

The occurrence of the selected *Fusarium* mycotoxins in Czech malting barley, harvested in 2012–2017

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Abstract: In 2012–2017, the occurrence of deoxynivalenol, zearalenone and T-2 toxin in 592 samples of malting barley from different regions of the Czech Republic was studied using the ELISA immunochemical method. On average, the total content of positive samples was 18.6% for deoxynivalenol (DON), 9.5% for zearalenone (ZEN) and 20.5% for T-2 toxin. The highest values measured were 917 µg/kg for DON (2012), 42 µg/kg for ZEN (2017) and 199 µg/kg for T-2 toxin (2013). The maximal DON and ZEN contents in cereals intended for food production are limited by the Commission Regulation (EC) No. 1881/2006, the EU limit from 2013 applies for the sum of T-2 and HT-2 toxins. Concentrations of any of the mycotoxins studied did not exceed the EU limit in any barley samples.

Keywords: malting barley; *Fusarium*; mycotoxins; ELISA

Mycotoxins are toxic secondary metabolites produced by microscopic filamentous fungi, namely *Fusarium*, *Aspergillus* and *Penicillium* sp. They are thermostable, low-molecular substances with different chemical structures and a number of negative effects on human and animal health. They commonly contaminate economic crops, food and feed (KRSKA *et al.* 2007), representing thus a major global economic problem (JI *et al.* 2016). With the ongoing climate change, an increased risk of the occurrence of mycotoxigenic fungi and mycotoxins is expected due to the adaptation of fungal pathogens to altered conditions (GEISEN *et al.* 2017; MEDINA *et al.* 2017). The spectrum of explored and described mycotoxins is expanding (VÁCLAVÍKOVÁ *et al.* 2013; BOLECHOVÁ *et al.* 2015; LUZ *et al.* 2017). Modified forms of mycotoxins represent new research trends (FREIRE & SANT'ANA 2018). Cereals may be simultaneously contaminated with two or more mycotoxins (BĚLÁKOVÁ *et al.* 2014; PLEADIN *et al.* 2013, 2017), detection of one mycotoxin may indicate the presence of another, and this contamination influences the cumulative toxic effect.

Fusarium fungi belong to the most important producers of mycotoxins, at the same time they are

important pathogens of agricultural crops (PLACINTA 1999; MORCIA *et al.* 2013). The occurrence of fusarioses and mycotoxins is greatly influenced by the course of weather. The occurrence, quantity and type of mycotoxin may depend on the environment, the type of fungi present, the severity of the infection and the cultivar or crop type. In malting barley, the presence of mycotoxins can be significantly affected by the storage and post-harvest treatment of the grain. Another factor that can significantly affect the intensity of the *Fusarium* occurrence is the technology of growing and susceptibility of the variety, pre-crop, soil cultivation and, also, by the application of fungicides. Relationships and interactions arising among the plant, pathogenes, microscopic fungi, environment, manner of plant protection and treatment are very complex and they significantly affect mycotoxin production and content (TERZI *et al.* 2014).

The most well-known toxins produced predominantly by filamentous *Fusarium* fungi include the group of trichothecene mycotoxins, zearalenones and the group of fumonisins (WOLF-HALL 2007; CAPRIOTTI *et al.* 2010).

The contamination of the organism with trichothecenes results in a wide variety of manifestations; various syndromes may include reduced intake or total rejection of food, skin irritation and dermal necrosis, vomiting, diarrhea and bleeding. Trichothecenes have also been described as immunosuppressants and inhibitors of protein and DNA synthesis (MOSTROM 2011). All trichothecenes, without exception, exhibit a higher or lower degree of toxicity for the animals; they also exhibit an insecticidal effect. Phytotoxic activity has also been described (ABBAS *et al.* 2013). Research into the acute and chronic toxicity of these substances is ongoing (GROOPMAN *et al.* 2013; ESCRIVÁ *et al.* 2015).

DON, the most observed trichothecene, is often detected in cereals. Its occurrence in cereals varies from year to year depending mainly on the weather in the given locality, type of pre-crop and resistance of the variety. It is an indicator of a possible contamination with other mycotoxins. DON often co-occurs with the acetylated isomers of 3-acetyldeoxynivalenol (3-ADON), 15-acetyldeoxynivalenol (15-ADON) and other *Fusarium* toxins, such as nivalenol (NIV), zearalenone (ZEN), T-2 toxin (T-2) and HT-2 toxin (HT-2) (PESTKA 2007; BRYLA *et al.* 2016).

In zearalenone and its metabolites, estrogenic effect, reproduction and developmental toxicity in animals have been proven; they pose hepatotoxic, hematotoxic, immunotoxic and genotoxic effects (ZINEDINE *et al.* 2007).

Controlling the health safety of consumed food and feed in terms of the presence of mycotoxins is an absolute prerequisite for the health protection of the population. Control programs for monitoring mycotoxins in food and feed have been introduced in many countries, including the European Union (EU). Commission Regulation (EC) No. 1881/2006, supplemented by Commission Regulation (EC) No. 1126/2007, set the Maximum Levels (ML) of selected mycotoxins in cereals and other foodstuffs. The currently valid European legislation stipulates the content of selected *Fusarium* mycotoxins in unprocessed cereals, including malting barley: 1250 µg/kg for DON and 100 µg/kg for ZEN. For the sum of T-2 and HT-2 toxins, the indicative limit of 200 µg/kg is used. The indicative levels are based on the occurrence data available in the EFSA database and they are not feed and food safety levels (EU Commission Recommendation 2013/165/EU).

This study summarizes the results of six-year (2012–2017) monitoring of the selected trichothecene mycotoxins (DON and T-2 toxin) and ZEN in malting barley from the 14 regions in the Czech Republic.

MATERIAL AND METHODS

Standards and chemicals. Methanol (analytical grade) for the mycotoxin extraction was purchased from Sigma-Aldrich (Germany). Certified reference materials (CRMs) – naturally contaminated wheat (for DON and T-2 toxin) and corn (ZEN) was purchased from Trilogy (USA). ELISA test kits (AgraQuant® Deoxynivalenol Assay 0.25/5.0, AgraQuant® Zearalenone Plus Assay 25/1000 and AgraQuant® T-2 Toxin Assay 20/500) were provided by Romer Labs (Tulln, Austria).

Barley samples. In 2012–2017, totally 592 samples of malting spring barley were analysed (*i.e.* the varieties KWS Ariane, Blanik, Bojos, Francin, Kangoo, KWS Irina, Laudis 550, Malz, Marthe, Overture, Prestige, Radegast, Sebastian, Sunshine, SY Tepee, Wintmalt, Xanadu, Manta, Pionier, RGT Plane), *i.e.* in 2012 ($n = 117$), 2013 ($n = 98$), 2014 ($n = 116$), 2015 ($n = 109$), 2016 ($n = 110$) and 2017 ($n = 42$). The samples were obtained directly from the growers from all regions of the Czech Republic as described at BĚLÁKOVÁ *et al.* (2014).

Determination of mycotoxins. Ground barley sample (20 g) was extracted with 100 ml of deionized H₂O (for the DON determination) or of 100 ml of MeOH : H₂O 70 : 30 (for ZEN and T-2 toxin), shaken for 50 min, centrifuged at 4000 rpm for 15 min, an aliquot of the supernatant (100 µl) was diluted according to the instructions or used directly for the analysis.

Mycotoxin concentrations (DON, ZEN and T-2 toxin) were determined using competitive ELISA test kits as instructed by the kit manufacturer. Each kit contains a microtiter plate with 96 wells coated with antibodies, standard solutions containing different concentrations of mycotoxins, an enzyme conjugate, anti-antibody, substrate and chromogen solution, stop solution, and washing and dilution buffers. The calibration curve was constructed according to the instructions in the set, repeatability – the relative standard deviation (% RSD) was calculated from 10 determinations. The LOD and LOQ values are given by the manufacturer. The quality control of the method was established using the appropriate CRMs. The parameters of the method for targeted mycotoxins are presented in Table 1.

RESULTS AND DISCUSSION

The occurrence of the individual mycotoxins in barley in the individual years. The contents of mycotoxins DON, ZEN and T-2 toxin in barley are summarized in Table 2. The minimal and maximal

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Table 1. Parameters of the ELISA method for targeted mycotoxins

Mycotoxin	LOD ($\mu\text{g}/\text{kg}$)	LO ($\mu\text{g}/\text{kg}$)	RSD (%)	CRM declared ($\mu\text{g}/\text{kg}$)	CRM measured ($\mu\text{g}/\text{kg}$)
DON	200	250	9.5	700 \pm 100	859.0 \pm 81.6
ZEN	20	25	10.2	454.2 \pm 37.6	419.8 \pm 42.8
T-2	10	20	7.9	57.1 \pm 10.5	60.9 \pm 4.8

LOD – limit of detection; LOQ – limit of quantification; CRM declared – given by the manufacturer; CRM measured – measured by the laboratory

values and percentage of positive samples in each harvest are given, samples where the mycotoxin content was detected above the quantification limit are considered positive. To calculate the average values in individual years, the mycotoxin content was taken into account only in positive samples. For the evaluation, the basic statistical characteristics were used (means, absolute and relative frequencies). Results are expressed in Figures 1–3.

The level of DON contamination ranged from 4.8% in 2017 to 29.1% in 2012. Since the ELISA immunochemical screening test for DON has a higher detection limit, the samples with any lower DON concentration were not captured. The highest determined DON content (917 $\mu\text{g}/\text{kg}$) was found in harvest 2012. In all other samples tested, DON ranged from 250 to 668 $\mu\text{g}/\text{kg}$ and was comparable to harvests in the next years when the average DON content moved around 300 $\mu\text{g}/\text{kg}$.

The lowest level of contamination was evident in mycotoxin ZEN, when in 2012, 2013, and 2015, no positive samples were found; in years 2014, 2016 and 2017 12–30% positive samples with the maximal ZEN content of 42 $\mu\text{g}/\text{kg}$ were detected

No sample positive for the presence of T-2 toxin was found in the years 2012 and 2017. In 2013–2016, the level of contamination moved in the scope from 11.8% to 46.9%, with the highest concentration (199 $\mu\text{g}/\text{kg}$) measured in 2013. Average mycotoxin contents are summarized in Figure 1.

The most contaminated harvests were those in 2014 and 2016, when all three monitored mycotoxins were detected in some samples. In 2012, no sample positive for ZEN and T-2 toxin contents was found and 29% of the samples were positive for DON content, including the above mentioned sample with the highest DON content. In 2017, no tested sample was positive for T-2 toxin, 11.9% of the samples contained ZEN above LOQ and only 4.8% of the samples were positive for the DON content.

From Figure 2 which summarizes the total occurrence of mycotoxins in barley in the period of

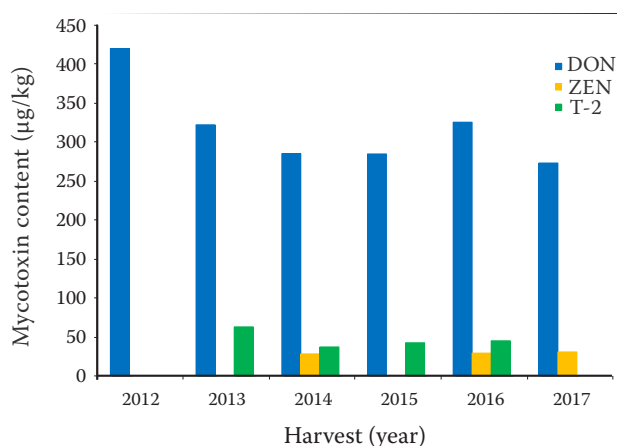


Figure 1. Average content of the studied mycotoxins in the individual years

Table 2. Occurrence of the studied mycotoxins ($\mu\text{g}/\text{kg}$) in barley in the Czech Republic in 2012–2017

Harvest	DON				ZEN				T-2			
	analysed/positive	(%)	min.	max.	analysed/positive	(%)	min.	max.	analysed/positive	(%)	min.	max.
2012	117/34	29.1	252	917	117/0	0	0	0	117/0	0	0	0
2013	9898/13	13.3	251	568	98/0	0	0	0	98/26	26.5	26	199
2014	116/23	19.8	250	577	116/18	15.2	25	39	116/39	33.6	25	90
2015	109/8	7.3	255	348	109/0	0	0	0	109/43	46.9	26	145
2016	110/30	27.3	251	668	110/33	30.0	26	39	110/13	11.8	25	129
2017	42/2	4.8	260	286	42/5	11.9	28	42	42/0	0	0	0
Total	592/110	18.6			592/56	9.5			592/121	20.4		

2012–2017, it is evident that on average the total content of the positive samples in the given years was 18.6%, 9.5%, and 20.5% of DON, ZEN, and T-2 toxin, respectively.

Some processes used in beer production act as a decontamination process, although some of the mycotoxins present in barley and malt may pass to the final product, nevertheless, the content found does not pose a significant health hazard to consumers (WOLF-Hall 2007; KARLOVSKY *et al.* 2016; PASCARI *et al.* 2017).

The effect of the variety and previous crop on the mycotoxin content. To evaluate the effect of a variety, only varieties with a sufficient number of samples (> 30) were selected, these were the varieties Bojos, Blaník, Laudis 550, and Malz. The numbers of the mentioned varieties were as follows: Bojos 208 samples, Laudis 550 61 samples, Blaník 39 samples and Malz 152 samples. Figure 3 shows that the variety did not have effect on the DON, ZEN and T-2 toxin content.

Maize, wheat, sugar beet, potatoes, spring barley, and rape were grown as a previous crop before malting barley. Maize affected the DON content most (detected average value 420.5 µg/kg), the adverse effect of maize as a pre-crop on *Fusaria* contamination and subsequently higher mycotoxin levels has already been confirmed by previous research (POLIŠENSKÁ *et al.* 2012; QIU *et al.* 2016), further, winter wheat (341.4 µg/kg), rape (324.8 µg/kg) and sugar beet (310.3 µg/kg). The effect of the previous crop on ZEN and T-2 toxin was not confirmed. Figure 3 shows the effect of the variety on mycotoxin content.

Comparison with the available data in the European countries. Barley contamination with *Fusarium* mycotoxins is quite common in the European countries and worldwide. In the period of 2005–2010, POLIŠENSKÁ

et al. (2012) analysed 327 samples of malting barley for the DON content using the ELISA assay. The level of contamination (when DON was > 40 µg/kg) varied from 63% in 2010 with the highest value of DON 227 µg/kg to 96% in 2009 when the highest measured value was 7050 µg/kg. In 2006, 2007, and 2010, the same authors also analysed ZEN content when in 2006, 100% positive samples were found (> 2 µg/kg) and the maximal detected value was 222 µg/kg. Conversely, in 2007 and 2010, 45%, and 22% of positive samples with a maximum of 48 and 14 µg/kg, respectively, were found (POLIŠENSKÁ *et al.* 2012). The results are practically comparable with BĚLÁKOVÁ *et al.* (2014), who monitored 325 samples of the malting barley harvested from 2008 to 2011. The maximum measured DON levels varied from 106.1 to 2213.5 µg/kg, ZEN from 21.4 to 59.4 µg/kg and ΣT-2, HT-2 toxins from 53.4 to 145 µg/kg (BĚLÁKOVÁ *et al.* 2014).

In 2012, monitoring of cereals including 20 samples of barley for the mycotoxin presence was conducted in Ireland (Food Safety 2015). Twelve samples contained DON at low concentrations (to 200 µg/kg), on the other hand, ZEN was found in 11 samples, the highest measured value was 150 µg/kg. T-2 and HT-2 were not found at quantifiable concentrations. The samples were analysed by the method of liquid chromatography with UV, FLD or MS detection.

In Switzerland, in 2013 and 2014, 280 and 160 barley samples for the presence of *Fusarium* mycotoxins were tested by the HPLC/MS method. DON was the prevailing mycotoxin in both harvests with the average concentration of 235 µg/kg in 2013 and 47 µg/kg in 2014. In 2013 the highest determined concentration was 4860 µg/kg, in 2014 of 1725 µg/kg. The average ZEN content was 3.7 µg/kg and 10.2 µg/kg in 2013

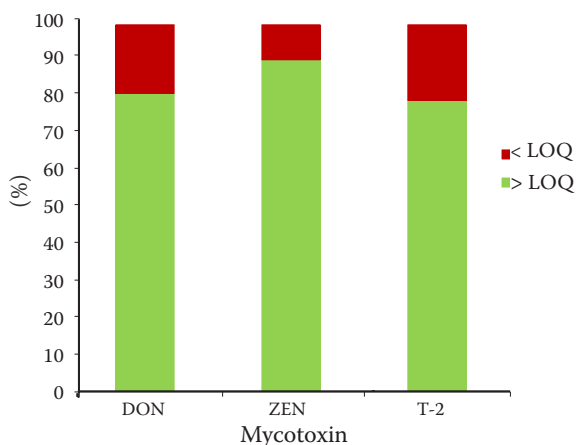


Figure 2. Total mycotoxin content in 2012–2017

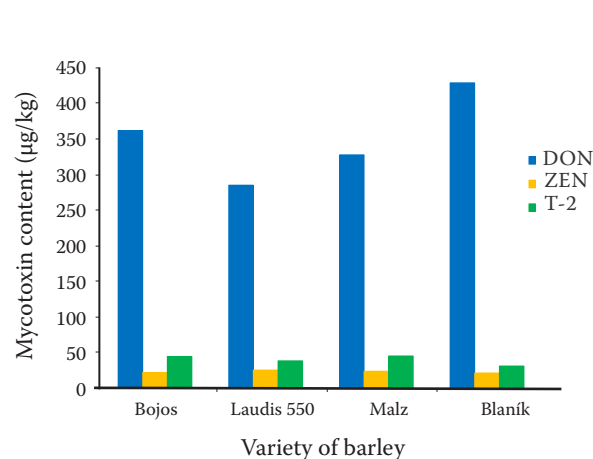


Figure 3. The effect of the variety on mycotoxin content

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and 2014, respectively. The highest concentration of 240 µg/kg was determined in 2014. T-2 and HT-2 toxins were detected in harvest 2013 in 16 (6%) and in harvest 2014 in 10 (6%) samples and the average concentration was 15.4% or 9.7% (SCHÖNEBERG *et al.* 2016).

In 2013–2015, research into mycotoxins in cereals in the area of Bosnia and Herzegovina investigated a total of 58 barley samples for the presence of mycotoxins DON, ZEN, and fumonisins by the ELISA method. The published data showed that DON was detected totally in 22 samples (38%) in quantifiable concentrations with the average content of 365 µg/kg and the highest detected values of 578 µg/kg, ZEN was found in 20 samples (34%) with the highest measured concentration of 84 µg/kg (PLEADIN *et al.* 2017). We can state that considering different weather conditions and used analytical methods the occurrence of the studied mycotoxins is comparable.

In Poland, BRYLA *et al.* (2016) analysed 26 mycotoxins in 147 various grain samples from the harvest 2014 included 8 samples of spring barley and 16 samples of winter barley. Four samples of spring barley contained DON with maximum concentration of 222 µg/kg and six samples contained ZEN with highest concentration of 31 µg/kg, all 16 samples of winter barley contained DON with maximum 1602 µg/kg and ten samples contained ZEN with maximum 19 µg/kg.

Similar data have been also presented in previously published research. For example, in 2001, eight samples harvested in Eastern Slovakia were analysed using LC/UV, five of which were contaminated with DON of the average concentration of 187 µg/kg with the highest concentration found 530 µg/kg (ČONKOVÁ *et al.* 2006). EDWARDS (2009) analysed in UK totally 446 barley samples from harvests 2002 to 2005 (approximately 25% of which were collected from organic growers) for the presence of 10 trichothecene mycotoxins by the GC/MS method. DON was found in 57% of the tested samples and only one of them from harvest 2005 exceeded ML EU with concentration of 1416 µg/kg. 12% of samples were T-2 and HT-2 positive of highest concentration of 138 µg/kg. The author stated that no significant differences in contamination between barleys grown in conventional and organic farming were found. PLEADIN *et al.* (2013) mapped concentration of *Fusarium* mycotoxins in cereals in six different Croatian regions, including 34 barley samples. They reported 53% samples contaminated by DON with an average content of 228 µg/kg and the highest content of 342 µg/kg, 9% of samples positive for ZEN with the average content of 32 µg/kg

and the highest content of 68 µg/kg. 32% of the samples contained T-2 with the average value of 13 µg/kg and the highest content of 26 µg/kg. On the other hand, TABUC *et al.* (2009) who within their research using ELISA examined totally 21 barley samples harvested in 2002–2004 in south eastern Romania, reported slightly higher concentrations of DON and ZEN. The authors found DON in the range from 0 to 4000 µg/kg and concentrations in 13 (62%) samples exceeded ML EU, and all samples were ZEN contaminated at the average concentration of 133 µg/kg and 71% of them exceeded ML EU.

Some of the cited authors also mapped in their studies the occurrence and representation of the individual pathogens of *Fusarium* species, they described local weather conditions in the given seasons and suggested that a great variability of the occurring pathogen or simultaneous co-occurrence of more pathogens and also occurrence of their metabolites was affected by these conditions. These facts indicate that the natural contamination of cereals, including malting barley by mycotoxins cannot be completely eliminated not even if proper farming practices are maintained.

CONCLUSION

In 2012–2017, screening of totally 592 samples of malting barley for the content of *Fusarium* mycotoxins by immunochemical ELISA method was conducted. On average, the total content of positive samples in all the studied mycotoxins was to 20.5% in the given years. The mycotoxin content was affected by a pre-crop, the least suitable was maize, and the effect of a barley variety was not proven. Mycotoxin contents did not exceed ML EU for unprocessed cereals in DON and ZEN and indicative limits for T-2 and HT-2 toxins in any of the studied samples. As mycotoxins may pass into malt and beer produced from the malting barley, further monitoring of quality of the input materials to protect consumers' health is necessary.

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