshold concentration), Dneprovsky 195 St. (concentration exceeded by 85%).

CONCLUSION

Thus, the secondary products of the metabolism of molds - mycotoxins are quite widespread among agricultural products, mold microflora is able to reproduce in a wide range of living conditions, as well as adapt to adverse effects. Therefore, the creation of appropriate conditions for production and storage, as

well as the improvement of control methods for agricultural raw materials and products produced from it, is an essential part of the system for ensuring safety and reducing the risk of food mycotoxicosis. In-depth studies on this problem will solve the problems of differentiated rationing and hygienic regulation of molds and new types of mycotoxins in food raw materials and food products.

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