

# MYCOTOXINS OCCURRENCE IN ORGANIC FARMING CEREAL CROPS\*

J. Petr, H. Reisnerová

*Czech University of Life Sciences, Faculty of Agrobiology, Food and Natural Resources, Prague, Czech Republic*

The contents of mycotoxins in cereal crops were tested during several seasons of a fifteen years experiment in an organic farming system. The experiment was conducted strictly according to IFOAM regulations (International Federation of Organic Agriculture Movement) and was regulated by national decrees for ecological agriculture. Contamination was compared in the same varieties from conventional intensive farming systems. Testing did not prove above-limit content of mycotoxins in any sample from ecological cultivation. Neither it has been confirmed that contamination by mycotoxins in ecological agriculture is higher than in intensive cultivation where fungicides are applied. Organic farming eliminated harmful pathogens by greater diversity of cultivated crops, their rotation, limitation of the share of cereal crops and application of intercrops for so-called green manure, which supplies a flow of organic matter into the soil. These are natural means to limit the occurrence of *Fusarium* sp. fungi and subsequent contamination by mycotoxins.

mycotoxins; organic farming; aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, G<sub>2</sub>; deoxynivalenol; ochratoxin A; wheat; barley; rye

## INTRODUCTION

Organic farming, also known as ecological agriculture, is a natural production system, which respects ecological cultivation and breeding interventions. It connects traditional agricultural practices with the innovations of modern agriculture. However, it does not use agrochemical inputs of synthetic pesticides and industrial fertilizers. Thus it avoids possible residua of these foreign substances, and this is then a decisive motivation for purchase of these bioproducts. Their quality has to reach the parameters, which are set by Czech standards and EU regulations but, moreover, it is specified by the manner in which the product was cultivated or the animal was bred and fed and how the products were processed. This means that the main criterion for the quality of ecological products (bioproducts) is the method with which they were produced. This method is given by domestic and national regulations the observance of which is controlled by institutions designated for the purpose. Thus the quality of bioproducts is guaranteed.

Exclusion of chemical control of cereal stands encouraged a suspicion that in ecological agriculture there is a greater possibility for the occurrence of fungal diseases, and hence also the likelihood of production of health-harmful mycotoxins. Such an opinion came mainly from agrochemical companies selling pesticide products for plant protection. These arguments are repeated continuously and it is, therefore, necessary to pay attention to the research of mycotoxins in biocereals.

In Czech legislation in the past much attention has been devoted to protection against foreign substances (Hygienic directions for foreign substances in foodstuffs, Aventinum 1986, vols. 61 and 69) that were rather strict

and presented the permitted amount of aflatoxin B<sub>1</sub> at 5 mg.kg<sup>-1</sup> generally, 1 mg.kg<sup>-1</sup> for baby food and in aflatoxin M<sub>1</sub> generally 5 mg.kg<sup>-1</sup> and in baby nutrition 0.5 mg.kg<sup>-1</sup>, in aflatoxin B<sub>2</sub>, G<sub>1</sub>, G<sub>2</sub>-sum generally 10 mg.kg<sup>-1</sup> and in baby nutrition 2 mg.kg<sup>-1</sup>.

In 2004 Law No. 305/2004 Coll. that sets the limits for aflatoxin in cereals at 4 µg.kg<sup>-1</sup> in total, in aflatoxin B<sub>1</sub> at 2.0 µg.kg<sup>-1</sup>, and for ochratoxin A at 10 µg.kg<sup>-1</sup>. For deoxynivalenol (DON) (Decree of the Ministry of Health No. 53/2002 Coll.) the limit is set at 500 µg.kg<sup>-1</sup>, and for zearalenon at 50 µg.kg<sup>-1</sup>.

After admission of the Czech Republic to the European Union, the Regulation of the European Commission No. 466/2001 became valid for us. It sets maximum limits for the content of deoxynivalenol in cereals for human nutrition at 1.25 mg.kg<sup>-1</sup>, i.e. 1250 µg.kg<sup>-1</sup>, and for zearalenon at 100 µg.kg<sup>-1</sup>. These values are not as strict as were the previous Czech limits. The latest maximum limits prescribed by the Regulation of the EU Commission No. 1881/2006 have been valid since 1<sup>st</sup> March 2007. The limit of aflatoxins B<sub>1</sub> for all kinds of cereals and products from cereals is 2.0 µg.kg<sup>-1</sup>, and for the sum of B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, G<sub>2</sub> it is 4.0 µg.kg<sup>-1</sup>. The limit of ochratoxin A for non-processed cereals is 5 µg.kg<sup>-1</sup>, and for products of non-processed cereals determined for human nutrition it is 3.0 µg.kg<sup>-1</sup>. In deoxynivalenol for non-processed cereals the limit remained unchanged at 1250 µg.kg<sup>-1</sup>, and for cereals intended for direct human nutrition the limit is 750 µg.kg<sup>-1</sup>. The limit of zearalenon is 100 µg.kg<sup>-1</sup> for non-processed cereals, while the limit for cereals for direct human consumption is 75 µg.kg<sup>-1</sup>.

Mycotoxins, secondary metabolites of microscopic fibrous fungi (moulds), are the result of the adaptation of fungus growth under stress conditions. Fungi can con-

\* The research project has been financed by NAZV MZe CR No. QG 50034 and with the support of the project MSM 6046070901.

taminate cereal crops and their grains during the ripening phase and subsequently cause health problems when the products from these cereals are consumed.

Under Czech climatic conditions the main producers of mycotoxins are the *Fusarium* spp. fungi (*F. graminearum* and *F. culmorum*, *F. avenaceum* and others), agents of fusarioses of the spike (Váňová et al., 2008).

As mentioned above with regard to the problems of mycotoxins there is a great collection of literature resources. The range of Czech publications that evaluate mycotoxins is also large (studies of Váňová et al., 2004, 2008; Hýsek et al., 2005, and others). At the Czech University of Life Sciences Prague we have been dealing with the occurrence of mycotoxins in conventional cultivation from as early as 1992 (Reisnerová et al., 1992; Sedmíková et al., 2001; Sedmíková, Reisnerová, 2003).

From the international point of view, the Flair Flow Europe 4 Project, which also focussed on information on mycotoxins was a great contribution for the countries of Central and Middle Europe. Quilleien (2002) prepared an overview on this topic. In 2004 the seminar of the Flair Flow Europe 4 Project at the Institute of Chemical Technology in Prague dealt primarily with the issue of quality of food originating from ecological agriculture. The risks of contamination by mycotoxins in ecological agriculture have been evaluated by van der Bulk from the Netherlands. He reported that from the present reports it is clear that there is a certain risk of contamination by mycotoxins, because synthetic pesticides are not applied. The author reminds that under insufficient concentration of fungicides the stress conditions for the growth of moulds are induced and this stimulates the production of mycotoxins. A report of the European Commission, the Scientific Committee for Plants, documents a number of cases where ineffectiveness of fungicides and, by contrast, stimulation of mycotoxin production, have been confirmed proved (Bulk, 2003). The Committee has also stated that there is a lack of sufficient data to show that pesticides have played an important and corresponding role in the prevention, inhibition or stimulation of mycotoxin products.

In recent years, several cases of the presence of mycotoxins have been described in conventional and ecologically cultivated grain. Pascale et al. (2000) in Italy discovered a greater infestation of plants in ecological than in conventional agriculture. However, on average, in ecological agriculture the level of contamination was lower. Berlech et al. (1998) in Germany did not find any differences between the contamination in the conventional and ecological grain samples. Váňová et al. (2008) did not observe significant differences in the deoxynivalenol (DON) content in winter wheat grain from eight-crop rotations either in ecological agriculture or in conventional cultivation. Her study emphasises the importance of individual crops representation and rotation for the occurrence of diseases, including fusarioses. The occurrence of diseases was lower in ecological crop rotations. The author explains that this is due to the lower

available nitrogen, which reduces susceptibility to diseases. The results of DON occurrence studied by Váňová et al. (2008) at different intensities of cultivation and ecological crop rotations, the lowest DON contents were significantly proved in nine observed winter wheat varieties just in ecological crop rotation.

Váňová et al. (2008) also quotes Schafsmat et al. (2005) who did not find differences between the ecological and conventional crop rotations in respect of mycotoxins occurrence. Some studies also bring the attention to the effect of tillage on the occurrence of mycotoxins. This issue concerns the negative influence of reduced tillage and the effect of post-harvest residues.

The ecological cultivation system, as prescribed by national and international regulations, has no agricultural practice elements that would support occurrence of fusarioses, and hence also contamination by mycotoxins. However, it is a system that does not create stress conditions, such as those due to the application of herbicides, growth regulators and liquid fertilizers, and thus it does not increase susceptibility to dangerous diseases. This has been confirmed by a separate study of the plant health status during the author's experiment (Prokinová et al. 2000). This study aims at summarising the continuous observations of the occurrence of mycotoxins and other toxic substances in winter wheat and spring barley.

## MATERIAL AND METHODS

Since 1994 we have carried out experiments in organic farming system of management in accordance with the IFOAM regulations (International Federation of Organic Agriculture Movement) and the decree of the Ministry of Agriculture concerning organic farming, at the Experimental Station of Czech University of Life Sciences Prague in Uhřetěves. The station is certified for this cultivation system in line with these regulations. These experiments involved varietal trials with winter wheat and spring barley, later also with winter rye and triticale, that were established with the same varieties as those used in the trials of the Central Institute for Supervising and Testing in Agriculture (ÚKZÚZ) for testing of registered varieties.

The plots are situated in a fertile sugar-beet growing region with an average altitude of 295 m above sea level. The arable land is medium to slightly humic with the content of 1.74–2.12%, and neutral reaction. The soil type is luvisols on the soilforming loess substrates. The production capacity of the soils reaches 84 points. Average annual temperature in the given region is 8.45 °C, average sum of precipitations is 575 mm. The most precipitations in long-term mean occur in June and July, the least in February. Comparative samples from intensive cultivation come from similar conditions, from the Breeding Station Stupice at a distance of 2 km, where the soil is cambisol, clay loam. Average temperature is 8.3 °C, and the sum of precipitations is 575 mm. Fertilizers were applied in the following amounts: 127 kg N, 35 kg P and 60 kg K per

1 ha. Dressed seed, the Glean + Agritox herbicide, Tango fungicide, Karate insecticide and the CCC growth regulator have been used. The pre-crops were clover for winter wheat and forage legumes for spring cereals.

Tests for the mycotoxins content were conducted after a three-year transitional period in the years of 1994 and 1995 in 22 varieties of winter wheat and 18 varieties of spring barley and then again in 1996 and 1997. P r o k i n o v á et al. (2000) observed the occurrence of fusarioses during the other periods.

The sequence of the crops was as follows: clover and winter cereals, followed in the next year by fodder legumes, oil bearing crops, green manure and spring cereals with undesown clover. Experiments with winter followed after clover, and spring cereals after fodder legumes mixture.

During the above-mentioned years the analyses were carried out in the RIA laboratory using the RIA kits of the Endocrinological Institute Lúčky, Slovakia. Mycotoxins were determined using the aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) radioimmunochemical method by P í c h o v á et al. (1981) and ochratoxin A (OA) after C h u et al. (1976). Rabbit serum against conjugate of bovine serum albumin and AFB<sub>1</sub> and radioligand marked I<sup>125</sup> were used. Bound and free radioligand after incubation with antiserum AFB<sub>1</sub> was used, separated using suspension of activated coal, coated by dextrane, in case of OA polyetyleneglycol was used for separation. The value of the specific bond at RIA was ranging between 30–40%. Unspecific bond was 8%. After measuring of impulses gamma-automatic machine NA-3601 Tesla Liberec was used. The results were evaluated on PC AT 386. Sensibility of the method for AFB<sub>1</sub> is 0.6 and OA 1.5 µg.kg<sup>-1</sup>.

This was followed by testing the mycotoxins contents in the cereals from organic farming system in a complete set of varieties of wheat, barley and other crops, using the HPLC method, suitable for the determination of aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, G<sub>2</sub>, M<sub>1</sub>, and ochratoxin A. The analyses were carried out by the CIA No. 1142 certified chemical and microbiological laboratory in the town of Písek. The AFB<sub>1</sub> are determined after purification of a sample and on the AFLAPREP immunofinite column using the method of high-efficient liquid chromatography with post-column derivation by the toxin using Kobra Celly and fluorescent detection. Qualitative evaluation of chromatograms has been performed by comparison of retention times with the

retention times of peaks of standard solutions. Quantitative evaluation of chromatograms has been done by the method of absolute calibration.

Ochratoxin A is determined after purification of samples on the OCHRAPREP immunoafinite chromatographic column, the same as for aflatoxin B. The standard materials used are as follows: Aflatoxin B,G (by Supelco), Ochratoxin A (by Serva), HPLC chromatography Kobra Celly for aflatoxins. Fusarium toxins (deoxynivalenol, zearalenon) were determined by the HPLC method.

The principle of the method consists of an extraction of deoxynivalenol from the sample by distilled water, zearalenon in the sample of the mixture methanol + distilled water (75 + 25) and purification on VICAM columns and their subsequent determination by the method of high-efficiency liquid chromatography. Qualitative evaluation of chromatograms is done by the comparison of retention times with the retention times of the peaks of standard solution (eventually comparison of UV spectra of these substances). Quantitative evaluation of chromatograms is done by the method of absolute calibration through the detector diode field (DAD) in deoxynivalenol and fluorescing detector (FLD) in zearalenon.

Standards of deoxynivalenol and zearalenon in the sample were made by Sigma. In 2008 analyses were conducted by the LC/MS/MS a UPLC/FLR methods.

## RESULTS AND DISCUSSION

We commenced our first observation of the mycotoxins occurrence in organic farming we started after three-year intermediate period of 1994/1995 using the IFOAM ecological agriculture instructions valid at the time. The results are outlined in Table 2.

All values of aflatoxins as well as ochratoxins contents in the cultivated varieties of winter wheat were below the valid limits of mycotoxins content of the current regulations of the EU Committee No. 1881/2006. In the varieties of winter rye contents aflatoxins were below the limit, and the values of ochratoxins in the varieties Albedo, KR 54 and Dankowskie Nowe were over 5 µg.kg<sup>-1</sup>. Only in the variety Rapid they were below the defined limit. A similar situation was found in achenes of buckwheat and millet grains.

Table 3 presents a comparison of the occurrence of mycotoxins from ecological and intensive agriculture in

Table 1. Weather pattern in 1994/1995, 2005/2006 and 2007/2008 years and long term average at the Uhřetěves Station

Year	Monthly average	IX	X	XI	XII	I	II	III	IV	V	VI	VII
1994/1995	temperature °C	14.4	6.9	6.7	-2.6	0.6	5.2	3.5	9.5	13.2	15.5	21.1
	precipitation mm	56.5	30.9	22.7	55.1	27.1	12.4	63.7	0	115.2	89.4	32.6
2005/2006	temperature °C	14.2	10.2	3.2	0.4	-4.8	-1.2	2.2	9.7	14.2	18.4	23.2
	precipitation mm	51.0	11.0	16.0	38.0	18.7	60.6	50.1	48.4	100.9	86.6	12.2
2007/2008	temperature °C	17.7	8.3	2.8	0.9	2.8	3.9	4.7	8.9	14.7	18.9	19.2
	precipitation mm	91.8	16.0	50.6	19.9	26.1	13.0	26.4	47.2	95.9	48.0	79.7
Long term average	temperature °C	14.0	8.6	3.2	-0.5	-2.1	-0.8	3.4	8.2	13.4	16.3	18.2
	precipitation mm	49.0	41.0	34.0	34.0	28.0	27.0	31.0	46.0	65.0	74.0	74.0

Table 2. Occurrence of mycotoxins ( $\mu\text{g}\cdot\text{kg}^{-1}$ ) in cereal samples from ecological farming

Varieties	Aflatoxin B <sub>1</sub>	Ochratoxin A	Varieties	Aflatoxin B <sub>1</sub>	Ochratoxin A
Varieties of winter wheat			Varieties of winter rye		
Rexia	< 0.6	< 1.5	Albedo	< 0.6	5.5
Livia	< 0.6	< 1.5	Rapid	< 0.6	< 1.5
Illona	< 0.6	< 1.5	Selgo	< 0.6	5.9
Hana	< 0.6	< 1.5	Dankowskie Nowe	< 1.2	6.0
Vlada	< 0.6	< 1.5			
Sparta	< 0.6	< 1.5	Buckwheat	< 0.6	< 1.5
Sida	< 0.6	< 1.5	Millet	< 0.6	< 1.5
Vega	< 0.6	< 1.5			
Blava	< 0.6	< 1.5			
Samanta	< 0.6	< 1.5			
Torysa	< 0.6	< 1.5			
Asta	< 0.6	< 1.5			
Siria	< 0.6	< 1.5			
Trana	< 0.6	< 1.5			
Bruta	< 0.6	< 1.5			
Regina	< 0.6	< 1.5			
Simona	< 0.6	< 1.5			
Mona	< 0.6	< 1.5			
Slavia	< 0.6	< 1.5			
Diana	< 0.6	< 1.5			
Mironovská	< 0.6	< 1.5			

Sensibility of the method AFB<sub>1</sub> is 0.6 and OA it is 1.5  $\mu\text{g}\cdot\text{kg}^{-1}$

11 varieties of winter wheat from the first period of ecological management. Even in these observations neither method of cultivation exceeded the specified limits of the contents of aflatoxins and ochratoxins, but in some varieties the measured values of both mycotoxins are higher in intensive cultivation.

Occurrences of aflatoxins and ochratoxins in spring barley were higher in 1995 and in some varieties (Orbit, Terno) they are reaching the limit values set by the Decree 305/2004. The highest contents of ochratoxin were found in the variety Terno – 5.9  $\mu\text{g}\cdot\text{kg}^{-1}$ , but even in this case the limit 10  $\mu\text{g}\cdot\text{kg}^{-1}$  was not exceeded.

Further study of the content of mycotoxins was carried out after 10 years of ecological management, when certain stability of the yield and content of nutrients in soil was manifested and we had acquired the knowledge of the dynamics of the release of mineral nitrogen as well as the plant health situation of the stand.

As it can be seen in Table 5, in none of the cases the permitted limit of the studied substances for cereals has been exceeded. The content of aflatoxins A was lower than 0.14  $\mu\text{g}\cdot\text{kg}^{-1}$  of aflatoxin B < 0.12, ochratoxin < 0.06, zearalenon < 0.05  $\mu\text{g}\cdot\text{kg}^{-1}$ . The content of deoxynivalenol had different values according to varieties, but in no case it had exceeded the value of the new EU regulation, i.e. 500  $\mu\text{g}\cdot\text{kg}^{-1}$ .

Aflatoxins uncertainty of measurement is 40%, ochratoxin uncertainty of is 70%, DON uncertainty of measurement is 20%, zearalenon – uncertainty of measurement is 13%.

In selected varieties of malting barley that are preferred by the breweries producing Czech trademarks of beer, we have studied the occurrence of mycotoxins from ecological and intensive farming. As can be seen in Table 5, all values of aflatoxins are below the limits set by the Decree of Ministry of Health and the Regulation of the EU Committee No. 1881/2006. Higher values of deoxynivalenol are also well below the limits.

We have received similar results (Table 7) in the tests for the content of mycotoxins in grain and malt in 2008, although a higher occurrence could have been expected due to greater precipitations in the period of the grain formation and ripening.

Observation of mycotoxins in cereals from controlled ecological cultivation in our long-term experiment did not prove above the limit contents of mycotoxins. It is known from conventional cultivation (Váňová et al., 2004; Hýsek et al., 2005; Váňová, 2006, and others), that the occurrence of these substances is affected by the structure of crops (representation of cereals on arable land), crop rotation and some forecrops, e.g. maize where its post harvest residues are a source of infection. In ecological agriculture the structure of the crops is very diversified with the share of crops below 50% and representation of improving, fertilizing crops, such as clover and legumes fixing atmospheric nitrogen. Another source of nutrients is green dressing that increases the phytosanitary effect in the soil. It is the diversity of crops, their rotation and application of green manure which, according to Váňová

Table 3. Occurrence of mycotoxins ( $\mu\text{g.kg}^{-1}$ ) in winter wheat varieties in ecological and intensive cultivation

Varieties	Ecological farming		Intensive farming	
	aflatoxin B <sub>1</sub>	ochratoxin A	aflatoxin B <sub>1</sub>	ochratoxin A
Hana	< 0.3	< 1.0	0.6	1.3
Siria	< 0.3	1.2	< 0.3	1.3
Ina	< 0.3	1.2	0.5	< 1.0
Alka	< 0.3	< 1.0	0.6	1.3
Siria	< 0.3	1.2	< 0.3	1.2
Asta	0.6	< 1.0	0.9	< 1.0
Trane	< 0.3	1.4	< 0.3	1.2
Regina	0.5	< 1.0	< 0.3	1.3
Torysa	0.9	1.5	0.8	1.4
Samanta	< 0.3	< 1.0	0.9	1.2
Astella	0.5	< 1.0	0.6	< 1.0

Sensibility of the method AFB<sub>1</sub> is 0.6 and OA it is 1.5  $\mu\text{g.kg}^{-1}$

Table 4. Occurrence of mycotoxins ( $\mu\text{g.kg}^{-1}$ ) in the grains of ecologically cultivated spring barley

Varieties	Aflatoxin B <sub>1</sub>	Ochratoxin A
Krystal	0.9	2.5
Rubín	0.9	2.0
Jaspis	1.0	1.9
Orbit	2.0	2.2
Jarek	0.8	3.1
Profit	0.9	2.0
Malvaz	1.5	2.0
Jubilant	1.0	2.4
Terno	2.2	5.9
Akcent	0.8	2.3
Sladko	1.1	2.5
Svit	1.0	< 1.5
Stabil	< 0.6	< 1.5
Pax (Kosan)	< 0.6	< 1.5
Novum	< 0.6	< 1.5
Radík	< 0.6	< 1.5
Forum	< 0.6	< 1.5
Viktor	< 0.6	< 1.5

Sensibility of the method AFB<sub>1</sub> is 0.6 and OA it is 1.5  $\mu\text{g.kg}^{-1}$

Table 5. The content of mycotoxins ( $\mu\text{g.kg}^{-1}$ ) in winter wheat grains cultivated in ecological agriculture in 2006

Varieties	Aflatoxin $\Sigma$	Aflatoxin B <sub>1</sub>	Ochratoxin	DON	Zearalenon
Samanta	< 0.14	< 0.12	< 0.06	< 0.08	< 0.05
Alana	< 0.14	< 0.12	< 0.06	< 0.08	< 0.05
Apache	< 0.14	< 0.12	< 0.06	< 0.08	< 0.05
Vlasta	< 0.14	< 0.12	< 0.06	0.17	< 0.05
Sulamit	< 0.14	< 0.12	< 0.06	0.11	< 0.05
Ludwig	< 0.14	< 0.12	< 0.06	0.15	< 0.05
Banquet	< 0.14	< 0.12	< 0.06	< 0.08	< 0.05
Rheia	< 0.14	< 0.12	< 0.06	< 0.08	< 0.05
Mladka	< 0.14	< 0.12	< 0.06	0.13	< 0.05
Maritto	< 0.14	< 0.12	< 0.06	< 0.08	< 0.05
Cubus	< 0.14	< 0.12	< 0.06	< 0.08	< 0.05
Barokko	< 0.14	< 0.12	< 0.06	0.24	< 0.05
PB01/1035	< 0.14	< 0.12	< 0.06	0.25	< 0.05
Ebi	< 0.14	< 0.12	< 0.06	0.13	< 0.05
Complet	< 0.14	< 0.12	< 0.06	0.28	< 0.05
Drifter	< 0.14	< 0.12	< 0.06	0.12	< 0.05
Batis	< 0.14	< 0.12	< 0.06	< 0.08	< 0.05
Ilias	< 0.14	< 0.12	< 0.06	0.12	< 0.05
Globus	< 0.14	< 0.12	< 0.06	< 0.08	< 0.05
Alibaba	< 0.14	< 0.12	< 0.06	< 0.08	< 0.05
Clarus	< 0.14	< 0.12	< 0.06	< 0.08	< 0.05
Rapsodia	< 0.14	< 0.12	< 0.06	0.12	< 0.05
Hedvika	< 0.14	< 0.12	< 0.06	0.22	< 0.05
Darwin	< 0.14	< 0.12	< 0.06	0.18	< 0.05
Akteur	< 0.14	< 0.12	< 0.06	0.14	< 0.05
Heroldo	< 0.14	< 0.12	< 0.06	< 0.08	< 0.05
Biscay	< 0.14	< 0.12	< 0.06	0.19	< 0.05

Table 6. The content of mycotoxins ( $\mu\text{g}\cdot\text{kg}^{-1}$ ) in grains of selected varieties of malting barley cultivated ecologically (ECO) and intensive (INT) farming in 2006

Varieties	Aflatoxin B <sub>2</sub>	Aflatoxin G <sub>1</sub>	Aflatoxin B <sub>1</sub>	Aflatoxin G <sub>2</sub>	Deoxynivalenol (DON)	Ochratoxin A
Malz ECO	< 0.1	< 0.1	< 0.1	< 0.1	< 100	< 0.35
Prestige ECO	< 0.1	< 0.1	< 0.1	< 0.1	< 100	< 0.35
Sebastian ECO	< 0.1	< 0.1	< 0.1	< 0.1	160	< 0.35
Malz INT	< 0.1	< 0.1	< 0.1	< 0.1	< 100	< 0.35
Prestige INT	< 0.1	< 0.1	< 0.1	< 0.1	< 100	< 0.35
Sebastian INT	< 0.1	< 0.1	< 0.1	< 0.1	< 100	< 0.35

Table 7. The contents of mycotoxins ( $\mu\text{g}\cdot\text{kg}^{-1}$ ) in grain and malt from barley from ecological farming in 2008

Varieties	Aflatoxin B <sub>1</sub>	$\Sigma$ Aflatoxins B <sub>1</sub> , B <sub>2</sub> , G <sub>1</sub> , G <sub>2</sub>	Deoxynivalenol	Ochratoxin A
Prestige grain	< 0.1	< 0.1	< 30.0	< 0.2
Sebastian grain	< 0.1	< 0.1	< 30.0	< 0.2
Prestige malt	< 0.1	< 0.1	< 30.0	< 0.2
Sebastian malt	< 0.1	< 0.1	< 30.0	< 0.2

et al. (2008) decrease the danger of mycotoxins being present. In ecological agriculture traditional tillage is applied. The different reduced systems of tillage and soil preparation are not used since they demonstrably contribute to the incidence of mycotoxins.

The effect of lodging of the stand on the occurrence of the above described toxins is also mentioned. In ecological agriculture this happens only as a result of extreme weather, because these stands are not overfertilized with mineral nitrogen fertilizers, and the stand density is lower than that under intensive cultivation.

Thus, one of the possible reasons for the plant infestation by *Fusarium* sp. is the weather pattern, particularly the daily sums of precipitations and air humidity associated with it, during the infection season.

Occurrence of *Fusarium* sp. fungi in malt barley also affects the quality of beer. A *Fusarium* outbreak exceeding 60% of infested grains increased the occurrence of overfoaming of beer, the so-called gushing. Using the results outlined in this study we can therefore support the suitability of ecological cultivation for the beer production, in our case bio-beer, not only for its sub-limit content of mycotoxins but also for the good quality parameters of malt barley and malt from barley that have been cultivated ecologically (P e t r , P s o t a , 2007).

## CONCLUSIONS

Repeated testing of the occurrence of mycotoxins in 91 varieties of cereals ecologically cultivated according to the IFOAM international rules and the Decree of the Ministry of Agriculture as well as the relevant EU regulations has not proved an above-limit occurrence of aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, G<sub>2</sub> deoxynivalenol and ochratoxin A.

Ecological cropping systems include most of the cultural practices that suppress infection and contamination by mycotoxins. They comprise a broad diversity of crops, their rotation, limited share of cereals in the cropping pro-

cedure, and include fertilizing crops – clover crops, legumes and intercrops for green fertilization. The basic systems of soil tillage are performed by traditional tillage. Such cropping systems have been significantly proven as a means for limitation of infestation by *Fusarium* spp. fungi and subsequent contamination by mycotoxins. The role of organic farming is also strengthened by the fact that mineral fertilizers are not used since they increase the sensibility to infections by diseases. Secondly, the plants are not stressed by application of pesticides. Therefore, in conclusion, our results confirm that in ecological agriculture where fungicides are not applied, the infestation by moulds of the genus *Fusarium* sp., as well as the content of mycotoxins, is not higher than in conventional cultivation system.

## REFERENCES

- BERLECH, M. – BACKES, F. – KRAMER, J.: Mould spectrum and mycotoxins (deoxynivalenol and ochratoxin A) in grain samples from ecological and integrated cultivated sites. *Agrobiol. Res.*, 51, 1998: 369–376
- BULK van der, R. W. : Mycotoxin risk in the organic production chain. In: Proc. Sem. „Kvalita potravin z ekologického zemědělství“ (Food quality from ecological agriculture), VŠCHT Praha, 7. 2. 2003, pp. 7–11.
- CHU, F. S.: Production of antibody against ochratoxin A. *Appl. Environ. Microb.*, 31, 1976: 813–836.
- HÝSEK, J. – BROŽOVÁ, L. – VÁŇOVÁ, M. – HAJŠLOVÁ, J.: Trichohecénové mykotoxiny hub rodu *Fusarium* na jarním ječmeni a ve sladu a ochrana proti nim (Trichothecene mycotoxines of the fungi of the genus *Fusarium* on spring barley and in the malt and protection against them). *Ječmenářská ročenka 2005* (Barley Yearbook 2005): 160–170.
- PASCALÉ, M. – de GIROLAMO, A. – VISCINTI, A. – PANCALDI, D.: Investigation on the occurrence of deoxynivalenol in cereals from Northern Italy in 1998. *Informatore Fitospatologo*, 50, 2000 (10): 68–73.
- PETR, J. – PSOTA, V.: Kvalita sladovnického ječmene z ekologického zemědělství agriculture (Quality of malting barley

from ecological agriculture). Ječmenářská ročenka 2007 (Barley Yearbook 2007): 219–225.

PÍCHOVÁ, D. – PÍCHA, J. – VEREŠ, K. – DOLEŽALOVÁ, V.: Stanovení aflatoxinu B<sub>1</sub> v krmivech a biologickém materiálu RIA postupem pomocí vlastních specifických protilátek a ligandu I<sup>125</sup> pro potřeby živočišné výroby (Determination of aflatoxin B<sub>1</sub> in feeds and biological material RIA by the procedure of specific antibodies and ligand I<sup>125</sup> for the needs of animal production). Realizační výstup dílčího úkolu VI-5-6/14, Praha-Uhřetěves, 1981. 26 pp.

PROKINOVÁ, E. – KAZDA, J. – PETR, J. – ALTMANOVÁ, G.: Health condition of winter wheat in conventional and ecological farming. *Scientia Agric. Bohem.*, 31, 2000: 25–40.

QUILLEIEN, J. F.: Mycotoxins. Flair-Flow 4 synthesis report. Paris, SMEs INRA 2002.

REISNEROVÁ, H. – SOVA, Z. – CAPOUCHOVÁ, I.: Sledování některých mykotoxinů u sladovnického ječmene a ve sladu (Investigation of some mycotoxins in malt barley and in malt). *Úroda*, 40, 1992: 303–304.

SEDMÍKOVÁ, M. – REISNEROVÁ, H.: Mykotoxiny v krmivech, I. a II. část (Mycotoxins in feedstuffs, parts I and II): Mikroskopické houby jako producenti mykotoxinů (Micro-

scopic fungi as producers of mycotoxins). Toxické účinky plísní rodu *Fusarium* a rizika jejich výskytu v krmivu (Toxic effects of the genus *Fusarium* and risks of their incidence in feeds). *Krmivářství*, 7, 2003: 26–29.

SEDMÍKOVÁ, M. – REISNEROVÁ, H. – DUFKOVÁ, Z. et al.: Potential hazard of simultaneous occurrence of aflatoxin B<sub>1</sub> and ochratoxin A. *Vet. Med.-Czech*, 46, 2001: 169–174.

VÁŇOVÁ, M.: Čím může být poškozeno zrno jarního ječmene? (What can cause damage to spring barley grain?). Ječmenářská ročenka 2006 (Barley Yearbook 2006): 189–203.

VÁŇOVÁ, M. – KLEM, K. – MÍŠA, P. – MATUŠÍNSKÝ, P. – HAJŠLOVÁ, J. – LANCOVÁ, K.: The content of *Fusarium mycotoxins*, grain yield and quality of winter wheat cultivars under organic and conventional cropping systems. *Plant Soil Environ.*, 54, 2008: 395–402.

VÁŇOVÁ, M. – HAJŠLOVÁ, J. – HAVLOVÁ, P. – MATUŠÍNSKÝ, P. – LANCOVÁ, K. – SPITZEROVÁ, D.: Effect of spring barley protection of *Fusarium* sp. mycotoxins in grain and malt using fungicides in field trials. *Plant Soil Environ.*, 50, 2004: 447–455.

Received for publication on August 11, 2009  
Accepted for publication on September 30, 2009

PETR, J. – REISNEROVÁ, H. (Česká zemědělská univerzita, Fakulta agrobiologie, potravinových a přírodních zdrojů, katedra rostlinné výroby a katedra veterinárních disciplín, Praha, Česká republika):

#### Výskyt mykotoxinů u obilovin z ekologického zemědělství.

*Scientia Agric. Bohem.*, 40, 2009: 189–195.

Produkty organického či ekologického zemědělství (EZ) jsou zvláště přísně kontrolovány z hlediska kvality a potravinové bezpečnosti. Velká pozornost se věnuje právě výskytu mykotoxinů, protože se předpokládalo, že porosty neošetřené fungicidy z ekologického pěstování budou podle názoru agrochemických firem silně napadeny plísněmi rodu *Fusarium* sp. Rizika výskytu nelze jistě vyloučit, např. vlivem počasí při dozrávání a při nevhodném skladování obilovin. Jak se ukázalo ze zprávy evropské komise – Scientific Committee on Plants EU, ani aplikace fungicidů nezaručuje zamezení výskytu chorob a kontaminace mykotoxiny.

V dlouhodobém ekologickém pokusu, vedeném podle mezinárodních pravidel IFOAM (Mezinárodní federace hnutí organického zemědělství) a vyhlášky MZe ČR a předpisů EU, jsme sledovali výskyt mykotoxinů u ozimé pšenice a jarního ječmene. Pokusy byly vedeny v úrodné řepařské oblasti v Uhřetěvsi. Ke stanovení mykotoxinů v počátečním období testování byla použita metoda RIA, v dalších letech metoda HPLC. Všechny hodnoty šetření obsahu aflatoxinů i ochratoxinů v prvním období sledování byly pod hranici tehdy platných limitů, předpisů i současných nařízení komise EU 1881/2006. I při srovnávání ekologického a intenzivního pěstování jarního ječmene byly prokázány nižší hodnoty toxinů z ekologického pěstování než z intenzivního pěstování. V dalším období testování moderními metodami HPLC nebyly u žádné odrůdy překročeny limity aflatoxinu A, aflatoxinů B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, G<sub>2</sub>, deoxynivalenolu, zearalenonu a ochratoxinu A. Podobně to bylo u odrůd jarního ječmene. Ekologický pěstitelský systém zahrnuje většinu agrotechnických prvků, které do značné míry eliminují infekci a kontaminaci mykotoxiny. Jde o širokou diverzitu plodin, jejich střídání, omezený podíl obilnin v osevním postupu, zastoupení zúrodnujících plodin – jetelovin a luskovin a meziplodin na zelené hnojení. Také základní způsoby zpracování půdy jsou dělány tradiční orbou. Takové pěstitelské postupy byly prokazatelně ověřeny jako prostředky k omezení napadení houbami rodu *Fusarium* sp. a následné kontaminace mykotoxiny. Roli také hraje, že se nepoužívá hnojení industriálními dusíkatými hnojivami, která zvyšují vnímavost k infekci chorob. Odolnost rostlin také zvyšuje to, že nejsou stresovány aplikací pesticidů. Můžeme tedy potvrdit, že v ekologickém zemědělství, kde se nepoužívají fungicidy, není napadení plísněmi rodu *Fusarium* sp. ani obsah mykotoxinů vyšší než v konvenčním pěstitelském systému, kde je použití fungicidů běžné.

mykotoxiny; ekologické zemědělství; aflatoxiny B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, G<sub>2</sub>; deoxynivalenol; ochratoxin A; pšenice; ječmen; žito

---

#### Contact Address:

Prof. Ing. Jiří Petr, DrSc., dr.h.c, Česká zemědělská univerzita v Praze, Fakulta agrobiologie, potravinových a přírodních zdrojů, Kamýcká 129, 165 21 Praha 6-Suchbát, Česká republika, e-mail: jpetr@af.czu.cz

---