MYCOTOXIN PREVENTION AND CONTROL IN LINSEED

MIKOTOKSĪNU NOVĒRŠANA UN KONTROLE LINSĒKLĀS

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Abstract. *The present paper analyses mycotoxin contamination in six oil flax seed of the flax varieties differing in the length of the growing season. The flax seed grown in precision field trials was analyzed for mycotoxin contamination at harvesting and during storage period.*

The analyses done at harvesting revealed the traces of aflatoxin were identified only in the seed samples of cvs. Lu-5 and Gold Merchant, while those 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 8 months of storage the levels of aflatoxin and ochratoxin A. in flax seed samples increased. Mycotoxin increasing immediately concerned with seed fungi contamination. These indicators could be determining by moisture control such as seed drying.

Key words: *Linum usitatissimum, linseed, fungi, mycotoxin, variety.*

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 seeds have been long-used in multi-grain cereals and snack foods they are increasingly used as an ingredient in feeds for improved animal and fish nutrition. The benefits of omega-3 fatty acids to pigs, cattle, horse ant other animals may be in preventing young animals from developing infections (Wirths et al., 1985; Mukhopadhyay, Ray, 2001; Ponter et al., 2006).

The quality of crop production produce during growing and storage is determined by the natural conditions and anthropogenic factors. The diversity of fungi occurring on oil flax seed is largely dependent on the growing conditions, however, some fungi species of *Colletotrichum, Fusarium*, *Rhizoctonia, Alternaria*, *Aspergillus*, *Penicillium* genera occur in all flax-growing countries (Mercer, Hardwick, 1991; Paul et al., 1991; Simay, 1994; Kumud et al., 1997).

Oil flax seed coat contains about 5.1–11.7 % carbohydrate-mucilage substances, cotyledons contain on average 25–45 % fat and up to 30 % protein. Apart from these substances, flax seed contains carbohydrates, phosphorus 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 and other factors. With the spread of fungi the chances of mycotoxin formation in food and feed occur. Mycotoxins are produced by fungi species of *Fusarium*, (De Nijs et al., 1996), *Peniciliium*, (Larsen et al., 2001), *Alternaria* (Stinson et al., 1980), *Aspergillus* (Abarca et al., 1994) genera. Mycotoxins are detrimental to human and animal health (Fink-Gremmels, 1999). These toxins in agricultural products cause health hazards to people and animals and economical problem. Prevention measures of mycotoxin contamination in flax seed are different. To design strategies for the reduction or elimination of mycotoxins,

knowledge about their fungal sources is needed. The growth of fungi in crops and agricultural products is the main cause of toxin formation and related to the concentration of the toxic substances. Many factors are involved in enhancing the formation of mycotoxins. They are plant susceptibility to fungi infestation, suitability of fungal substrate, temperate climate, moisture content and physical damage of seeds, etc. (Semple et al.). The knowledge on variety influence on seed mycotoxin contamination is limited.

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. The six varieties with different maturity were tested in the trial –Helmi (early), Szaphir (medium early), Symphonia (early), Gold Merchant (medium early), Blue Chip (medium late), Lu-5 (late).

Fungal infection level of flax seed was analysed at the laboratories of the Lithuanian Institute of Agriculture and the Upytė Research Station.

Analyses of seed microflora were done following Samson R.A. et al. (1992), Mathur S.G., Kongsdal O. (2003) methodology. Identification was carried out using Malone J.P., A.E. Muskett (1997), Саттон Д. et al., (2002), Mathur S.B., Kongsdal O. (2003) descriptors.

Seed samples were analyzed 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 the reading of immunoenzymic micro strips.

Results and discussion

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). All the tested cultivars matured at different time. The cultivars Helmi, Symphonia and Szaphir reached yellow maturity stage the earliest, on August 3, and their growing period lasted for 82 days. At that time cvs. Blue Chip, Gold Merchant and Lu-5 were at green maturity stage. 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, and the length of its growing period was 100 days (Table 1).

Table 1.

The 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

Having analysed the seed for internal contamination at harvesting, we identified fungal propagules of *Alternaria, Fusarium, Penicillium* and *Aspergilus* genera (Figures 1-3). The most abundant fungi were of *Alternaria* genus (from 20.0 to 42.5 % of infected seed) (Figure 1) and *Fusarium* genus (infected up to 50.0 % of seed) (Figure 2). The highest infection level

with these fungi was identified on the seed of cv. Lu-5, although seed surface contamination level was low. The seed of cv. Helmi had the lowest content of *Fusarium* propagules (7.5 %). Of the other fungi identified in seed the following ones are worth mentioning: *Coletotrichum lini, Drechslera sp., Rhizoctonia sp., Botrytis cinerea*, however, their total content did not exceed 5 %.

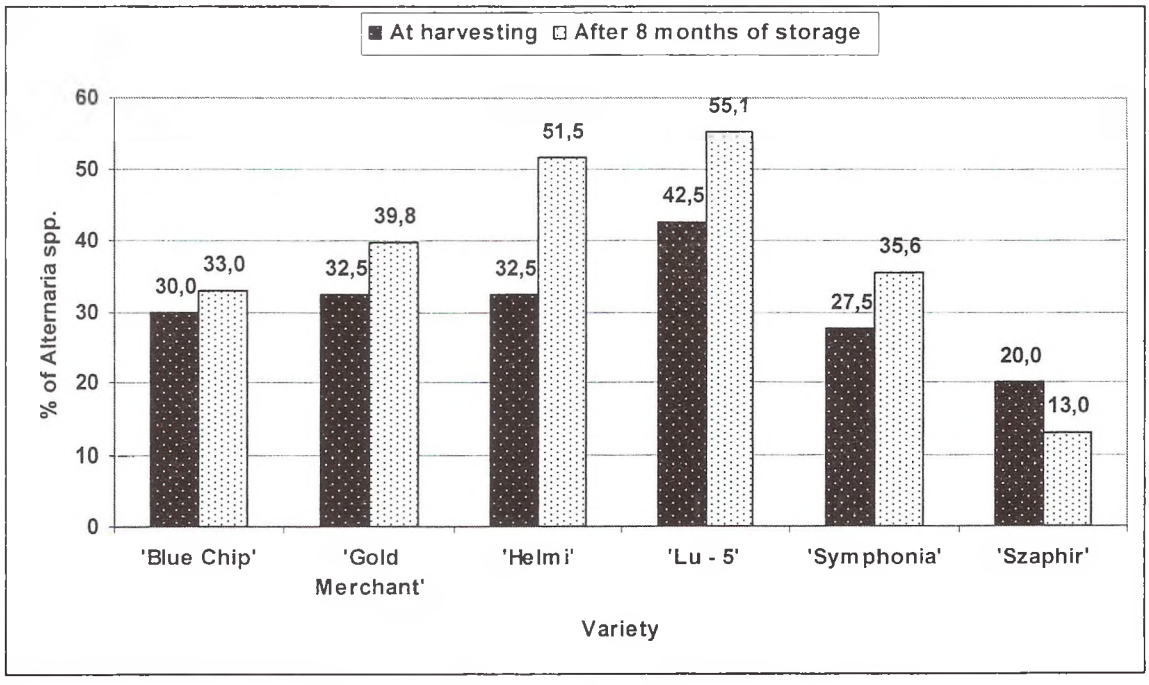

Figure 1. **The level of Alternaria spp. on flax seed at harvesting and during storage** Upytė 2005–2006

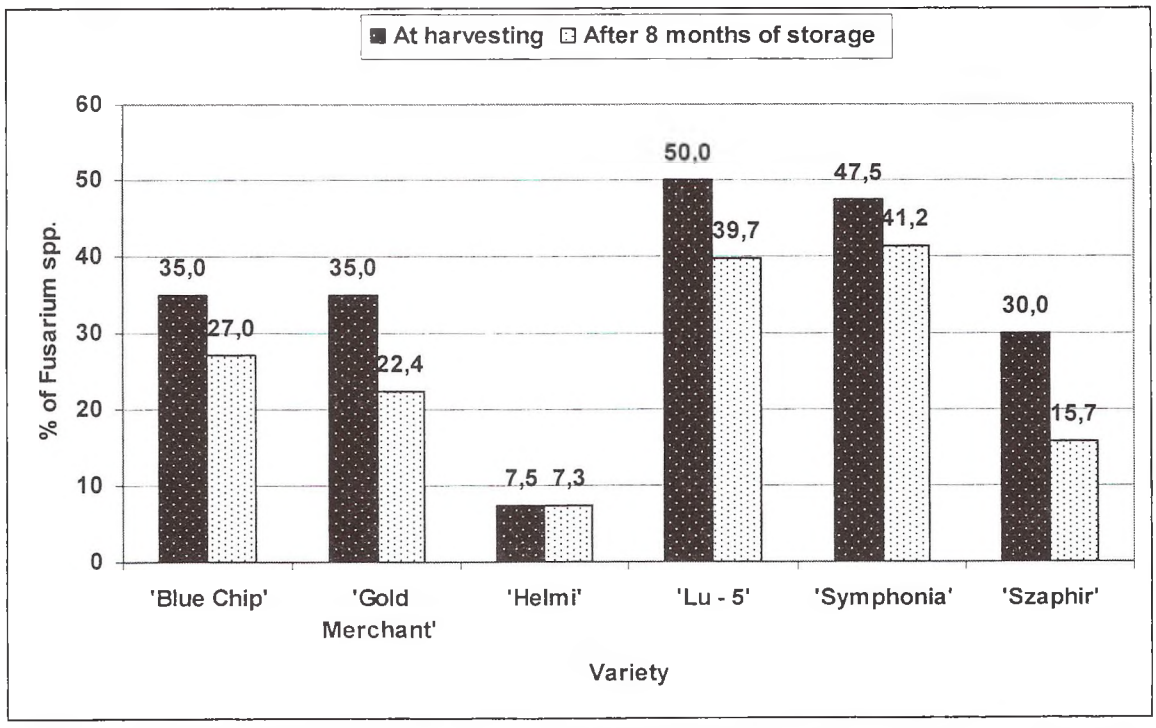

Figure 2. **The level of Fusarium spp. on flax seed at harvesting and during storage** Upytė 2005–2006

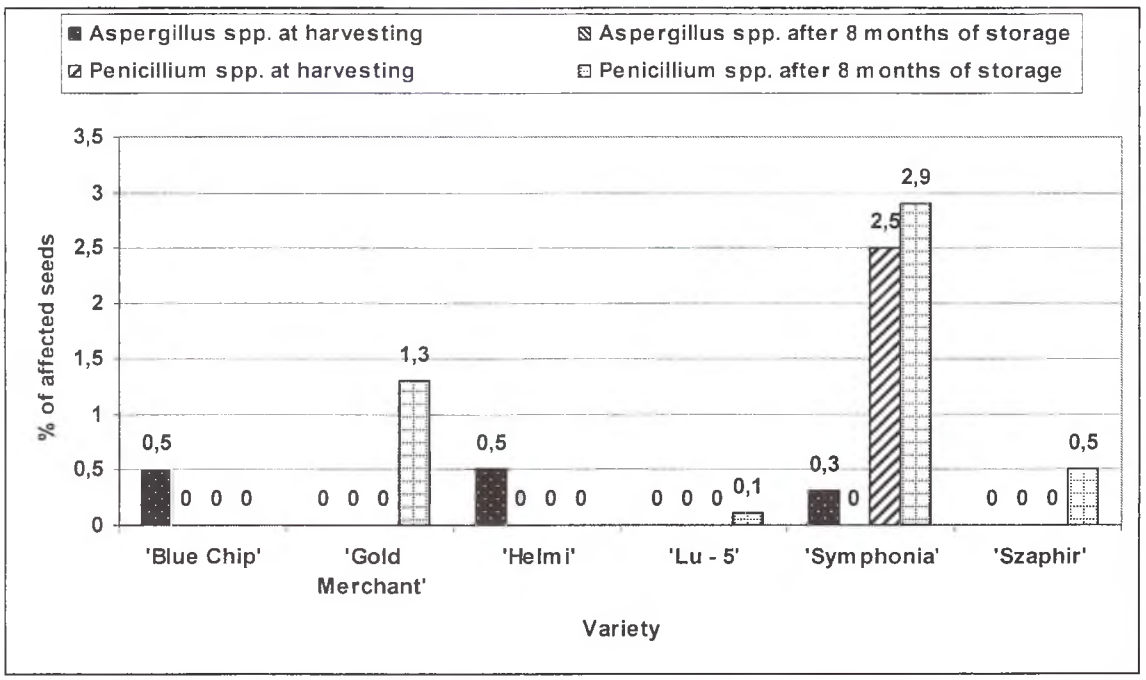

Figure 3. **The level of Aspergillus sp. and Penicilium sp. on flax seed at harvesting and during storage**
Upytė 2005–2006

Flax seed was stored for 8 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 it increased especially markedly: in the seed of cv. Szaphir by 89.7 %, in Symphonia by 92.8 % (Figure 1-3). 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.

Analysis of seed contamination with fungi capable of producing mycotoxins of the six oil flax cultivars at harvesting and during storage suggests that fungal contamination of seed was more dependent on the weather conditions at harvesting and was less dependent on the genotype of variety.

In 2005 flax seed contamination with mycotoxines at harvesting was low. Traces of aflatoxin were identified only in the seed samples of cvs. Lu-5 and Gold Merchant, ochratoxin A (2.3µg kg^{-1}) in the seed sample of cv. Szaphir (Table 2). DON was identified in all samples tested, except for cv. Blue Chip, but the contents identified were very low.

Table 2.

Mycotoxin contamination of flax seed of various flax cultivars at harvesting and during storage (LIA, 2005)

Cultivar	*Mycotoxins µg kg^{-1}*				
	At harvesting			*After 8 months of storage*	
	DON	*Aflatoxin (total)*	*Ochratoxin A*	*Aflatoxin (total)*	*Ochratoxin A*
Blue Chip	0	0	0	trace	1,1
Gold Merchant	trace	trace	0	1,2	0
Helmi	trace	0	trace	0	1,1
'Lu - 5'	trace	trace	0	2,1	0
Symphonia	trace	0	0	1,1	1,0
Szaphir	trace	0	2,3	2,5	1,2

Aflatoxin content in flax seed increased after 8 months of storage. The highest content of aflatoxin (2.5μg kg^{-1}) was identified in cv. Szaphir seed (Table 2). During storage ochratoxin A contamination level in seed increased. Small contents of ochratoxin A were identified not only in the seed samples of cv. Szaphir (1.2 μg kg^{-1}) but also in those of Blue Chip (1.1μg kg^{-1}) and Symphonia (1.0 μg kg^{-1}).

The contents of mycotoxins identified in the flax seed of various cultivars were very low; however, mycotoxin increasing trends were identified during storage. Mycotoxin increasing immediately concerned with seed fungi contamination. In our opinion we can decrease mycotoxin content when stop fungus spreading on seed during storage. However, these indicators could be determining by seed moisture control at drying.

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. Analyses of flax seed internal fungal infection level at harvesting and during storage showed the fungi of *Alternaria* genus (up to 42.5 % of seed infected) and *Fusarium* genus (up to 50.0 % of seed infected) to be the most prevalent ones.
3. Fungal contamination of seed was more dependent on the weather conditions at harvesting and was less dependent on the genotype of variety
4. Seed surface and internal infection with fungal propagules increased during the eight months of storage. Seed surface contamination increased by 89.7 % and 92.8 %, respectively of early-maturing cvs. Szaphir and Symphonia. Seed surface contamination increased by 30.4-67.9 % of later-maturing cvs.
5. The contents of mycotoxins identified in the flax seed of various cultivars were very low; however, mycotoxin increasing trends were identified during storage.
6. If we suspend fungus spreading on seed during storage mycotoxin content would be lower.

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