Fusarium mycotoxin content of Slovakian organic and conventional cereals

Obsah fuzáriových mykotoxínov ekologicky a konvenčne pestovaných obilnín na Slovensku

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Abstract

Many species of *Fusarium* fungi can produce a number of different mycotoxins including trichothecenes such as deoxynivalenol (DON), nivalenol (NIV), zearalenone (ZEA) and others. The farming system have an effect on quality of grown plants, including mycotoxin contamination. The contamination of winter wheat (*Triticum aestivum* L.) and rye grain by toxins with focus on the genus *Fusarium* was monitored within the years 2009 – 2011 under the official control according to EC Regulation 401/2006 and 178/2010 on the territory of the Slovak Republic in organic and conventional grains. The concentration of deoxynivalenol (DON) was determined by HPLC/DAD detector and concentration of zearalenone (ZEA) by HPLC/FLD detector. Results of analysed samples showed that DON was the most prevalent *Fusarium* toxin. In the analysed samples were observed significant differences between organic and conventional wheat samples. Results showed that samples from organic production contained significantly lower contamination of DON and ZEA mycotoxins than from conventional production. In four wheat samples from conventional production was the content of DON over the EC Regulation no. 1881/2006 about setting the maximum levels for certain contaminants in foodstuff. Within the observed period, the highest DON concentrations were observed in the year 2010, in both wheat and rye samples, and organic and conventional production. The year 2010 was characterised by constant rain, moist soil on the most area of Slovakia. Since lower contamination of *Fusarium* toxins was found in wheat and rye from organic farming, it can be concluded, that factors related to agricultural practices in this system can reduce the risk of *Fusarium* mycotoxin contamination.

Keywords: conventional farming, deoxynivalenol, organic, rye, wheat, zearalenone

Abstrakt

Mnoho druhov húb z rodu *Fusarium* môže produkovať celý rad rôznych mykotoxínov, vrátane trichotecénov ako deoxynivalenol, nivalenol, zearalenon a ďalších. Systém hospodárenia má vplyv na kvalitu pestovaných rastlín, vrátane kontaminácie mykotoxínmi. Kontaminácia ozimnej pšenice a raže fuzáriovými toxínmi bola sledovaná v rokoch 2009-2011 v rámci úradných kontrol podľa nariadenia ES 401/2006 a 178/2010 na území Slovenskej republiky v ekologickom a konvenčnom systéme hospodárenia. Koncentrácia deoxynivalenolu bola stanovená metódou HPLC/DAD a koncentrácia zearalenonu metódou HPLC/FLD. Výsledky analyzovaných vzoriek ukázali, že DON bol prevládajúci fuzáriový toxín. V analyzovaných vzorkách boli zaznamenané preukazné rozdiely medzi ekologickými a konvenčnými vzorkami. Z výsledkov vyplýva, že vzorky z ekologickej produkcie boli preukazne menej kontaminované DON a ZEA mykotoxínmi ako vzorky z konvenčnej produkcie. V štyroch vzorkách pšenice bola koncentrácia DON mykotoxínov nad maximálnu povolenú hodnotu podľa EC č. 1881/2006 ktorou sa ustanovujú maximálne hodnoty obsahu niektorých kontaminantov v potravinách. V sledovanom období najvyššia koncentrácia DON bola pozorovaná v roku 2010 v pšenici a raži v ekologickom aj konvenčnom hospodárení. Rok 2010 bol charakteristický vytrvalými dažďami, hmlami a vlhkou pôdou na väčšine územia Slovenska. Keďže nižšia koncentrácia fuzáriových toxínov bola pozorovaná v pšenici a raži z ekologického systému, možno konštatovať, že postupy ekologického poľnohospodárstva môžu znížiť riziko kontaminácie fuzáriovými toxínmi.

Kľúčové slová: deoxynivalenol, ekologický, konvenčný systém, pšenica, raž, zearalenon

Introduction

As of the end of 2011, 10.6 million hectares of agricultural land in Europe were managed organically on almost 290000 farms. In Europe, 2.2 percent of the agricultural area, and in the European Union, 5.4 percent of the agricultural area is organic. In Slovakia organically managed land represented 8.61 percent of all agricultural land in 2011 and 365 producers were involved in this sector (Willer and Kilcher, 2013). Organic agriculture is of particular interest with regard to healthy, ecologically friendly produced food, because inputs of chemicals such as synthetic fertilisers and pesticides are not allowed (Nelson et al., 2004).

Significant criterion of winter wheat quality is also its safety. Food safety issues due to mycotoxins of *fusarium* infections can be very important for cereal market and food and feed chain. All these quality traits can be influenced by the environment, genotype, crop rotation and management practices (Váňová et al., 2008). Organic disease management strategies should, from principle, be largely preventive and depend on the whole system approach, with maintenance of soil health, as a central component of any strategy (Davies et al., 2002). The soil fertility and soil health are basic elements of organic cropping systems. A break of two to four years is sufficient to reduce inoculum to a level that will allow the production of a healthy crop (Wolfe, 2002). Along with food issues due to mycotoxins, effects of *Fusarium* infections on wheat quality can be disastrous. Some of the fusarium head blight mycotoxins, such as deoxynivalenol (DON), present in infected wheat may be lost during the process of cleaning. The level of DON mycotoxin can differ considerably in food products and in raw material (Lancová et al., 2008).

To minimize the problems caused by *Fusarium*, it is important to investigate the occurrence of *fusarium* moulds and *fusarium* mycotoxins and factors influencing the infection and the formation of toxins (Döll et al., 2002). Oldenburg et al. (2008) ranked the factors influenced the infection of grain, especially wheat. The author found the climate to have the greatest impact, followed by infection pressure, tillage, maize as preceding crop, plant protection, cultivars and plant nutrition. In consideration of these influencing factors, the crop production system of organic farming seems to hold some advantages concerning crop rotation and tillage as compared to conventional farming. Chu (1977) presented, that contamination of cereals by DON can be reduced by keeping good agricultural practice and the use of appropriate agro-technical measures and products for plant protection.

The European Commission has set legislative limits for the *Fusarium* mycotoxins including the trichothecene, DON and ZEA in cereal grains and cereal based products intended for human consumption. Commission regulation (EC) no. 1881/2006 set the maximum level of DON at 1250 $\mu g*kg^{-1}$ in unprocessed cereals other than durum wheat, oat and maize. For ZEA was set the maximum level at 100 $\mu g*kg^{-1}$ in unprocessed cereals other than maize (EC no. 1881/2006).

The objective of this study was the evaluation of *Fusarium* mycotoxin contaminations in organically and conventionally managed cereals in the Slovak republic, focused on the trichothecenes deoxynivalenol and zearalenone.

Materials and Methods

The contamination of winter wheat (*Triticum aestivum* L.) and rye (*Secale cereale* L.) grains by toxins with focus on the genus *Fusarium* were monitored within the years 2009 – 2011 under the official control according to EC Regulation no. 401/2006 and 178/2010 on the territory of the Slovak Republic in ecological and conventional production systems. In total, 189 wheat and 39 rye samples were analyzed for mycotoxin determination. The concentration of mycotoxins was determined by HPLC method.

Deoxynivalenol determination method

Extraction: A total of 25 g of the ground samples was extracted with 200 ml deionized water for 20 min in shaker, then the extract was filtered through a folded filter and purified on immunoaffinity column. An aliquot of the purified extract was concentrated to dryness by rotary evaporation at 60°C, redissolved with 1 ml of mobile phase and mixed.

HPLC conditions: HPLC/DAD system with RP column LC-18, 150 mm x 4.6 mm was used as analytical column. The mobile phase consisted of H_2O/methanol/acetonitrile (90/5/5, v/v/v). All separations were carried out at 40°C, applying a flow rate of

1 mL*min^{-1}. The injection volume was 50 µl. Deoxynivalenol was detected with DAD detector at 218 nm.

Zearalenone determination method

Extraction: A total of 20 g of the ground samples was extracted with 100 ml extraction solvent acetonitrile/water (75/25 v/v) and homogenized. The suspension was centrifuged at 1600 g for 10 min, filtered through a folded filter and purified on immunoaffinity column. HPLC conditions: HPLC/FLD system with RP column LC-18, 250 mm x 4.6 mm was used as analytical column. The mobile phase consisted of acetonitrile/H_2O/acetic acid (51/47/2 v/v/v). All separations were carried out at 30°C applying a flow rate of 1 mL*min^{-1}. The injection volume was 50 µl. Zearalenone was detected with fluorescence detector at 274/455 nm.

Methods of analysis used for food control were in accordance with the provisions of items 1 and 2 of Annex III of Regulation (EC) no. 882/2004. Laboratory complies the provisions of Article 12 of Regulation (EC) no. 882/2004 for official controls performed to ensure the verification of compliance of feed and food law, animal health and animal welfare.

Obtained results were analysed in the program Statgraphic, and evaluated using the analysis of variance ANOVA ($p = 0.05$). Due to the fact, that different numbers of samples were analysed for each year, it was necessary to carry out Bartlett's test of homogeneity. Duncan's test of contrasts was used for unbalanced numbers of the measurements.

Results and Discussion

In conditions of Central Europe fusariosis occur annually at 70% of cereal crops. Attacks and economically significant crop losses occur about every third year (Wakulinski, 1990). This claim was also confirmed in past in Slovakia by Šrobárova and Vašková (1987). In their experiments, the most often isolated species in Slovakia were: *F. culmorum*, *F. graminearum*, *F. avenaceum*, *F. oxysporum*, *F. moniliforme* and *M. nivale*. Current range of *Fusarium* species in winter cereals is similar, only the order of their dominance and frequency is changed: *F. avenaceum*, *F. culmorum*, *F. graminearum*, *F. poae* and *M. nivale* (Hudec, 2006). *F. graminearum* and *F. culmorum* are the most important *Fusarium* head blight pathogens of wheat as they are more pathogenic than other species and produce higher levels of mycotoxins, in particular DON (Edwards, 2004).

Results of wheat samples analysed for DON, from organic and conventional production systems are shown in Table 1. In total, 165 wheat samples were analysed from conventional production and 24 samples from organic production system. Within the relevant years in the analysed samples were observed significant differences between organic and conventional wheat samples. In samples from organic production, results show significantly lower contamination of DON mycotoxins than in conventional production. Average DON content within observed period in wheat from organic production ranged from 34.88 µg*kg^{-1} in 2009 to 336.04 µg*kg^{-1} in 2010.

Table 1. Comparison of deoxynivalenol content in samples of winter wheat from organic and conventional production (2009-2011)

Tabuľka 1. Obsah deoxynivalenolu vo vzorkách pšenice letnej formy ozimnej z ekologickej a konvenčnej produkcie (2009-2011)

Year	Farming system	LOQ (μg*kg^{-1})	Mean (μg*kg^{-1})	The highest concentration (μg*kg^{-1})
2009	Conventional	20.00	158.96 b	2220.00
	Organic	20.00	34.88 a	117.00
2010	Conventional	20.00	502.91 b	2438.22
	Organic	20.00	336.04 a	826.46
2011	Conventional	20.00	327.68 b	2651.79
	Organic	20.00	135.05 a	387.00
2009-2011	Conventional	20.00	335.11 b	2651.79
	Organic	20.00	185.03 a	826.46

Legend: LOQ - limit of quantification; Values marked by the same letter indicate that the values are not significantly different in column and year at p = 0.05

In wheat coming from the conventional production was found significantly higher concentrations of DON than from organic production. The average contents of DON in conventional production ranged from 158.96 μg*kg^{-1} in 2009 to 502.91 μg*kg^{-1} in 2010. The experiment also showed that four (two samples in year 2010, and one sample in year 2009 and 2011) wheat samples of conventional production contained the DON concentration over the EC Regulation no. 1881/2006, setting the maximum levels for certain contaminants in foodstuff. In wheat from ecological production was not detected any sample with DON contamination over the legislation limit. The same results for DON mycotoxin as in wheat were observed in rye, listed in Table 2.

Table 2. Comparison of deoxynivalenol content in samples of rye from organic and conventional production (2009-2011)

Tabuľka 2. Obsah deoxynivalenolu vo vzorkách raže z ekologickej a konvenčnej produkcie (2009-2011)

Year	Farming system	LOQ (μg*kg^{-1})	Mean (μg*kg^{-1})	The highest concentration (μg*kg^{-1})
2009	Conventional	20.00	65.25 b	239.00
	Organic	20.00	22.86 a	25.00
2010	Conventional	20.00	289.00 b	289.00
	Organic	20.00	50.50 a	81.00
2011	Conventional	20.00	79.16 b	175.00
	Organic	20.00	27.00 a	34.00
2009-2011	Conventional	20.00	83.14 b	289.00
	Organic	20.00	29.18 a	81.00

Legend: LOQ - limit of quantification; Values marked by the same letter indicate that the values are not significantly different in column and year at p = 0.05

In observed period, 28 rye samples were analysed from conventional production and 11 samples from organic production system. Average DON content for conventional rye ranged from 65.25 μg*kg^{-1}, in 2009 to 289 μg*kg^{-1} in 2010. Average DON concentrations in rye from organically managed farms were found several times lower in each of observed year, ranged from 22.86 μg*kg^{-1} in 2009 to 50.5 μg*kg^{-1} in 2010. In rye was not detected any sample with DON contamination over the legislation limit neither in conventional nor in organic production system.

Within the study, the concentration of ZEA trichothecene was also evaluated. Concentrations of ZEA mycotoxins detected in samples of wheat coming from organic and conventional production are shown in Table 3. In total, 149 wheat samples were analysed from conventional production and 27 wheat samples from organic production. Analysed samples demonstrated the significantly lower concentrations of ZEA in organic production than in conventional in years 2009 and 2010. In year 2010 the concentration of ZEA was almost three times higher in conventional (14.56 μg*kg^{-1}) compared to organic (5.18 μg*kg^{-1}) production. In year 2011 was not observed significant differences in ZEA concentration. Within observed period, wheat coming from conventional production contain almost two times higher average ZEA concentration, significantly affected by year 2010.

Table 3. Comparison of zearalenone content in samples of winter wheat from organic and conventional production (2009-2011)

Tabuľka 3. Obsah zearalenonu vo vzorkách pšenice letnej formy ozimnej z ekologickej a konvenčnej produkcie (2009-2011)

Year	Farming system	LOQ (μg*kg^{-1})	Mean (μg*kg^{-1})	The highest concentration (μg*kg^{-1})
2009	Conventional	1.00	7.01 b	22.00
	Organic	1.00	5.00 a	10.00
2010	Conventional	1.00	14.56 b	288.00
	Organic	1.00	5.18 a	20.00
2011	Conventional	1.00	6.90 a	18.00
	Organic	1.00	7.00 a	10.00
2009-2011	Conventional	1.00	9,84 b	288.00
	Organic	1.00	5.44 a	20.00

Legend: LOQ - limit of quantification; Values marked by the same letter indicate that the values are not significantly different in column and year at p = 0.05

The same results were observed in a Belgian survey of Pussemier et al. (2006) in organic and conventional wheat in 2002 and 2003. Pussemier et al. (2006) reported higher and more frequent ZEA contamination in conventionally than organically cultivated wheat. In 2002 the incidence rates of zearalenone were 85 and 52% in conventional compared to organic samples, respectively. One sample of conventional wheat showed ZEA concentration over the limit of legislation. From our results followed, that one sample from conventional production system exceeded the legislation limit for ZEA concentration in wheat. Hoogenboom et al. (2008) examined organically and conventionally cultivated wheat samples in the Netherlands in 2003 and 2004. The high concentrations of ZEA were detected in wheat after heavy rainfall in the autumn 2004. But no significant differences were observed in ZEA concentrations between organic and conventional wheat. Bernhoft et al. (2012) examined 602 samples of organically and conventionally grown barley, oats and wheat during years 2002-2004 in Norway. Agronomic and climatic factors explained 10–30% of the variation in *Fusarium* species and mycotoxins. Significantly lower *Fusarium* infestation and concentrations of important mycotoxins were found in the organic cereals.

Kuzdralinski (2013) investigated the influence of organic and conventional farming systems on mycotoxin levels in oats in Poland within years 2006-2008. In his study, statistical analysis did not reveal any influence of the cropping system on the concentrations of remaining mycotoxins. The number of mycotoxin-positive samples was higher in organic farming in comparison with conventional production. Furthermore, considering only the mycotoxin-positive samples, the concentrations of deoxynivalenol, T-2, HT-2, diacetoscirpenol, nivalenol, and aflatoxins (B1, B2, G1 and G2) were slightly higher, but not statistically significant, in samples from conventional farming.

Twarużek et al. (2013) evaluated 98 samples of oat grain and products from organic and conventional farming system, during a three-year period (2009–2011) and analyzed for the presence of trichothecenes (deoxynivalenol, nivalenol, 3-acetyldeoxynivalenol, T-2 toxin, HT-2 toxin, monoacetoxyscirpenol, diacetoxyscirpenol) and zearalenone. All mycotoxins were found at different frequency and levels in the tested oat samples; but higher intensity of distribution of DON, T-2 and HT-2 toxins was found in the conventional system of farming than in organic. On the other side, the study realized on organic and conventional wheat in United Kingdom did not show significant difference in the ZEA concentration in positive samples. Also there were no significant differences in the DON concentration of organic and conventional samples (Edwards, 2009).

The *Fusarium* head blight is affected also by several factors, primarily climatic conditions, particularly rain and the temperature at flowering stage, but also agronomic factors, such as soil cultivation, nitrogen fertilization, fungicides, crop rotation, and host genotype (Bottalico and Perrone, 2002). Dry weather in late spring promotes sporulation, and conidia are splash dispersed by rainfall. Small-grain cereals are susceptible to head infection under warm, wet conditions from head emergence onwards, but particularly during flowering (Edwards, 2004). Hooker et al. (2002) showed the importance of weather conditions around flowering by developing a model that accounted for 73% of the variation in the concentration of DON based on weather conditions during this period.

The impact of weather conditions were reflected also in our experiment. Within the observed period, the highest DON and ZEA concentrations were observed in the year 2010, in both wheat and rye samples. Within years 2009-2011, the highest concentration of DON contamination in both conventional and organic wheat was observed in the year 2010. Concentrations of DON in wheat samples in years 2009 and 2011 are considered as low, compared to the year 2010. The reason of this finding is probably a low incidence of rainfall during the flowering of the cereals in the year 2009. Hudec (2009) presented that higher *Fusarium* head blight occurrence was only on a few places of Slovakia in 2009, but mostly unimportant. During flowering of winter wheat, at most areas of the Slovakia was dry and warm weather, which ultimately led to negligible significance of *Fusarium* head blight and low incidence in 2009.

Weather conditions in year 2010 were quite different. Hudec (2010) described the year 2010 as favourable for fungal diseases because of the constant rain, foggy weather, and moist soils created optimal conditions for the spreading of fungi. Among the cereals, most destructive fungal diseases were on wheat from the beginning of spring, where clear dominance of *Septoria* disease (*Septoria tritici)* over the entire vegetation was recorded. Very high dominance of Fusarium head blight was evident. The author predicted higher fungal disease incidence in organically managed farms. In this context, higher mycotoxin contamination in organic wheat samples was not observed in our study. Although it was not the aim of the experiment, in our opinion it can be due to several above mentioned factors, particularly: more resistant varieties to Fusarium head blight used in organic system, implementation of crop rotations, tillage practices, intercrops (catchcrops) and others. At the most of Slovakian conventional farms are used market oriented crop rotations with high of cereals.

These claims confirmed our previous findings from experimental field in western Slovakia, with integrated and organic six course crop rotations (50% of cereals in integrated, 33% of cereals in organic system). In this experiment were evaluated DON and ZEA mycotoxin contamination in winter wheat, and concentrations were below the level considered as safe for consumption. In organic system, the concentration of DON was lower by 46% compared to integrated system. Results determined pre-crop effect on DON concentration, when peas increased the level of DON in winter wheat grains (Lacko-Bartošova et al., 2011). Similar results were observed by Bernhoft et al. (2012) who presented that crop rotation with non-cereals significantly reduce mycotoxin concentrations as well as various *Fusarium* infestations (Bernhoft et al. 2012).

A combination of different factors, including cultivation of maize as previous crop, no-till farming, and cultivar selection (*Fusarium* resistant varieties) can be responsible for the higher contamination of cereals in integrated cultivation. Organic cultivation is characterized by the omission of maize as previous crop, traditional tillage, and by selection of relatively *Fusarium*-resistant cultivars. Furthermore, the ban of plant protectants and other chemicals such as fungicides or growth regulators (retardants) which are applied in integrated cultivation could contribute to the relatively low *Fusarium* contamination (and thus of the low toxin contents) in organically cultivated cereals (Meier et al., 2000; Obst et al., 2000; Oldenburg, 2004; Meister, 2009).

Döll et al. (2002), reported that in the case of non fungicide treatment, wheat from organic farming is more tolerant to *Fusarium* infection than wheat from conventional farming systems. The regime of fertilization have higher impact on *Fusarium* infection than the using of pesticides. Relatively low and/or slow delivery of nutrients in organic farming system helps to induce the plant's natural defences against infection. As the plant is allowed to grow abnormally fast through excessive inputs of nutrients, accumulation of defensive compounds is reduced, as well as resistance to pests and diseases (Brandt and Mølgaard, 2006).

The year effect on the *Fusarium* head blight occurrence underline the result of Bíliková and Hudec (2013). In study realized in years 2011-2012 in south Slovakia in organic and conventional farms, was the amount of rainfall the main factor which influenced the occurrence of Fusarium head blight in each year. The high amount of rainfall was related to high value of Fusarium head blight index in organic farm.

Fusarium mycotoxins (DON, NIV and ZEA) content in cereals depends on both, year and cropping system. The severity of Fusarium head blight and toxin levels differ considerably between years, reflecting climatic effects. In the work of Champeil et al. (2004), no clear relationship was found between disease severity and levels of contamination with DON, NIV or ZEA under conditions of natural contamination. Mycotoxin levels were the highest in the year with the highest disease severity and under the direct drilling system.

Since lower contamination of *Fusarium* toxins was found in wheat from organic farming, it can be concluded, that factors related to agricultural practices can reduce the risk of *Fusarium* contamination. Contamination of *Fusarium* mycotoxins, as DON and ZEA, can be reduced by good agricultural practice and the use of appropriate agro-technical measures in organic production system. Factors associated with agricultural practice according to Council Regulation (EC) no. 834/2007 may reduce the risk of contamination by *Fusarium* toxins.

Results of analysed samples indicated that DON was the most prevalent *Fusarium* toxin. Measured concentrations in samples of winter wheat and rye indicated significantly lower mycotoxin contamination in organic than in conventional production. In the conditions of the Slovak Republic, organic farming contributed to the reduction of mycotoxins in cereals. Within the observed period, the highest DON and ZEA concentration was observed in the year 2010 in both winter wheat and rye. The year 2010 could by characterised by constant rain, foggy weather and moist soils what created conditions for the spreading of fungi.

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