

Mycotoxin contamination of grain of selected winter wheat genotypes

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Abstract. This study defines several mycotoxin (Aflatoxin B1, Aflatoxin B2, Aflatoxin G1, Aflatoxin G2, Ochratoxin A, Deoxynivalenol, Zearalenone, Toxin T-2, Toxin HT-2, Nivalenol, Fusarenon X, 3-Acetyl-deoxynivalenol) contamination of winter common, durum, spelt and einkorn wheat genotypes. The compared species (*Triticum aestivum* ssp. *vulgare*, *T. durum*, *T. aestivum* ssp. *spelta* and *T. monococcum*) have different susceptibility to *Fusarium* and toxin accumulation. Durum wheat (cv. Komnata) was the most susceptible to contamination with mycotoxins. In durum grain the highest level of contamination was detected, especially with Deoxynivalenol (2–4 times over the allowed level for unprocessed grain). *T. aestivum* ssp. *spelta* (cv. Schwabenkorn) and *T. monococcum* (EN 5003) showed the lowest mycotoxin level. *Triticum aestivum* ssp. *vulgare* (cv. Tonacja) was less contaminated with mycotoxins than *Triticum durum* but more than *T. aestivum* ssp. *spelta* and *T. monococcum*. Other mycotoxins in grain of the examined genotypes occurred in trace amounts.

keywords: common wheat, durum wheat, einkorn wheat, grain quality, mycotoxins, spelt wheat

INTRODUCTION

Mycotoxins are food contaminants, of natural origin, highly dangerous to humans and animals. Their presence and level in cereal grain are extremely important parameters of its quality. They are products of metabolism of mould fungi, e.g. *Fusarium*, *Aspergillus*, *Penicillium*. Mycotoxins can be the cause of serious, acute or chronic, alimentary toxicoses, due to the accumulation of the toxins in the organism, as they have toxic, immunosuppressive, carcinogenic and teratogenic effects, their common feature being a low molecular weight (Grajewski, 2006). Toxin

accumulation is favoured by high moisture, favourable for fungal growth, as well as temperature and nutrient availability. It is estimated that approximately 25% of world cereal production is contaminated with mycotoxins (Chanaiyah, 2011).

In our climate the most important mycotoxins contaminating cereals are Deoxynivalenol (DON, also called vomitoxin), Zearalenone, Ochratoxin A, and T-2/HT-2 toxin. Mycotoxins can appear on cereal plants during their growth in the field, as well as due to improper storage of cereal grain. Trichothecenes, including e.g. DON, are formed during the vegetation of cereals as a result of the activity of such *Fusarium* fungi as *Fusarium culmorum*, *F. poae*, *F. gramineum*, *F. crookwellense*, *F. acuminatum*, *F. sporotrichoides* (Grajewski, 2006). In Poland two *Fusarium* species are most often encountered: *F. culmorum* and *F. avenaceum* (Bottalico, Perrone, 2002). Higher levels of Deoxynivalenol are often detected in maize, wheat (especially in winter wheat cultivars) and in barley, while lower levels emerge in oat, rye, sorghum and rice (Binder et al., 2007). *Fusarium* spp. attacks heads and panicles of cereals and maize cobs worldwide. On post-harvest residues or in the soil, *Fusarium* survives contaminating cultivations in the following season (Selwet, 2010). Food and feed obtained using contaminated raw material will contain mycotoxins (Chelkowski, 1985). In the European Union as much as 57% of food of cereal origin is contaminated with DON (Nielsen et al., 2009).

The production of mycotoxins is affected by various environmental factors, such as the deficit or presence of an important nutrient in the substrate or the occurrence, at a given location, of other competitive species of mould fungi or bacteria (Miller, 1994).

The collection of *Fusarium* species isolated from kernels of hulled wheat cultivars varies in relation to the geographic region, year of study, weather conditions during the vegetation or the production technology applied, simi-

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larly as in the case of common wheat (Oerke et al., 2010). The species composition of fungi causing head fusariosis depends on the atmospheric conditions, and especially on precipitation and temperature in the blooming phase of cereals and on production technology factors such as soil tillage, nitrogen fertilisation, use of fungicides, crop rotation, and genotype of the host plant (resistant cultivars) (Bottalico, Perrone, 2002). The growth of *Fusarium* is stimulated by air temperature above 20°C during wheat blooming, with high air humidity (above 85-90%), for a minimum of one day (Podolska, 2013). Although, Packa et al. (2013) report that while *F. gramineum* infects plants at temperature of ca. 25°C and high humidity, *F. culmorum*, *F. avenaceum* or *F. poae* can attack plants already at 15°C and suitable high humidity. Another factor conducive to the growth of *Fusarium* is water activity above $aw=0.87$. A head infected during the period of maturation of the cereal becomes covered with a salmon-coloured bloom, the kernels are often wrinkled and poorly developed (Podolska, 2013). Unfortunately, the typical fusariosis is hard to identify with the naked eye as it is usually accompanied by other fungal and bacterial diseases (Gašiorowski, 2004).

It has been found that certain (hitherto unknown) factors may cause the loss of the ability of mycotoxin synthesis by fungi (Schrödter, 2004).

The high toxicity of *Fusarium* toxins results from their structure, and namely from the presence of an epoxy ring which is highly reactive (Tamm, Breitenstein, 1980). Generally, DON is toxic and immunotoxic. According to some researchers, Deoxynivalenol, through changes at the level of chromosomes and cells, may cause cancers in mammals (Packa, 2006). High levels of Deoxynivalenol in wheat grain are a problem for many producers of food (flours and cereal products), DON is resistant to technological processing, including heat treatment.

The particular wheat species (*Triticum aestivum*, *durum*, *spelta*, *monococcum*) vary in terms of their susceptibility to fusarioses and the levels of accumulation of the toxins. Hulled wheat species grown in the past are also infected by fungi from the genus *Fusarium*, as indicated by kernel colonisation and accumulation of fusarium toxins in grain of hulled wheats (Packa, 2013, Oliver et al., 2007; Kurowski, Wysocka, 2009; Sadowski et al., 2010; Filoda, Wickiel, 2010; Suchowilska et al., 2008; Konvalina et al., 2011). Gašiorowski (2004) reports that in new cultivars attention is frequently paid to the trait of resistance to *Fusarium nivale*, although it has been noted that the degree of fusariosis infection is not always reflected in the amount of mycotoxins in grain. During the storage of cereals and their products under proper conditions the content of DON does not change. Unfortunately, mycotoxins are compounds with a high stability (Żakowska, Stobińska, 2000).

In sustainable agricultural systems, species are grown that are not commonly cultivated in intensive agriculture, such as durum, spelt, einkorn wheat etc. (Cyrkler-Degulis,

Bulińska-Radomska, 2006, Rachoń et al., 2013). Due to the above it appears justified to study those species in terms of their suitability for the production of high quality food.

The objective of the study was to compare the grain of four winter wheat genotypes with regard to mycotoxin contamination.

MATERIAL AND METHODS

The study was conducted with the use of grain harvested in the years 2012 and 2013 from a field experiment at the Felin Experimental Farm of the University of Life Sciences in Lublin. The research material included winter cultivars and populations of common wheat (*Triticum aestivum* ssp. *vulgare*) – cv. Tonacja, durum wheat (*Triticum durum*) – cv. Komnata, spelt wheat (*Triticum aestivum* ssp. *spelta*) – cv. Schwabencorn, and einkorn wheat (*Triticum monococcum*) – PL 5003 (seeds were acquired from the National Centre of Plant Gene Resources).

Grain of the wheat species under study was brought to a moisture content of 15%, and after that mycotoxins contamination was assayed. The following mycotoxins were assayed: Aflatoxin B1, Aflatoxin B2, Aflatoxin G1, Aflatoxin G2, Ochratoxin A, Deoxynivalenol, Zearalenone, Toxin T-2, Toxin HT-2, Nivalenol, Fusarenon X, 3-Acetyl-deoxynivalenol. The method of the assays consisted in sample extraction with a water/acetonitrile mixture and purification on a MycoSep 226 AflaZon (Romer Labs) column. The extract obtained was evaporated till dry in an air stream, and then recreated with a methanol/water mixture with an addition of ammonium. Samples prepared in that manner were subjected to LC-MS/MS analysis. In accordance with the principle of validated method of mycotoxin assay with the method of liquid chromatography with tandem mass spectrometry detection (LC-MS/MS) the measurements were made in the MRM mode (Multiple Reaction Monitoring) with the use of positive and negative ionisation (ESI+/ESI-) of the electrospray type (ESI). Mycotoxins such as Aflatoxin B1, B2, G1, G2, Ochratoxin A, Toxin T-2, Toxin HT-2 were assayed in mode ESI+, while Fusarenon X, 3-Acetyl-deoxynivalenol, Nivalenol, Deoxynivalenol, Zearalenone in mode ESI-. The results of the assays were expressed with expanded uncertainty, U. The uncertainty was estimated taking into account the expansion coefficient $k=2$ which ensures a confidence level of approximately 95%. The assays were performed at the Central Laboratory of Agroecology of the University of Life Sciences in Lublin.

The weather conditions in the vegetation seasons in which the study was performed are presented in Table 1. In 2013, high temperatures and large amounts of precipitation during the blooming phase (May–June) created favourable conditions for wheat infection with *Fusarium*. This found confirmation in the analysis of the results obtained. The level of infection with this pathogen and, consequently, the

Table 1. Characteristics of the weather conditions.

Year	Months											
	IX	X	XI	XII	I	II	III	IV	V	VI	VII	VIII
	Rainfalls [mm]											
2011/2012	5.4	28.5	1.0	34.5	33.6	22.1	28.6	34.0	56.3	62.8	52.3	37.6
2012/2013	35.5	88.8	29.8	28.8	57.7	28.5	60.8	51.1	101.6	105.9	126.1	17.8
Mean for 1951–2010	53.7	40.1	38.2	31.4	23.4	25.8	28.0	39.0	60.7	65.9	82.0	70.7
	Air temperature [°C]											
2011/2012	15.2	8.0	2.4	2.0	-1.8	-7.1	4.3	9.5	15.0	17.3	21.5	19.2
2012/2013	15.0	8.0	5.5	-3.8	-3.8	-1.0	-2.4	8.1	15.3	18.5	19.2	19.2
Mean for 1951–2010	12.6	7.6	2.6	-1.6	-3.7	-2.8	1.0	7.4	13.0	16.3	18.0	17.2

content of mycotoxins in wheat grain were distinctly higher in 2013 compared to 2012.

RESULTS AND DISCUSSION

The study demonstrated that the grain of durum wheat had the highest level of contamination with mycotoxins, and especially with deoxynivalenol – 3860 $\mu\text{g}\cdot\text{kg}^{-1}$ in 2012 and 8460 $\mu\text{g}\cdot\text{kg}^{-1}$ in 2013 (Table 2, Table 3). A lower concentration of DON was noted in the grain of common wheat (225–791 $\mu\text{g}\cdot\text{kg}^{-1}$), and the most resistant were spelt wheat (122–605 $\mu\text{g}\cdot\text{kg}^{-1}$) and einkorn wheat (51–448 $\mu\text{g}\cdot\text{kg}^{-1}$). In the grain of durum wheat the permissible level of deoxynivalenol, according to the Regulation 1881/2006, was exceeded approx. 2–4-fold.

For the sake of consumer health, the current EU legislation regulates the maximum permissible levels of mycotoxins, including DON, in cereal food products (Table

4). In accordance with the Commission Regulation (EC) No. 1881/2006 the content of DON in non-processed durum wheat cannot exceed 1750 $\mu\text{g}\cdot\text{kg}^{-1}$, and in common wheat or einkorn wheat 1250 $\mu\text{g}\cdot\text{kg}^{-1}$. In products such as dry pasta the content of deoxynivalenol cannot exceed 750 $\mu\text{g}\cdot\text{kg}^{-1}$.

The EC Scientific Committee on Food established values of TDI (Tolerable Daily Intake), i.e. safe intake doses the daily intake of which, over the entire lifetime, does not cause any negative effects on human health. For DON the TDI value has been set at 1 $\mu\text{g}\cdot\text{kg}^{-1}$ of body mass per day (Committee Regulation (EC) No. 856/2005 of 6th June, 2005, revising the Regulation (EC) No. 466/2001 with relation to *Fusarium* toxins). Fusarioses, apart from the production of mycotoxins, cause also poorer yielding of cereals resulting from reduced weight of 1000 kernels, as well as a reduction of the number and weight of kernels per head. According to certain sources, the concentration

Table 2. Mycotoxin content in wheat grain in 2012 [$\mu\text{g kg}^{-1}$].

Type of mycotoxin	<i>Triticum aestivum</i> ssp. <i>vulgare</i>	<i>Triticum durum</i>	<i>Triticum aestivum</i> ssp. <i>spelta</i>	<i>Triticum monococcum</i>
Aflatoxin B1	< LOQ = 1.0	< LOQ = 1.0	< LOQ = 1.0	< LOQ = 1.0
Aflatoxin B2	< LOQ = 1.0	< LOQ = 1.0	< LOQ = 1.0	< LOQ = 1.0
Aflatoxin G1	< LOQ = 1.0	< LOQ = 1.0	< LOQ = 1.0	< LOQ = 1.0
Aflatoxin G2	< LOQ = 1.0	< LOQ = 1.0	< LOQ = 1.0	< LOQ = 1.0
Ochratoxin A	< LOQ = 10.0	< LOQ = 10.0	< LOQ = 10.0	< LOQ = 10.0
Deoxynivalenol	225.0	3860.0	122.0	51.0
Zearalenone	< LOQ = 1.0	9.8 \pm 3.5	< LOQ = 1.0	< LOQ = 1.0
Toxin T-2	< LOQ = 1.0	< LOQ = 1.0	< LOQ = 1.0	< LOQ = 1.0
Toxin HT-2	12.8 \pm 5.2	4.5 \pm 1.8	< LOQ = 1.0	1.2 \pm 0.5
Nivalenol	209.0	1270.0	46.0	133.0
Fusarenon X	< LOQ = 1.0	< LOQ = 1.0	< LOQ = 1.0	< LOQ = 1.0
3-Acetyl-deoxynivalenol	2.6 \pm 0.6	2.1 \pm 0.5	< LOQ = 1.0	< LOQ = 1.0

LOQ – limit of quantification; LOQ for deoxynivalenon and nivalenol was 1.0 $\mu\text{g kg}^{-1}$

Table 3. Mycotoxin content in wheat grain in 2013 [$\mu\text{g kg}^{-1}$].

Type of mycotoxin	<i>Triticum aestivum</i> ssp. <i>vulgare</i>	<i>Triticum durum</i>	<i>Triticum aestivum</i> ssp. <i>spelta</i>	<i>Triticum monococcum</i>
Aflatoxin B1	< LOQ = 1.0	< LOQ = 1.0	< LOQ = 1.0	< LOQ = 1.0
Aflatoxin B2	< LOQ = 1.0	< LOQ = 1.0	< LOQ = 1.0	< LOQ = 1.0
Aflatoxin G1	< LOQ = 1.0	< LOQ = 1.0	< LOQ = 1.0	< LOQ = 1.0
Aflatoxin G2	< LOQ = 1.0	< LOQ = 1.0	< LOQ = 1.0	< LOQ = 1.0
Ochratoxin A	< LOQ = 10.0	< LOQ = 10.0	< LOQ = 10.0	< LOQ = 10.0
Deoxynivalenol	791.0	8460.0	605.0	448.0
Zearalenone	2.2 \pm 0.8	138.0	18.9 \pm 6.8	< LOQ = 1.0
Toxin T-2	1.2 \pm 0.4	49.0	< LOQ = 1.0	< LOQ = 1.0
Toxin HT-2	11.9 \pm 4.9	148.0	6.0 \pm 2.5	5.4 \pm 2.2
Nivalenol	425.0	675.0	46.0	131.0
Fusarenon X	< LOQ = 1.0	< LOQ = 1.0	< LOQ = 1.0	< LOQ = 1.0
3-Acetyl-deoxynivalenol	12.5 \pm 2.9	68.0	8.1 \pm 1.9	3.7 \pm 0.9

LOQ – limit of quantification

Table 4. Maximum levels for certain mycotoxins according to Commission Regulation (EC) No 1881/2006 of 19 December 2006.

Type of mycotoxin	Mycotoxin content [$\mu\text{g kg}^{-1}$]
Aflatoxin B1	2
Aflatoxin B2	4
Aflatoxin G1	4
Aflatoxin G2	4
Ochratoxin A	5
Deoxynivalenol	1250 (<i>Triticum aestivum</i> , <i>T. monococcum</i>) 1750 (<i>Triticum durum</i>)
Zearalenone	100
Toxin T-2	–
Toxin HT-2	–
Nivalenol	–
Fusarenon X	–
3-Acetyl-deoxynivalenol	–

of mycotoxins can increase in the course of grain storage. In products from grain with fusariosis, e.g. in flour, one can observe a low content of protein and low quality gluten (Gašiorowski, 2004).

Our own study is in support of the observations of COBORU which show that einkorn wheat (*Triticum monococcum*) was the least susceptible to *Fusarium*, the most infested being cv. Komnata (*Triticum durum*). Among common wheat cultivars there were differences, the least infested being the winter wheat cultivars Arkadia, Fidelius, Torrild and Jantarka (Gleń, 2013).

Packa et al. (2013) analysed the resistance of three hulled wheat species – *T. monococcum* (einkorn wheat), *T. dicoccum* (emmer wheat) and *T. spelta* (spelt wheat) – to infection with *Fusarium culmorum*, comparing them to *T. aestivum*. *T. monococcum* responded to the inoculation the most strongly among the hulled forms, while the re-

sponse of *T. spelta* was the weakest (the lowest decrease in the biometric traits under study). The authors indicate that the negative effect of the infection of the cereals with *Fusarium* is a decrease of their food and feed values.

In 2014, at the University of Bologna, Italy, a study was conducted in which two tetraploid wheat cultivars – *Triticum turgidum* spp. *durum*, Claudio and *Triticum turgidum* spp. *turancium*, Kamut – were compared in terms of their content of DON which in both cases did not exceed the level of contamination permissible in the EU. However, the study showed that the application of cleaning procedures before milling, such as grain washing and cleaning, notably decreased the content of mycotoxins, especially in the case of cv. Kamut (46–93% reduction of DON), which indicates surface contamination of grain in that cultivar (Dinelli et al., 2014).

If we want to reduce the formation of mycotoxins we should concentrate on several factors favouring fungi. To start with we need to focus on the selection of wheat cultivars with greater resistance to fusarioses, and on methods of cultivation. Podolska (2013) reports that with the application of an early first ploughing followed by deep ploughing one can destroy fungal spores. Other helpful measures include fertilisation which enhances the resistance of plants to diseases, traditional crop rotation (*Fusarium* may survive in soil for over 5 years, while crop rotation will reduce the fungal populations), proper preparation of sowing material (cleaning and priming), plant protection agents (application of fungicides directly prior to infection), protection against soil pests that, damaging the grain sown, may make it more susceptible to infections, limitation of the number of insects which can transport contaminating material, organic residues being a source of infections, and after harvest – correct conditions of transport, drying and storage, and suitable methods of separation of mould-infested kernels with the use of optical sorting devices (Chełkowski, 1989, 1991). Perkowski (1999) arrived at the conclusion

that in the finer grain fractions (below 2.5 mm) the number of infected kernels increases notably, and especially in the finest fraction, <2.2 mm, the amount of *Fusarium* toxins is the highest. Suitable sowing time is also important to reduce the coincidence of the phase of the greatest sensitivity of the cereal with the attack of pathogens, as well as correct (not too big) density of sowing and proper time of harvest (Podolska, 2013). Without observation of the rules of GAP, GPP and GHP (Good Agricultural, Production and Hygienic Practice) one cannot guarantee that products will be not only of high quality but also safe for consumer health. An Italian study (Quaranta et al., 2010) indicates that the most important factors affecting the level of contamination with deoxynivalenol include the year (weather conditions) and the location of sowing, as well as the choice of suitable cultivars (among durum wheat cultivars those authors indicate Creso as the most resistant to contamination and the best suited for areas where elevated levels of DON appear the most frequently, i.e. Central Italy). The study also concludes that the system of cultivation (ecological and conventional with the use of plant protection agents) appears not to have any effect on the level of DON that would be supported with research results.

The problem of mycotoxins, and of deoxynivalenol in particular, appears to intensify, and researchers suggest that climate change may have a direct bearing on the increase of DON contamination of wheat cultivations in Europe, as the levels of contamination increased up to 3-fold in most of the studied regions of North-Western Europe over less than the last twenty years (Van der Fels-Klers et al., 2012). Moreover, as indicated by Šlivkova et al. (2014), analyses of the quality and technological parameters of common wheat cultivars in 2010 and 2011 show that even wheats with a high technological quality can contain high and dangerous levels of DON, which undoubtedly forces the producers of cereal products to remain alert and to conduct systematic analyses.

CONCLUSIONS

1. Grain of durum wheat (cv. Komnata) was the most susceptible to mycotoxin contamination. In the grain of that wheat cultivar contamination with the largest number of mycotoxins was found, and especially with deoxynivalenol (values 2–4-fold higher than the permissible norm).

2. Grain of spelt wheat (cv. Schwabekorn) and einkorn wheat (PL 5003) displayed the highest resistance to contamination with mycotoxins.

3. Grain of common wheat (cv. Tonacja) was less contaminated with mycotoxins compared to durum wheat, but more than the grain of spelt and einkorn wheat.

4. Other detected mycotoxins appeared in trace amounts in the grain of the wheat genotypes under study.

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