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Audrone Mankeviciene[1], Skaidre Suproniene[1], Irena Brazauskiene[1], Elvyra Gruzdeviene[2]

[1]Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry, Instituto alėja 1, Akademija, LT-58344 Kėdainiai distr.; [2]Upytė Experimental Station of the Lithuanian Research Centre for Agriculture and Forestry Linininkų 3, Upytė LT- 38294, Panevėžys distr.

NATURAL OCCURRENCE OF *FUSARIUM* MYCOTOXINS IN OIL CROP SEED

ABSTRACT

Oilseeds are a perfect medium for microfungi spread and mycotoxin production. With increasing demand for oil crop produce such research has gained a special relevance since research evidence on this issue is scarce. During 2007-2009, prevalent fungi genera, including *Fusarium* genus, potential producer of deoxynivalenol (DON), zearalenone (ZEA), T-2 toxin (T-2) etc. were determined in our tests. The ELISA immunoenzymatic method and Veratox Fast kits were used to identify and quantify mycotoxins, while *Fusarium* fungi species were identified using conventional fungi determination techniques. Higher *Fusarium* fungi contamination level was found on linseed compared with that on spring or winter rapeseed. The difference was even more obvious in different experimental years, however, having identified *Fusarium* species, *F. avenaceum* and *F. oxysporum* were found to be prevalent in the seed of all crop species tested. In 2009, spring rape samples were found to contain *F. dimerum* which is a significant human pathogen.

The presence of DON was identified in 18.2-100%, ZEA in 40 -100%, and T-2 toxin in 100% of seed samples of all oil crop species tested. From the food safety viewpoint, the concentrations determined did not exceed the levels hazardous for health, laid out in the EU regulations, however, the effect of low toxin concentrations is slow and the negative consequences manifest themselves only after some time and in various forms, which poses a serious health risk for humans and animals.

Key words: deoxynivalenol, *Fusarium* spp., linseed, rapeseed, T-2 toxin, zearalenone,

INTRODUCTION

Fusarium spp. fungi control and mycotoxin content analyses are mostly done on various cereal samples, and there is little evidence on the occurrence of these fungi and their metabolites on oil crops' seed. It is known that linseed, winter and spring rape crops are badly damaged by Fusarium

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wilt, whose causal agents *F. oxysporum* and *F. avenaceum* are also detected on the seed of these crops (Lange 2002, Ehrensing 2008). It was found that apart from *F. oxysporum, F. avenaceum,* in separate years linseed was infested with *F. poae, F. heterosporum, F. proliferatum, F. graminearum* (Gruzdevienė *et al.* 2006), while rapeseed was found to infested with *F. culmorum, F. equiseti, F. moniliforme, F. poae* (Lugauskas 2005), which are producers of deoxynivalenol (DON), zearalenone (ZEA), T-2 toxin (T-2), HT-2 toxin (HT-2), fumonizin (FUM) and other mycotoxins (D'Mello *et al.* 1999, Bennett and Klich 2003). Some authors suggest that low DON levels are found in both rapeseed and linseed samples (Brazauskienė *et al.* 2006, Gruzdevienė *et al.* 2006), however ZEA and T-2 toxin incidence on the seed of these plants was not identified. Mycological analyses of rapeseed meal and cake revealed that *F. graminearum*, *F. moniliforme, F. oxysporum* fungi and DON and ZEA traces were present in practically each sample tested (Tabuc and Stefan 2005).

This paper assessed the frequency of *Fusarium* fungi observed in winter and spring oil seed rape and linseed analysed at the Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry during 2007-2009 and identified and quantified the mycotoxins detected in the samples.

MATERIALS AND METHODS

Sample handling

A total of 348 representative samples of oil crops' seeds produced during 2007-2009 were analyzed: 152 winter rapeseed, 120 spring rapeseed, 69 linseed and 7 rapeseed cake samples. They were collected in the central region of Lithuanian (Kėdainiai and Panevėžys distr.) immediately after seed harvesting. Oil crops were grown following the conventional technology.

Fusarium spp. analysis

The internal seed infestation with *Fusarium* fungi was tested on winter rape, spring rape and linseed samples (341 per crop) by an agar plate method (Mathur and Kongsdal 2003). The surface-sterilized seeds (400 per sample) were plated on a Potato Dextrose Agar (PDA) and incubated at 26 ± 2ºC in the dark. The infection level of seed was evaluated in percent (0 – all seeds healthy, 100% – all seeds infected). Microscopic studies of *Fusarium* fungi were carried out after 7–8 days. The purified single spore cultures of *Fusarium* species were identified on the basis of their cultural and morphological characteristics according to Gerlach and Nirenberg (1982), Nelson *et al.* (1983) and Leslie *et al.* (2006).

Analysis of mycotoxins

Oil crop seed samples (103 per crop) were collected at harvesting and analysed for deoxynivalenol (DON), zearalenone (ZEN), and T-2 toxin (T-2) contamination. The analysis was done by the CD-ELISA (competitive direct enzyme-linked immunosorbent assay) method (Wilkinson et al. 1992). The Veratox® quantitative test kits (Neogen corporation, Food Safety Diagnostics), approved by the AOAC Research Institute (Certificate N 950702) were used for the analysis. Mycotoxin extraction and tests were performed according to manufacturer's instructions. The optical densities of samples and controls from standard curve were estimated by a photometer Multiskan Ascent, using filter of 650 nm. The data were computed using the Ascent Software. Measured absorbances were automatically converted to the mycotoxin concentration units - $\mu g \times kg^{-1}$. The results were estimated taking into account the lowest calibration curve's mycotoxin concentration value (LOD-limit of detection), which is for: DON – 100.0 $\mu g \times kg^{-1}$ (ppb); ZEN – 10.0 $\mu g \times kg^{-1}$ (ppb); T-2 – 7.5 $\mu g \times kg^{-1}$ (ppb).

Statistical analysis

ANOVA was applied for the statistical processing of data. For data significance the Fisher test was used. Averages for the other data were calculated (Tarakanovas and Raudonius 2003).

RESULTS AND DISCUSSION

No *Fusarium* fungi were found in internal tissue of winter rapeseed in 2007 – 2008 (Table 1), and in 2009, the contamination level amounted to 0.43 %. In 2007 – 2009, spring rapeseed internal infection with *Fusarium* fungi varied from 0.41 % to 1.86 %. *F. oxysporum and F. avenaceum* species prevailed. In 2009, spring rape samples were found to contain *F. dimerum* which is a significant human pathogen (Marom *et al.* 2008). A higher *Fusarium* contamination level was identified on linseed (3.5 – 14.5 %). *F. oxysporum, F. avenaceum, F. poae* and other species prevailed in the samples. In 2007, contamination with the above-mentioned fungi was more than twice as high as that in 2008 or 2009. The higher *Fusarium* contamination of linseed might have been determined by the weather conditions, harvesting timing, or later maturity (Gruzdeviene *et al.* 2006).

Mycotoxin tests showed that irrespective of the fact that *Fusarium* spp. fungi were not found in internal tissue of winter and spring rapeseed, in 2007 DON was identified in 75 - 100 %, ZEA 92.3 - 100 % samples tested, and traces of T-2 toxin were found in all winter and spring rapeseed samples tested (Table 2). The concentrations of T-2 identified were low (8.5-10.2 $\mu g\ kg^{-1}$). Linseed samples were 100 % contaminated with DON, ZEA, and T-2 toxin. Although the levels detected are not high, the risk lies in the fact that co-occurrence of all the three toxins was determined in each

sample tested and the effects of their interaction on human and animal health have not been studied, since all of them affect different body systems (Bennett and Klich 2003, Tabuc and Stefan 2005).

Table 1.

***Fusarium* contamination in oil crops' seed in 2007-2009**

Oil crops	Internal infection of oilseeds		
	2007	2008	2009
Winter oilseed rape			
No. analyzed samples	32	60	60
Mean of infection %	0	0	0.43
Standard deviation	0	0	0.22
Min values %	0	0	0
Max values %	0	0	3.0
Coefficient of variation %	0	0	199.22
Spring oilseed rape			
No. analyzed samples	32	44	44
Mean of infection %	1.11	0.41	1.86
Standard deviation	0.77	0.11	0.94
Min values %	0	0	0
Max values %	7.0	1.0	10.50
Coefficient of variation %	208.37	91.76	167.16
Linseed			
No. analyzed samples	24	40	5
Mean of infection %	14.5	3.5	5.2
Standard deviation	1.65	0.42	2.65
Min values %	8.0	2.0	0.5
Max values %	19.0	5.5	15.5
Coefficient of variation %	27.84	38.92	114.10

In 2008, all winter and spring rapeseed samples tested (100%) were contaminated with ZEA and T-2 toxin (Table 3), however a lower DON contamination (18.2%) was identified in spring rapeseed samples. For winter rapeseed DON contamination was 4 times as high as that for spring rapeseed (84 %). Mycotoxin contamination on linseed differed from that on rapeseed . DON was identified in 100 % of samples tested, ZEA in 47.4 %,

T-2 in 30.4 %. Other researchers also identified low contents of DON and ZEA in all samples of rapeseeds and rapeseed meal tested using ELISA method (Tabuc and Stefan 2005).

Table 2.

Mycotoxin contamination in the seeds of various oil crops in 2007

Level of contamination and concentration	Mycotoxins [µg × kg^{-1}]		
	DON	ZEA	T-2
Winter oilseed rape			
No. of analyzed samples	8	12	8
Sample contamination %	100	100	100
Mean of contamination	165.0	18.6	9.2
Min values	153.5	10.6	8.5
Max values	176.5	25.6	10.2
Standard deviation	2.97	1.88	0.19
Coefficient of variation %	5.10	34.92	5.96
Spring oilseed rape			
No. of analyzed samples	8	13	8
Sample contamination %	75.0	92.3	100
Mean of contamination	133.0	19.6	9.7
Min values	0	0	8.2
Max values	181.0	25.10	10.1
Standard deviation	29.05	2.08	0.22
Coefficient of variation %	61.76	38.34	6.43
Linseed			
No. of analyzed samples	6	6	6
Sample contamination %	100	100	100
Mean of contamination	155.3	19.4	15.9
Min values	146.0	11.3	9.8
Max values	163.5	24.1	22.1
Standard deviation	2.78	2.40	2.60
Coefficient of variation %	4.39	30.37	40.10

Table 3.

Mycotoxin contamination in the seeds of various oil crops in 2008

Level of contamination and concentration	Mycotoxins [μg × kg^{-1}]		
	DON	ZEA	T-2
Winter oilseed rape			
No. of analyzed samples	25	25	19
Sample contamination %	84.0	100	100
Mean of contamination	209.7	14.3	<LOD*
Min values	0	<LOD	<LOD
Max values	278.3	32.3	11.6
Standard deviation	18.85	1.45	0.72
Coefficient of variation %	44.94	50.49	51.43
Spring oilseed rape			
No. of analyzed samples	11	11	9
Sample contamination %	18.2	100	100
Mean of contamination	<LOD	14.3	<LOD
Min values	0	<LOD	<LOD
Max values	226.0	25.1	10.1
Standard deviation	27.49	1.67	0.85
Coefficient of variation %	222.49	38.74	43.26
Linseed			
No. of analyzed samples	19	19	23
Sample contamination %	100	47.4	30.4
Mean of contamination	209.7	<LOD	<LOD
Min values	110.5	0	0
Max values	271.7	22.5	8.6
Standard deviation	13.21	1.69	0.56
Coefficient of variation %	27.46	154.23	82.73

*LOD - limit of detection

In 2009, mycotoxin tests were done in rapeseed and rapeseed cake samples (Table 4). DON and T-2 was found in 100 % of linseed samples tested, ZEA in 40 %. It was noticed that in rapeseed cake DON concentrations were nearly twice as high as those in rapeseed samples (267.3-590.5 μg kg^{-1}). For rapeseed cake ZEA and T-2 contamination was present in 86 % and 66.6 % of samples

tested. Using rapeseed cake with such a high contamination level as an additive in animal feed can be detrimental to animal health.

Table 4.

Mycotoxin contamination in linseed and rapeseed cake in 2009

Level of contamination and concentration	Mycotoxins μg kg^{-1}		
	DON	ZEA	T-2
Linseed			
No. of analyzed samples	10	10	10
Sample contamination %	100	40	100
Mean of contamination	115.5	<LOD*	9.3
Min values	109.8	0	8.7
Max values	124.1	10.5	9.9
Standard deviation	1.38	1.69	0.14
Coefficient of variation %	3.79	129.11	4.70
Rapeseed cakes			
No. of analyzed samples	7	7	3
Sample contamination %	100	86	66.6
Mean of contamination	387.0	12.3	11.8

*LOD - limit of detection

CONCLUSIONS

Monitoring of *Fusarium* fungi and DON, ZEA, T-2 mycotoxins in the seed and cake of Lithuania-grown oil crops showed that in separate experimental years (depending on the weather conditions, plant species and other factors) favourable conditions for their spread can occur , which, in terms of food safety, may result in a chain of risk factors. More attention should be paid to linseed contamination with *Fusarium* fungi and mycotoxins under field conditions, since our research found a higher contamination level in it. A higher *Fusarium* species diversity was established, which creates conditions for the spread of metabolites of more varied chemical composition.

Rapeseed cake used as an additional protein source intended to enrich feed-stuffs can be a risk factor since research indicated practically every sample to be contaminated with DON, ZEA, and T-2 toxin. For mycotoxin risk assessment, the importance of *Fusarium* fungi and trichotecenes and zearalenone contamination of oil crop seeds warrants further study.

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