

The effect of environmental conditions on the variation of fungi and mycotoxin contents in oil flax seed

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The present paper analyses fungal and mycotoxin contamination of oil flax seed of the flax cultivars ‘Symphonia’, ‘Blue Chip’, ‘Szaphir’, ‘Gold Merchant’, ‘Lu-5’ and ‘Helmi’ differing in the length of the growing season. The flax seed grown in precision field trials was analysed for fungal and mycotoxin contamination at harvesting and during the storage period. Analyses at harvesting revealed the fungi of the genera *Alternaria* (from 20.0 to 42.5% of infected seed) and *Fusarium* (up to 50.0% of infected seed) to be the most prevalent. Flax seed was stored for 8 months in dry and cool premises. Seed surface contamination during the storage period of the early cv. ‘Symphonia’ increased by 92.8% and that of cv. ‘Szaphir’ by 89.7%. The fungal contamination of seed of the later-ripening cvs. ‘Blue Chip’ and ‘Gold Merchant’ increased by 30.4 and 41.2%, respectively. During storage, the content of propagules of the genera *Alternaria* and *Penicillium* increased as compared with their contents at harvesting, whereas that of *Fusarium* declined. Four species of *Alternaria* were identified: *A. alternata*, *A. linicola*, *A. dianthi* and *A. pluriseptata*. The greatest number of fungi species (11) were identified on the seed of cv. ‘Gold Merchant’. Traces of aflatoxin was identified only in the seeds samples of cvs. ‘Lu-5’ and ‘Gold Merchant’ and of ochratoxin A (2.3 µg kg⁻¹) in the seed sample of cv. ‘Szaphir’. All the seed samples tested were found positive for DON contamination, except for cv. ‘Blue Chip’, but the levels identified were very low. After eight months of storage the levels of aflatoxin and ochratoxin A. in flax seed samples increased.

Key words: *Linum usitatissimum*, *Alternaria* spp., *Penicillium* spp., *Fusarium* spp., mycotoxins, seeds

INTRODUCTION

Flax (*Linum usitatissimum* L.) is unique in its high alpha-linolenic fatty acid content in seeds. Alpha-linolenic fatty acid is an omega-3 fatty acid which contributes to good human and animal health (Wensing et al., 1999). Flax adds a pleasant nutty taste to products. The attractive, oval reddish-brown seeds of flax add taste, extra texture and good nutrition to breads and other baked goods. Flax has been long used in multi-grain cereals and snack foods (Wirths et al., 1985). Flax also delivers the benefits of its soluble fibre, lignans, omega-3 fatty acid mix and protein. Flax is increasingly used as an ingredient in feeds for improved animal and fish nutrition. The benefits of omega-3 fatty acids to pigs, cattle, horse and other animals may be in preventing young animals from developing infections (Mukhopadhyay, Ray, 2001; Ponter et al., 2006).

Flax seed and its products are used in food and feed preparation technologies, therefore their microbiological contamination is a very important quality indi-

cator. The quality of crop produce during growing and storage is determined by natural conditions and anthropogenic factors. The diversity of fungi occurring on oil flax seeds is largely dependent on the growing conditions, however, some fungi species of the genera *Colletotrichum*, *Fusarium*, *Rhizoctonia*, *Alternaria*, *Aspergillus*, *Penicillium* occur in all flax-growing countries (Mercer, Hardwick, 1991; Paul et al., 1991; Simay, 1994; Kumud et al., 1997). Flax seed gets infected with fungi during ripening of capsules, since at the end of ripening the capsules of many flax cultivars crack and thus favour fungal infection. Weather conditions, especially a contrasting air temperature regime and moisture, are the most important factors for fungal development on flax (Лучина, 1981). No less important factors are pathogen aggressiveness, moisture content on infected surface, host-plant genotype and plant development, cultivar, sowing timing, preceding crop, crop stand density (Paul et al., 1991).

In India, there were identified the following fungi species on oil flax seed: *Alternaria alternata*, *A. linicola*,

Aspergillus flavus, *Colletotrichum linicola*, *Curvularia lunata*, *Fusarium moniliforme*, *F. oxysporum* f. sp. *lini*, *F. pallidoroseum*, *Rhizoctonia bataticola*, *R. solani*, *Phoma exigua* var. *linicola*, *Botrytis cinerea*, *Drechslera tetramera* (Kumud et al., 1997).

In the USA, the following fungi species were identified on flax seed: *Pleospora herbarum* (Pers.) Rabenh., *P. stenospora* J. Schröt., *Comoclathris permunda* (Cooke) E. Müll. and *Mycosphaerella linicola* Naumov, *Curvularia geniculata* (Tracy & Earle) Boedijn (Farr et al., 1989).

Oil flax seed coat contains about 5.1–11.7% carbohydrate (mucilageous substances), cotyledons contain on average 25–45% fat and up to 30% of protein. Apart from these substances, flax seed contains carbohydrates, phosphorous compounds that are similar to fat in their composition, pigments, carotene, glycoside linamarine, enzymes (lipase, protease, etc.) and other substances (Stramkale et al., 2003). Flax seed is very hygroscopic, which makes it a good medium for the occurrence of various fungi. During storage, flax seed fungal contamination level can vary due to various factors, such as moisture, changes or variations in heat regime, etc. The best storability of seeds is achieved when the seed moisture does not exceed 10%. During storage it is recommended to protect the seeds from direct sunlight. Very large temperature variations are also undesirable during storage. The recommended ambient temperature for seed storage should not exceed 15 °C and relative air humidity 40%.

With the spread of fungi, mycotoxin formation in food and feed occurs. Mycotoxins are produced by fungi species of the genera *Fusarium* (De Nijs et al., 1996), *Penicillium* (Larsen et al., 2001), *Alternaria*, *Aspergillus* (Abarca et al., 1994). Mycotoxins are detrimental to human and animal health (Fink-Gremmels, 1999).

There are a lot of data in the literature on mycotoxin contamination in other oil crops (sunflower, cotton seed, various kinds of nuts), however, the experimental evidence of oil flax contamination with these toxic metabolites as affected by the environmental conditions, plant genotype and other factors is rather limited.

The present paper analyses fungal and mycotoxin contamination levels at harvesting and during storage in flax seeds of the varieties differing in the length of the growing season.

MATERIALS AND METHODS

The oil flax tested was grown in a crop rotation at the Upytė Research Station of the Lithuanian Institute of Agriculture in 2005. Soil tillage, weed and pest control practices were used in compliance with the recommendations for oil flax cultivation in Central Lithuania (Mikelionis, 2001). Oil flax was sown at a seed rate of 8 million viable seed per hectare with an SL-16 sowing machine, at 10 cm row spacing. The seeds were treated with the fungicide fludioxonil 18.75 g l⁻¹ + cyproconazole

6.25 g l⁻¹ (Maxim Star 025FS at a 1.5 l t⁻¹ rate) five days before sowing. Six varieties with different maturity were tested in the trial. These were 'Helmi' (early), 'Szaphir' (medium early), 'Symphonia' (early), 'Gold Merchant' (medium early), 'Blue Chip' (medium late), 'Lu-5' (late).

At the early yellow maturity stage, flax was pulled and dried in shocks in the field. Then it was thrashed with a ML-60 thrasher. The seeds were cleaned, weighed and put into paper bags. For analyses, seeds were sampled 300 g per treatment shortly after thrashing and cleaning. Flax seed samples were kept in paper bags in a dry (relative humidity 40 ± 5%) and cool (air temperature +10 ± 2 °C) place.

The fungal infection level of flax seed was analysed at the laboratories of the Lithuanian Institute of Agriculture, Institute of Botany and the Upytė Research Station.

Oil flax seed testing methods. Analyses of seed microflora were done following the methodology of Samson et al. (1992), Mathur et Kongsdal (2003). The initial observation of fungal colonies was conducted using a microscope at the lowest magnification (×10) in plates. For further morphological analyses microscopic preparations were prepared. The fungal colonies were re-sown and pure cultures of causal agents were isolated. The fungi were isolated and identified according to the cultural and morphological properties of the colonies. For determination of internal fungal infection the seeds were disinfected with 70% ethyl alcohol for 0.5 min, then rinsed three times in distilled water, dried on sterile filter paper and sown on malt agar medium. For the evaluation of one treatment or variety, 400 seeds were analysed. Ten flax seeds were placed per 9 mm Petri dish. After 5–7 days of incubation in a thermostat at a temperature of 25 °C the plates were inspected and checked for seed fungal contamination. Identification was carried out using Malone, Muskett (1997), Саттон и др., (2002), Mathur, Kongsdal (2003) descriptors.

Evaluation of seed surface contamination. For the evaluation of seed surface contamination with fungi the dilution plating technique was applied. 10 g of seeds was placed in 100 ml of sterile water, shaken for 10 min, dilutions (1:100, 1:1000, 1:10000) were made and 1 ml of suspension was drawn into a Petri dish and poured over with malt agar medium with chloramphenicol (50 mg/l). The analysis of each sample was performed in three replications (Samson et al., 1992). Fungi were cultivated for 7–10 days at a temperature of 28 °C. Colony forming units (cfu) of fungi per 1 gram of seeds and the distribution frequency (as a ratio of the number of samples where a species was found to the total number of examined samples in %) of the prevailing species were calculated (Mirchink, 1988). Pure fungal strains were isolated on standard Czapek, malt and corn extract media and identified according to manuals (Domsch et al., 1980; Ellis, 1971; 1976; Samson et al., 2000).

Mycotoxin analysis. Oil flax seed samples (200 g) were stored at -18 °C prior to examination for mycotoxins. The mycotoxins deoxynivalenol (DON), ochratoxin A, aflatoxin (total) concentration was determined on sub-samples of 50 g of seed per each plot. Seed samples were analysed by the ELISA (enzyme-linked immunosorbent assay) method (Bennet et al., 1994; Wilkinson et al., 1992). The Veratox® aflatoxin, Veratox® DON 5/5, Veratox® ochratoxin A test kits (Neogen, USA) were used for the analysis. Mycotoxin extraction and tests were performed according to manufacturer's instructions. Multiskan MS was used for reading immunoenzymic microstrips.

Statistical analysis. The data were processed by the analysis of variance (ANOVA) according to Fisher's protected least significant difference (PLSD) test at $P = 0.05$ and 0.01 to indicate statistically significant differences between treatments (Tarakanovas, Raudonius, 2003). Means were calculated for the other data.

RESULTS AND DISCUSSION

The weather conditions in 2005 were unfavourable for oil flax cultivation. There was a shortage of rainfall in the first and third ten-day periods of May, the first and second ten-day periods of July, and the second and third ten-day periods of August. There was very heavy rain in the second ten-day period of May and the first ten-day period of August. Conducive conditions to linseed fungal infection occurred in August, when rainy and wet weather prevailed.

Oil flax was sown on May 6 in 2005. The plants of oil flax varieties 'Symphonia', 'Blue Chip', 'Szaphir', 'Gold Merchant', 'Lu-5' and 'Helmi' started to emerge on May 13–18, and fully emerged on May 20–25 (Table 1). In July there was a shortage of rainfall because of which flax crops flowered rather rapidly. All the cultivars tested matured at different time. The cultivars 'Symphonia' and 'Szaphir' reached yellow maturity stage the earliest, on August 3, and their growing period lasted for 82 days, whereas cv. 'Helmi' was at early yellow maturity stage at that time and cvs. 'Blue Chip', 'Gold Merchant' and 'Lu-5' at green maturity

stage. The rainfall that fell on August 4 and 6 amounted to 6 and 12.5 mm and on August 8 – as much as 44 mm. Later, rainfall occurred daily until August 14, which resulted in prolonged flax maturation when some plants grew extra branches and produced flowers. The flax of cvs. 'Helmi', 'Blue Chip' and 'Gold Merchant' was harvested on August 18, when most of the capsules had matured until yellow maturity. The cultivar 'Lu-5' matured the latest, on August 23, the length of its growing period was 100 days (Table 1).

Having pulled, dried and thrashed flax, seed samples were taken and were analysed for surface and internal contamination with fungus capable of producing mycotoxins. Having analysed oil flax seed surface infection with fungi in the six oil flax cultivars the lowest contents of fungal propagules (0.5 and $0.7 \text{ cfu g}^{-1} \times 10^4$) were identified on seeds of cvs. 'Symphonia' and 'Lu-5' (Table 2), whereas the highest contents (1.6 and $1.7 \text{ cfu g}^{-1} \times 10^4$) were identified on seeds of cvs. 'Blue Chip' and 'Helmi'. It is possible that flax seed fungal infection at harvesting was determined by the weather conditions. The rainy period at the end of flax maturation stage was conducive to the occurrence of fungi. The flax of cvs. 'Szaphir' and 'Symphonia' matured earlier than the other cultivars, but after pulling and putting them into shocks for drying there was a heavy rainfall and the shocks began drying only after the rainy period had passed. The flax of cv. 'Lu-5' was still at green maturity stage at this time, therefore the fungal contamination of seed surface was lower than in other cultivars.

On analysing seeds for internal contamination at harvesting, we identified fungal propagules of the genera *Alternaria*, *Fusarium*, *Penicillium* and *Aspergillus*. The most abundant were *Alternaria* (from 20.0 to 42.5% of infected seed) and *Fusarium* (infected up to 50.0% of seeds) (Table 3). The highest infection level with these fungi was identified on the seed of cv. 'Lu-5', although the level of seed surface contamination was low. Seeds of cv. 'Helmi' had the lowest content of *Fusarium* propagules (7.5%). Of the other fungi identified in seeds, the following are worth mentioning: *Coletotrichum lini*, *Drechslera* sp., *Rhizoctonia* sp.,

Table 1. Data on the growing period of oil flax varieties Upytė, 2005

Cultivar	Growth stage					Length of growing period (days)
	Start of germination*	Flowering	Green maturity	Yellow maturity	Pulling time	
'Blue Chip'	18 05	09 07	19 07	15 08	18 08	92
'Gold Merchant'	17 05	09 07	19 07	14 08	18 08	93
'Helmi'	13 05	06 07	15 07	18 08	18 08	97
'Lu-5'	15 05	13 07	22 07	23 08	23 08	100
'Symphonia'	13 05	07 07	18 07	03 08	03 08	82
'Szaphir'	13 05	07 07	18 07	03 08	03 08	82

* All cultivars were sown on May 6.

Table 2. Content of fungi propagules on flax seed at harvesting and during storage (cfu g⁻¹)
Lithuanian Institute of Agriculture, 2005–2006

Cultivar	Content of fungi propagules (cfu g ⁻¹ × 10 ⁴)		
	At harvesting	After 8 months of storage	Increase during storage %
'Blue Chip'	1.6 ± 0.5	2.3 ± 1.5	30.4
'Gold Merchant'	1.0 ± 0.5	1.7 ± 0.6	41.2
'Helmi'	1.7 ± 0.9	5.3 ± 0.6	67.9
'Lu-5'	0.7 ± 0.7	2.0 ± 0	65.0
'Symphonia'	0.5 ± 0.1	7.0 ± 1.7	92.8
'Szaphir'	1.0 ± 0.5	9.7 ± 0.6	89.7

Table 3. Percentage of fungi capable of producing mycotoxins on flax seed at harvesting and during storage
Uptytė, 2005–2006

Cultivar	<i>Alternaria</i> spp.	<i>Fusarium</i> spp.	<i>Aspergillus</i> spp.	<i>Penicillium</i> spp.
Content of fungi-affected flax seed (%) at harvesting				
'Blue Chip'	30.0	35.0	0.5	0
'Gold Merchant'	32.5	35.0	0	0
'Helmi'	32.5	7.5	0.5	0
'Lu-5'	42.5	50.0	0	0
'Symphonia'	27.5	47.5	0.3	2.5
'Szaphir'	20.0	30.0	0	0
LSD ₀₅	3.14	2.83	2.81	-
Content of fungi-affected flax seed (%) after 8 months of storage				
'Blue Chip'	33.0	27.0	0	0
'Gold Merchant'	39.8	22.4	0	1.3
'Helmi'	51.5	7.3	0	0
'Lu-5'	55.1	39.7	0	0.1
'Symphonia'	35.6	41.2	0	2.9
'Szaphir'	13.0	15.7	0	0.5
LSD ₀₅	3.69	3.54	-	2.78

Botrytis cinerea, however, their total content did not exceed 5%.

Flax seed was stored for eight months in dry and cool premises and seed contamination tests were done again in the spring of 2006. Seed surface contamination increased during storage. For some varieties the increase especially notable: in the seed of cv. 'Szaphir' by 89.7%, in 'Symphonia' by 92.8% (Table 2). It is noteworthy that fungal seed contamination of later-maturing flax cultivars 'Blue Chip' and 'Gold Merchant' increased by 30.4 and 41.2%, respectively.

Analyses of seed for internal contamination revealed the presence of fungal propagules of the genera *Alternaria*, *Fusarium*, *Penicillium* (Table 3). Our experimental evidence suggests that during storage the contents of propagules of *Alternaria* and *Penicillium* on seeds and under the seed coat increased, and those of *Fusarium* decreased as compared with their contents identified at harvesting. No fungal propagules of the genus *Aspergillus* were found on flax seed after 8 months of storage.

Surface and internal flax seed fungal infection after 8 months of storage was estimated at the Institute of

Botany (Tables 4, 5). Various fungi species were identified, some of which occurred on most of the cultivars tested. These were *Alternaria* spp., *Cladosporium herbarum*, and *Mycelia sterilia*. *Alternaria* were most numerous in terms of species, there were identified as many as four species: *A. alternata*, *A. linicola*, *A. dianthi* and *A. pluriseptata* (Table 5). The greatest number of species (11) was identified on the seeds of cv. 'Gold Merchant'. Fewer fungi species (5–8) were identified on the seeds of earlier or later-maturing cultivars. Meteorological observations showed that when cv. 'Gold Merchant' reached early yellow maturity it was raining for several days, so the conditions for the development of fungi were favourable. This might have determined a more abundant incidence of fungi on flax capsules as well as on seed. Further tests are needed to verify this assumption. By the same token, after eight months of storage external seed contamination at early maturity was lower for cvs. 'Szaphir' and 'Symphonia' as compared with the other cultivars tested.

Analysis of seed contamination with fungi capable of producing mycotoxins on six oil flax cultivars at harvesting and during storage suggested that fungal

Table 4. **Fungi identified in flax seed washings after 8 months of storage**
Institute of Botany, 2006

Cultivar	Fungi identified in flax seed washings after 8 months of storage
'Blue Chip'	<i>Alternaria alternata</i> , <i>A. linicola</i> , <i>Aureobasidium lini</i> , <i>Cladosporium macrocarpum</i> , <i>Embellisia chlamydospora</i> , <i>Melampsora fallax</i> , <i>Olphidium brassicae</i> , <i>Mycelia sterilia</i> (8 species)
'Gold Merchant'	<i>Acremonium murorum</i> , <i>Acremoniella verrucosa</i> , <i>Alternaria linicola</i> , <i>Eladia saccula</i> , <i>Fusarium poae</i> , <i>Gelasinospora reticulospora</i> , <i>Gymnoascus roseus</i> , <i>Paecilomyces lilacinus</i> , <i>Thamnidium elegans</i> , <i>Torula herbarum</i> , <i>Mycelia sterilia</i> (11 species)
'Helmi'	<i>Alternaria alternata</i> , <i>Arthroderma tuberculata</i> , <i>Cladosporium cladosporioides</i> , <i>Ulocladium oudemansii</i> , <i>Mycelia sterilia</i> (5 species)
'Lu-5'	<i>Acremonium cerealis</i> , <i>Acremonium strictum</i> , <i>Alternaria alternata</i> , <i>A. linicola</i> , <i>Mycelia sterilia</i> (5 species)
'Symphonia'	<i>Alternaria alternata</i> , <i>Arthrinium phaeospermum</i> , <i>Gilmaniella humicola</i> , <i>Sclerotinia sclerotiorum</i> , <i>Thielaviopsis basicola</i> , <i>Mycelia sterilia</i> (6 species)
'Szaphir'	<i>Acremonium cerealis</i> , <i>Aureobasidium pullulans</i> , <i>Penicillium album</i> , <i>Gilmaniella humicola</i> , <i>Cladosporium herbarum</i> , <i>C. cladosporium</i> , <i>Torula herbarum</i> , <i>Mycelia sterilia</i> (8 species)

Table 5. **Fungi identified on flax seed of various flax cultivars after 8 months of storage**
Institute of Botany, 2006

Cultivar	Internal fungal infection of flax seed after 8 months of storage
'Blue Chip'	<i>Alternaria alternata</i> , <i>A. linicola</i> , <i>Aureobasidium lini</i> , <i>Cladosporium herbarum</i> , <i>C. macrocarpum</i> , <i>Embellisia chlamydospora</i> , <i>Fusarium heterosporum</i> , <i>F. proliferatum</i> , <i>Olphidium brassicae</i> , <i>Sclerotinia sclerotiorum</i> , <i>Sporotrichum olivaceum</i> , <i>Mycelia sterilia</i> (12 species)
'Gold Merchant'	<i>Alternaria alternata</i> , <i>A. dianthi</i> , <i>A. linicola</i> , <i>Drechslera repens</i> , <i>Embellisia chlamydospora</i> , <i>Fusarium oxysporum</i> , <i>Penicillium verrucosum</i> , <i>Phoma exiqua</i> , <i>Sclerotinia sclerotiorum</i> , <i>Ulocladium oudemansii</i> , <i>Mycelia sterilia</i> (11 species)
'Helmi'	<i>Alternaria alternata</i> , <i>A. linicola</i> , <i>Aureobasidium pullulans</i> , <i>Embellisia chlamydospora</i> , <i>Fusarium graminearum</i> , <i>F. proliferatum</i> , <i>Fusarium sp.</i> , <i>Olphidium brassicae</i> , <i>Scytalidium lignicola</i> , <i>Ulocladium chartarum</i> , <i>U. oudemansii</i> , <i>Mycelia sterilia</i> (12 species)
'Lu-5'	<i>Acremonium strictum</i> , <i>Alternaria alternata</i> , <i>A. pluriseptata</i> , <i>Botryotrichum piluliferum</i> , <i>Colletotrichum lini</i> , <i>Drechslera sorokiniana</i> , <i>Embellisia chlamydospora</i> , <i>Humicola grisea</i> , <i>Oidiodendron tenuissimum</i> , <i>Penicillium verrucosum</i> , <i>Tilachlidium spp.</i> , <i>Mycelia sterilia</i> (12 species)
'Symphonia'	<i>Alternaria alternata</i> , <i>A. linicola</i> , <i>Fusarium graminearum</i> , <i>F. oxysporum</i> , <i>Gilmaniella humicola</i> , <i>Phoma exiqua</i> , <i>Stemphylium spp.</i> , <i>Trichoderma viride</i> , <i>Mycelia sterilia</i> (9 species)
'Szaphir'	<i>Aureobasidium lini</i> , <i>Cladosporium herbarum</i> , <i>Fusarium oxysporum</i> , <i>Gilmaniella humicola</i> , <i>Penicillium corylophilum</i> , <i>Ulocladium oudemansii</i> , <i>Mycelia sterilia</i> (7 species)

Table 6. **Mycotoxin contamination of flax seed of various flax cultivars at harvesting and during storage**
Lithuanian Institute of Agriculture, 2005

Cultivar	Mycotoxins $\mu\text{g kg}^{-1}$				
	At harvesting			After 8 months of storage	
	DON	Aflatoxin (total)	Ochratoxin A	Aflatoxin (total)	Ochratoxin A
'Blue Chip'	0	0	0	traces	1.1
'Gold Merchant'	34.0	traces	0	1.2	0
'Helmi'	traces	0	traces	0	1.1
'Lu-5'	39.0	traces	0	2.1	0
'Symphonia'	23.0	0	0	1.1	1.0
'Szaphir'	54.0	0	2.3	2.5	1.2

contamination of seeds was more dependent on the weather conditions at harvesting and was less dependent on the genotype of a variety.

In 2005, flax seed contamination with fungi at harvesting was low. Traces of aflatoxin were identified only in the seed samples of cvs. 'Lu-5' and 'Gold

Merchant', ochratoxin A ($2.3 \mu\text{g kg}^{-1}$) in the seed sample of cv. 'Szaphir' (Table 6). DON was identified in all the samples tested, except for cv. 'Blue Chip', but the contents identified were very low.

After 8 months of storage, aflatoxin contents in flax seed increased. The highest content of aflatoxin (2.5

$\mu\text{g kg}^{-1}$) was identified in seeds of cv. 'Szaphir' (Table 6). During storage, ochratoxin A contamination in seed increased. Small contents of ochratoxin A were identified not only in seed samples of cv. 'Szaphir' ($1.2 \mu\text{g kg}^{-1}$), but also in those of 'Blue Chip' ($1.1 \mu\text{g kg}^{-1}$) and 'Symphonia' ($1.0 \mu\text{g kg}^{-1}$).

CONCLUSIONS

1. The flax cultivars 'Symphonia' and 'Szaphir' matured the earliest, the length of their growing period was 82 days. The cultivar 'Lu-5' was found to be the latest-maturing, the length of its growing period was 100 days.

2. At harvesting, the lowest contents of fungal propagules (0.5 and $0.7 \text{ cfu g}^{-1} \times 10^4$) were identified on seeds of cvs. 'Symphonia' and 'Lu-5', whereas the highest content of fungal propagules (1.6 and $1.7 \text{ cfu g}^{-1} \times 10^4$) were found on seeds of cvs. 'Blue Chip' and 'Helmi'. The fungal infection level on flax seeds was determined by the weather conditions.

3. Analysis of flax seed internal fungal infection level at harvesting and during storage showed fungi of the genera *Alternaria* (up to 42.5% of seed infected) and *Fusarium* (up to 50.0%) to be the most prevalent ones.

4. During the eight months of storage, the external and internal infection of seeds with fungal propagules increased. In the early-maturing cvs. 'Szaphir' and 'Symphonia' seed surface contamination increased by 89.7% and 92.8%, respectively and in later-maturing cvs. by 30.4–67.9%. However, these indicators could be modified by moisture at drying.

5. The contents of mycotoxins identified in the flax seed of various cultivars were very low, however, mycotoxin-increasing trends were identified during storage, when also seed contamination with ochratoxin A increased.

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References

- Abarca M. L., Bragulat M. R., Castella G., Cabañes F. J. 1994. Ochratoxin A production by strains of *Aspergillus niger* var. *niger*. *Applied and Environmental Microbiology*. Vol. 60. P. 2650–2652.
- Bennett J. W., Klich M. 2003. Mycotoxins. *Clinical Microbiology Reviews*. Vol. 16. No. 3. P. 497–516.
- Bennett G. A., Nelsen T. C., Miller B. M. 1994. Enzyme-linked immunosorbent assay for detection of zearalenone in corn, wheat and pig feed: collaborative study. Vol. 77. P. 1500–1509.
- De Nijs M., Rombouts F., Notermans S. 1996. *Fusarium* molds and their mycotoxins. *Journal of Food Safety*. Vol. 16. No. 1. P. 15–58.
- Domsch K. H., Gams W., Anderson T.-H. 1980. *Compendium of Soil Fungi*. Vol. 1. London: Academic Press. 859 p.
- Ellis M. B. 1971. *Dematiaceous Hyphomycetes*. C.A.B. International. 608 p.
- Ellis M. B. 1976. *More Dematiaceous Hyphomycetes*. C.A.B. International. 507 p.
- Fink-Gremmels J. 1999. Mycotoxins: their implications for human and animal health. *Vet. Q.* Vol. 21. No. 4. P. 115–120.
- Krysinska-Traczyk E., Skorska C., Prazmo Z., Sitkowska J., Cholewa G., Dutkiewicz J. 2004. Exposure to airborne microorganisms, dust and endotoxin during flax scutching on farms. *Ann. Agric. Environ. Med.* Vol. 11. No. 2. P.309–326.
- Kumud K., Jitendra S., Yadav M. D. 1997. Fungi associated with linseed seeds, their effect and chemical control. *Annals of Plant Protection Sciences*. No. 5. P. 179–183.
- Larsen T. O., Svendsen A., Smedsgaard J. 2001. Biochemical characterization of ochratoxin A-producing strains of the genus *Penicillium*. *Appl. Environ. Microbiol.* Vol. 67. P. 3630–3635.
- Malone J. P., Muskett A. E. 1997. *Seed-Borne Fungi. Description of 77 fungus species*. Sheppard J. W. (Ed.). Zurich: ISTA. P. 191.
- Mathur S. B., Kongsdal O. 2003. *Common Laboratory Seed Health Testing Methods for Detecting Fungi*. Copenhagen. 425 p.
- Mikelionis S. 2001. *Aliejiniai linai*. Akademija. 34 p.
- Moss M. O. 2000. Mycotoxins in Agriculture and Food Safety. *International Journal of Food Science & Technology*. Vol. 35. No. 3. P. 354–355.
- Mukhopadhyay N., Ray A. K. 2001. Effects of amino acid supplementation on the nutritive quality of fermented linseed meal protein in the diets for rohu, *Labeo rohita*, fingerlings. *Journal of Applied Ichthyology*. Vol. 17. No. 5. P. 220–226.
- Paul V. H., Sultana C., Jouan B., Fitt B. D. L. 1991. Strategies for control of diseases on linseed and fibre flax in Germany, France and England. *Production & Protection of Linseed*. No. 1. P. 65–69.
- Ponter A. A., Parsy A. E., Saade M., Mialot J. P., Ficheux C., Duvaux-Ponter C., Grimard B. 2006. Effect of a supplement rich in linolenic acid added to the diet of post partum dairy cows on ovarian follicle growth and milk and plasma fatty acid compositions. *Reproduction, Nutrition, Development*. Vol. 46. No. 1. P. 19–29.
- Samson R. A., Hocking A. D., Pitt J. I. 1992. *Modern Methods in Food Mycology*. Amsterdam. 388 p.
- Stramkale V., Sulojeva J., Serzhane R., Janshevskis E., Gudriniece E. 2003. Flax – the perspective crop for fiber and oil manufacturing in Latvia. *Environment. Technology. Resources. Proceedings of the International Conference*. Rezekne. P. 251–257.
- Tarakanovas P., Raudonius S. 2003. *Agronominių tyrimų duomenų statistinė analizė taikant kompiuterines programas Anova, Stat, Split-plot iš paketo Selekcija ir Irristat*. Akademija. P. 6–26.
- Wensing A. G. C. L., Mensink R. P., Hornstra G. 1999. Effects of dietary ω -3 polyunsaturated fatty acids from plant and marine origin on platelet aggregation in healthy elderly

- subjects. *The British Journal of Nutrition*. Vol. 82. P. 183–91.
23. Wilkinson A. P., Ward C. M., Morgan M. R. A. 1992. Immunological analysis of mycotoxins. In: Lins-Kens H.F., Jackson J. F. (Eds.). *Plant toxin analysis*. Berlin: Springer-Verlag. P. 185–225.
24. Wirths W., Berglar T., Dieckhues A., Bauer G. 1985. Fiber-rich snacks with reference to their effect on the digestive activity and blood lipids of the elderly. *Zeitschrift für Gerontologie und Geriatrie*. Vol. 18. No. 2. P. 107–117.
25. Лучина Н. Н. 1981. *Болезни льна*. Ленинград: Колос. 88 с.
26. Мирчинк Т. Г. 1988. *Почвенная микология*. Москва: Изд-во МГУ. 220 с.
27. Саттон Д., Фотергилл А., Ринальди М. 2001. *Определитель патогенных и условно патогенных грибов*. Москва: Мир. 468 с.

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**APLINKOS SĄLYGŲ ĮTAKA MIKROMICETŲ IR
MIKOTOKSINŲ KIEKIO KITIMUI ALIEJINIŲ LINŲ
SĖMENYSE**

S a n t r a u k a

Straipsnyje analizuojamas tiksluosiuose lauko bandymuose išaugintų skirtingos vegetacijos trukmės aliejinių linų veislių ‘Symphonia’, ‘Blue Chip’, ‘Szaphir’, ‘Gold Merchant’, ‘Lu-5’ ir ‘Helmi’ sėmenų užterštumas mikromicetais bei mikotoksinais derliaus nuėmimo metu bei sandėliuojant. Ištyrus vidinį sėklų užterštumą derliaus nuėmimo metu, daugiausiai buvo aptikta *Alternaria* genties (apsikrėtę nuo 20,0 iki 42,5% sėklų) ir *Fusarium* genties (apsikrėtę iki 50,0% sėklų) grybų. Linų sėklos 8 mėnesius buvo laikytos sausoje ir vėsioje patalpoje. Paviršinis sėklų užterštumas laikymo metu ankstyvos veislės ‘Symphonia’ sėklose padidėjo 92,8%, o ‘Szaphir’ – 89,7%. Vėlyvesnės brandos veislės ‘Blue Chip’ ir ‘Gold Merchant’ sėklų užterštumas mikromicetais padidėjo atitinkamai 30,4 ir 41,2%. Laikymo metu genčių *Alternaria* ir *Penicillium* pradų kiekis, palyginus su kiekiu derliaus nuėmimo metu, pagausėjo, *Fusarium* genties – sumažėjo. *Alternaria* genties rūšių buvo aptikta gausiausiai – net keturios: *A. alternata*, *A. linicola* ir *A. dianthi*, *A. pluriseptata*. Daugiausiai grybų rūšių (11) identifikuota ant veislės ‘Gold Merchant’ sėklų. Aflatoksino pėdsakai aptikti tik ‘Lu-5’ ir ‘Gold Merchant’ sėmenų mėginiuose, ochratoksino A (2,3 μg kg⁻¹) – ‘Szaphir’ mėginyje. DON užteršti visi tirti mėginiai, išskyrus ‘Blue Chip’, tačiau nustatyti kiekiai yra labai maži. Po 8 mėnesių laikymo aflatoksino ir ochratoksino A kiekiai sėmenų mėginiuose padidėjo.

Raktažodžiai: *Linum usitatissimum*, *Alternaria* spp., *Penicillium* spp., *Fusarium* spp., mikotoksinai, sėklos