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OCCURRENCE OF MYCOTOXINS IN CEREAL GRAINS FROM ORGANIC FARMING AFTER A LONG STORAGE TIME

Summary

Mycotoxin contamination of food and feed is a big problem, also with regard to cereal products from organic farming. For that reason screening of mycotoxins occurrence in cereal grains from organic farming after prolonged storage was performed. The work presents monitoring of the contamination of organic spring wheat, spelt, oat, rye and spring barley harvested in 2017, by the most important mycotoxins: deoxynivalenol (DON), zearalenone (ZEA), ochratoxin A (OTA), sum of aflatoxins (AFL) and the sum of T-2 and HT-2 toxins after nine months of grain storage in different storage systems. Content of mycotoxins was defined by enzyme-linked immunosorbent assay ELISA. Concentration of mycotoxins was very diverse and high in relation to OTA in spring barley and rye and in relation to Fusarium toxins (DON, ZEA, T-2/H-T2) despite low moisture of the grains (below 15%). Among analyzed cereals samples, rye was the grain that was the most frequently contaminated with all kinds of mycotoxins.
***Key words**: mycotoxins, cereal grain, organic farming, moisture, molds*

MIKOTOKSYNY W DŁUGO PRZECHOWYWANYM ZIARNIE ZBÓŻ Z UPRAW EKOLOGICZNYCH

Streszczenie

Zanieczyszczenie żywności i pasz mikotoksynami stanowi duży problem, również w odniesieniu do produktów zbożowych pochodzących z rolnictwa ekologicznego. Z tego powodu przeprowadzono monitoring występowania mikotoksyn w ziarnie zbóż z upraw ekologicznych po dłuższym okresie jego przechowywania. W pracy przedstawiono wyniki oznaczeń pięciu najważniejszych mikotoksyn: deoksyniwalenolu (DON), zearalenonu (ZEA), ochratoksyny A (OTA), sumy aflatoksyn (AFL) oraz sumy toksyn T-2 i HT- 2 w pszenicy jarej, orkiszu, owsie, życie i jęczmieniu jarym po dziewięciu miesiącach przechowywania ziarna w różnych systemach magazynowych. Zawartość mikotoksyn została określona za pomocą testu immunoenzymatycznego ELISA. Stężenie mikotoksyn było bardzo zróżnicowane i wysokie w odniesieniu do ochratoksyny A w jęczmieniu jarym i życie oraz w odniesieniu do toksyn fuzaryjnych (DON, ZEA, T-2/HT-2), pomimo niskiej wilgotności składowanego ziarna (poniżej 15%). Spośród przebadanych zbóż, próbki żyta były najbardziej zanieczyszczone wszystkimi oznaczanymi mikotoksynami.
***Słowa kluczowe**: mikotoksyny, ziarno zbóż, rolnictwo ekologiczne, wilgotność, grzyby pleśniowe*

1. Introduction

Storage of agricultural produce is a key stage in the agri-food production chain. Improper storage conditions of cereals favor the growth of microorganisms, including molds [14]. Molds are known for their ability to producing mycotoxins – toxic, carcinogenic, immunosuppressant, teratogenic etc. substances contaminating food and feed products. These compounds are mainly formed by molds of the genera *Aspergillus, Penicillium, Fusarium, Alternaria and Claviceps.* The most important mycotoxins present in cereals and created in our climatic conditions are: ochratoxin A (OTA), deoxynivalenol (DON), zearalenone (ZEA), T-2 / HT-2 toxin, as well as aflatoxins (AFL). Ochratoxin A and aflatoxins are produced during grain storage, other mycotoxins are formed during plant vegetation by *Fusarium* spp. [4, 6, 19]. In Poland, mycotoxins produced by *Fusarium* fungi are the most often detected [7]. Contamination of raw materials, food and feed with mycotoxins is a serious health, as well as economic problem. For instance DON is known as a immunosuppressant and may cause kidney problems. When consuming DON-contaminated grain, vomition syndrome could occur. Zearalenone has estrogenic effects [15]. Moreover, contaminated grain is not suitable for further processing because mycotoxins are not decomposed during technological processes [1, 3, 10]. Water activity and temperature are two main factors which affect the growth of molds and the production of mycotoxins [8, 15]. For that reason it is so important to preserve Good Storage Practice which aims to prevent grain contamination with mycotoxins by creating conditions unfavorable to molds growth (water activity below 0.85 which corresponds to grain moisture below 15% and storage temperature below 15°C) [8]. Research conducted in recent years in Poland has shown that mycotoxin contamination of food and feed is a big problem, also with regard to cereal products from organic farming [17, 18].

The aim of the study was to assess the degree of mycotoxin contamination of cereal grains from organic farming after a long period of storage.

2. Materials and methods

A total of 27 samples of cereals grains cultivated in different regions of Poland were analyzed. The grain harvested in 2017 came from organic farms with a crop area up to 10

ha. The cereal has been stored in various ways (Table 1). The samples of different types of cereals from the same voivodeship and county came from the same agricultural farm. Samples were collected for analysis after nine months of storage. A representative sample of the grain was grinded so that 95% will pass through a 20-mesh sieve, then the sample portion was thoroughly mixed. 20 g of grounded sample was extracted with 70% methanol for 15 minutes and then filter through a Whatman #1 filter. pH of extracts were regulated to values 6-8 and then mycotoxins, such as deoxynivalenol (DON), zearalenone (ZEA), total aflatoxin (AFL), ochratoxin A (OTA) and the sum of T-2 and HT-2 toxins (T-2/HT-2) were analyzed by direct competitive enzyme-linked immunosorbent (ELISA) method with the usage of AgraQuant® Assays produced by Romer Labs®. Limit of detection of DON, ZEA, AFL, OTA and T-2/HT-2 was 250; 20; 1,4; 2,0 and 10 μg kg^{-1} respectively.

Grain moisture was determined according to PN-EN ISO 712:2012. The total number of molds was determined by the plate method according to PN-EN ISO 4833:2004+Ap1:200 and PN-ISO 21527-2:2009. Sample weight was diluted 10 times with saline and then further diluted from 10^{-1} to 10^{-5}. From the last three dilutions 0,1 mL was plated on agar YGC medium (in triplicate) and then incubated in 25°C for seven days.

3. Results and discussion

Moisture of grains did not exceed 15% and ranged between 9.0-12.6% and was not beneficial for molds growth (Table 1). Grain moisture is crucial in the process of storage. Moisture at 13-13.5% limits the formation of toxins [14, 16]. Molds spores germinate at grain moisture above 15%. The developing mycelium causes an increase in humidity and temperature, which promotes the further development of fungal colonies. In areas of intense mycelium development, the most common phenomenon is self-heating and caking of grain [8].

The number of molds was very diverse and amounted to 3,00 - 5,38 log CFU g^{-1} regardless of grain moisture (Table 1). The number of molds in the marked range is natural for this type of plant material. For comparison in wheat grain stored in two different silos the number of molds amounted to 2,4-5,0 log CFU g^{-1} and belonged mainly to *Aspergillus* and *Penicillium* genera, however molds from *Fusarium* genera were also found [11]. According to other authors the degree of contamination by *Fusarium* molds in relation to the total number of molds decreases during cereal storage, while the number of stored fungi of *Aspergillus* and *Penicillium* species increases [9, 16].

In this study mycotoxin contamination of cereal grains was varied and high in relation to *Fusarium* toxins (Table 2).

In the case of ochratoxin A, spelt and oat were free from contamination with this kind of toxin (concentration of OTA was below limit of quantification). OTA content was very high in spring barley and rye. This toxin was not detected in most samples of spring wheat, but in three samples for nine OTA concentration was above the allowable limit, which is 5 μg kg^{-1} according to (WE) Nr 1881/2006 (Table 2).

Table 1. Moisture and the number of molds in organic cereal grains from different parts of Poland
Tab. 1. Wilgotność oraz liczba grzybów pleśniowych w ziarnie zbóż z upraw ekologicznych z różnych części Polski

Cereal	Number of the sample	Voivodeship / county	Storage method	Moisture (%)	Number of molds (log CFU g^{-1})
Sping wheat	1	lubelskie / krasnostawski	big bags	12,9	4,65
	2	dolnośląskie / lwówecki	big bags	11,2	4,30
	3	lubelskie / parczewski	loosely on the concrete floor	11,3	4,30
	4	mazowieckie / zwoleński	wooden chest	11,7	4,00
	5	warmińsko-mazurskie / działdowski	silos	12,6	3,60
	6	małopolskie / proszowicki	wooden chest	9,1	3,70
	7	łódzkie / zduńskowolski	loosely on the concrete floor	10,0	3,65
	8	lubelskie / łęczyński	big bags	12,0	3,00
	9	wielkopolskie / międzychodzki	poliethylene bag	9,6	4,70
Rye	1	podkarpackie / brzozowski	silos	11,6	4,54
	2	mazowieckie / zwoleński	wooden chest	11,2	4,54
	3	podlaskie / białostocki	loosely on the concrete floor	10,0	4,40
	4	łódzkie / zduńskowolski	loosely on the concrete floor	10,0	4,11
	5	wielkopolskie / międzychodzki	poliethylene bag	10,8	4,48
Spring barley	1	wielkopolskie / międzychodzki	poliethylene bag	11,8	4,00
	3	małopolskie / dąbrowski	silos	11,3	5,04
	4	małopolskie / proszowicki	wooden chest	9,4	3,30
	5	lubelskie / bialski	loosely on the concrete floor	9,9	5,30
Spelt	1	mazowieckie / zwoleński	wooden chest	11,4	4,30
	2	kujawsko-pomorskie / mogileński	silos	10,6	3,00
	3	dolnośląskie / lwówecki	big bags	9,4	5,04
	4	lubelskie / łęczyński	big bags	9,0	4,90
Oat	1	dolnośląskie / lwówecki	big bags	12,3	4,20
	2	zachodniopomorskie / słowieński	loosely on the concrete floor	10,7	4,60
	3	mazowieckie / zwoleński	wooden chest	9,1	5,38
	4	wielkopolskie / międzychodzki	poliethylene bag	6,5	4,48
	5	lubelskie / bialski	loosely on the concrete floor	9,6	4,54

Source: own study / Źródło: opracowanie własne

Table 2. Mycotoxins in organic cereals grains
Tab. 2. Mikotoksyny w ziarnie zbóż z upraw ekologicznych

Cereal	Number of the sample	Mycotoxins (μg kg^{-1})				
		OTA	AFL	DON	ZEA	T-2/HT-2
Spring wheat	1	<2,0	1,2	132,1	50,4	56,2
	2	<2,0	1,4	578,8	37,6	65,9
	3	<2,0	1,5	663,0	63,8	74,0
	4	<2,0	1,5	622,7	27,5	51,9
	5	<2,0	1,4	682,9	40,5	69,7
	6	<2,0	1,7	598,4	32,0	58,3
	7	**9,3**	1,4	572,2	34,4	61,6
	8	**10,1**	1,6	518,0	28,4	53,3
	9	**12,7**	1,3	223,2	**108,3**	**127,7**
Rye	1	3,5	2,1	902,8	55,5	**241,6**
	2	**5,4**	3,0	**1461,7**	**105,6**	**215,5**
	3	**5,8**	3,2	849,7	**216,9**	89,2
	4	**17,5**	**6,5**	**1468,3**	**424,0**	**647,6**
	5	**10,9**	**4,1**	19,3,9	**221,2**	**157,1**
Spring barley	1	**11,3**	1,5	294,6	**134,8**	<10,0
	2	**28,8**	1,6	699,9	**148,2**	25,1
	3	**12,2**	1,8	783,0	**153,5**	14,9
	4	**20,0**	1,5	**1281,2**	**135,3**	44,9
Spelt	1	<2,0	3,2	994,7	59,4	27,1
	2	<2,0	<1,0	766,5	<20,0	**282,1**
	3	<2,0	<1,0	1047,3	**100,4**	**398,4**
	4	<2,0	2,1	1002,3	**545,5**	**110,8**
Oat	1	<2,0	<1,0	**1967,0**	69,8	63,7
	2	<2,0	<1,0	**2550,3**	24,9	**363,0**
	3	<2,0	<1,0	**2040,2**	36,3	**852,2**
	4	<2,0	<1,0	532,6	<20,0	<10,0
	5	<2,0	<1,0	**2077,0**	<20,0	83,2

Source: own study / Źródło: opracowanie własne

In the case of other mycotoxins produced by molds during storage of grain – aflatoxins – their occurrence was not detected in oat. In the rest of analyzed grains concentration of AFL was at acceptable level, except of two samples or rye, where the concentration of AFL was over 4 μg kg^{-1} (WE Nr 1881/2006) (Table 2).

As far as deoxynivalenol is concerned, its occurrence was detected primarily in oat grains, where it was above the acceptable limit (1750 μg kg^{-1}). In the case of spring wheat and spelt, the level of DON contamination was at the acceptable level (below 1250 μg kg^{-1}). Exceeded level of DON content was recorded in some samples of rye and spring barley grains (Table 2).

The content of zearalenone was above acceptable limit 100 μg kg^{-1} (WE Nr 1881/2006) in all samples of spring barley and in four for five samples of rye grains. In one sample of spelt grains ZEA was not detected, in another concentration of ZEA was five times higher than acceptable maximum (Table 2).

In the case of T-2/HT-2 toxins maximum acceptable limit in cereals has not been defined so far. However, Commission Recommendation of 27 March 2013 on the presence of T-2 and HT-2 toxin in cereals and cereal products defines indicative levels for the sum of T-2 and HT-2, whose investigations should be performed, certainly in case of repetitive findings. In this study acceptable level (200 and 1000 μg kg^{-1} respectively) of the sum of T-2 and HT-2 toxins was not exceeded only in the case of barley and oat (Table 2).

Mycotoxins are commonly found in cereals around the world. In Poland, mycotoxins produced by *Fusarium* fungi are the main threat to food/feed safety [2]. DON and ZEA are produced by *Fusarium graminearum* and *F. culmorum* [5, 20]. High level of *Fusarium* toxins detected in grains in this study indicates that the plants must have been infected by *Fusarium* molds during the vegetation. *Fusarium* mycotoxins are naturally present in cereals in the amount of 200-300 μg kg^{-1}. However, in conditions conducive to the development of molds (warm weather and high humidity) the content of *Fusarium* mycotoxins may increase to a dangerous level of 2-4 mg kg^{-1} [8].

In other study different mycotoxins were determined in 99 samples of winter wheat and 48 samples of barley, oat and triticale cultivated in five regions of Poland in 2014. Mycotoxins produced by *Fusarium* fungi were the major contaminants [2]. In subsequent study on mycotoxins in fodder based on grain (including maize) cultivated between 2006 and 2009 in various regions of Poland, DON, ZEN, OTA, T-2 and HT-2 were identified as the most frequently-occurring toxins [7]. In 2010, the common occurrence of fusaria mycotoxins such as: deoxynivalenol, T-2 toxin and zearalenone in organic cereal products was found. No presence of the storage mycotoxins, (aflatoxins and ochratoxin A) was found in any tested product [17].

The presence of mycotoxins in cereal grain is affected by the biodiversity of molds that develop on plants during their vegetation, as later in the stored raw material [4]. Proper drying and cooling of the grain does not ensure a long storage period, without qualitative changes in the grain. After loading into the warehouse, the grain should also be periodically ventilated in order to limit the temperature difference in the stored grain, and thus prevent changes

in grain moisture [8]. Methods of grain storage used in small farms, especially based on big bags do not allow active ventilation of the grain, which could be the reason of high mycotoxins content after the long storage period. For that reason it seems important for organic farming to use biopreparations which, used on plants during their vegetation, inhibit the development of toxinogenic molds [12, 13].

4. Conclusion

The results of screening analysis shows that contamination of cereals from organic farming with mycotoxins is a current problem. Coexistence of different mycotoxins creates a synergistic action of these toxins. The content of *Fusarium* mycotoxins: DON, ZEA and T-2/H-T2 toxins in cereals from the harvest conducted in 2017 is an evidence that there were favorable weather conditions for the development of *Fusarium* molds. The grain storage system, which does not provide active grain ventilation, could also affect development of molds during prolonged storage period.

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