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The influence of storage conditions on bee pollen contamination by microscopic fungi and their mycotoxins

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Abstract

The aim of the experiment was to determine the effect of storage duration at different ambient temperatures on bee pollen contamination by microscopic fungi and their mycotoxins. The dilution plate technique was used for isolation of fungi from the samples. The contents of mycotoxins aflatoxin (AFL), ochratoxin A (OTA), deoxynivalenol (DON), zearalenone (ZEN) and T-2 toxin (T-2) were analysed by the direct competitive enzyme-linked immunosorbent assay (ELISA) method. Toxin-producing fungi, belonged to *Penicillium*, *Aspergillus* and *Fusarium* spp., isolated during bee pollen storage with a total amount varying from 1×10 to 3.5×10^3 cfu g^{-1}. The amount of toxin-producing fungi was the lowest in the bee pollen stored for 1 month at 8–9°C temperature: it varied from 1×10 to 2.1×10^3 cfu g^{-1}. After 4 months of storage at 20–22°C temperature, the highest contamination of bee pollen was found: it ranged from 2×10 to 3.5×10^3 cfu g^{-1}. Contamination with toxins AFL, OTA and T-2 in all bee pollen samples was found to be below the limit of detection. Mycotoxins ZEN and DON after 1 month of storage at 8–9°C temperature were not detected. The highest DON concentration (185 μg kg^{-1}) was ascertained after 4 months of storage at 8–9°C temperature; the highest ZEN concentration (830 μg kg^{-1}) was found after 1 month of storage at 20–22°C temperature.

Key words: bee pollen, contamination, fungi, mycotoxins, storage.

Introduction

Bee pollen is a diet product presented as a healthy food supplement (Kostić et al., 2020). Bee pollen is a favourable medium for microorganisms to grow and multiply (Bennet, Klich, 2003). The most abundant fungi of genera *Aspergillus*, *Fusarium*, *Penicillium*, *Cladosporium* and *Alternaria* are widely spread globally (Pitt, Hocking, 2009). One of the most important criteria for bee pollen quality standard is microbiological contamination. It is highly dependent on the environmental factors that affect the activity of microscopic fungi (Xue et al., 2014).

Microscopic fungi, which are always abundant in the environment, begin to develop as soon as favourable humidity and temperature conditions occur (Petrović et al., 2014). Toxic fungi may have a significant influence on the quality of bee pollen (Kostić et al., 2019).

Mycotoxins are secondary metabolites produced by *Aspergillus*, *Fusarium* and *Penicillium* spp. fungi (Belhadj et al., 2012). Factors contributing to the presence of mycotoxins in food are associated with storage and environmental conditions controlled by humans (Hussein, Brasel, 2001). The cause of occurrence of mycotoxins depends on factors such as humidity, temperature, storage time and level of contamination (Bennet, Klich, 2003). Mycotoxins cannot be removed from food raw materials; therefore, it is very important to avoid food contamination with mould (Gompa, 2013). People can be negatively affected by different fungal species, fungal spores or toxic fungal metabolites such as mycotoxins by direct consumption of contaminated foodstuffs (Belhadj et al., 2014).

Mycotoxins may be acutely toxic, carcinogenic and teratogenic. Contamination of bee pollen by storage fungi (*Aspergillus* and *Penicillium*) presents a potential risk for human health (Pitt, Hocking, 2009; Nardoni et al., 2016). Aflatoxins (AFL) are the product of the metabolism of different fungi species, which belong to *Aspergillus* genus with *A. flavus* and *A. parasiticus* strains as the main producers. Both aflatoxins B_1 and B_2 are carcinogenic for humans and are listed in Group I of carcinogenic substances according to the International Agency for Research on Cancer (IARC) (Petrović et al., 2014; Vidal et al. 2018). The main ochratoxin A (OTA) producers are different *Aspergillus* species and one species *P. verrucosum* belonging to *Penicillium* genus (Bennet, Klich, 2003). OTA is known for its immunosuppressive and teratogenic properties (Pitt et al., 2000) and belongs to the IARC 2B group, which means that it is a possible

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carcinogen for humans. Special attention should be given to the group of mycotoxins zearalenone (ZEN) and deoxynivalenol (DON) produced by *Fusarium* spp. ZEN is mycoestrogen with limited toxicity and belongs to the IARC 3 (Vidal et al., 2018). This toxin may have a role in hormonal balance and mammary cancer (Pitt et al., 2000). T-2 and DON belong to trichothecene compounds, the sesquiterpenoid metabolites obtained after microbiological activity of *Fusarium*, *Trichoderma*, *Myrothecium*, etc. Various trichothecenes may be immunosuppressive (Bennet, Klich, 2003).

Due to the considerable time elapsed between bee pollen harvesting and its use as a food supplement, microscopic fungi, including some toxigenic fungi, are highly likely to spread (Kačániová et al., 2011). In Lithuania, monitoring of mycotoxins is mainly carried out on imported goods, while there is insufficient research on contamination with mycotoxins of collected and stored bee pollen. Very little has been reported regarding toxin-producing fungi in bee pollen and their capability to produce mycotoxins under some circumstances (Rodríguez-Carrasco et al., 2013). Studies examining the genus and species composition of fungi, their relationship to environmental conditions and storage duration may offer new solutions to control the contamination of bee pollen by microscopic fungi and the toxins produced during storage.

Therefore, to ensure food safety, the aim of the research was to determine the influence of storage duration at different ambient temperatures on bee pollen contamination by toxigenic fungi and mycotoxins AFL, OTA, DON, ZEN and T-2.

Materials and methods

The experiment was carried out during 2018–2019 at Vytautas Magnus University Agriculture Academy. Mycotoxin analysis was carried out in the Mycotoxicology Laboratory of Veterinary Academy of Lithuanian University of Health Sciences.

Preparation of bee pollen samples for storage. In mid-July 2018, a total of 46 bee pollen samples (50 g each) were collected from one apiary in Central Lithuania at the same time. The multifloral bee pollen was collected using a high-bottom trap, mounted inside hives, 40 cm above the bottom. Then, having removed the fresh bee pollen on the same day, it was purified and dried to 8% moisture content. The time of drying in a drying oven was 8 hours. The maximum temperature was 35°C.

Determination of bee pollen moisture content. The moisture content of bee pollen was determined according to European Commission Regulation EC 152.2009 (Mayda et al., 2020).

Bee pollen storage duration and conditions. Dried bee pollen samples were placed in sterile plastic zip-lock bags and stored in controlled climate chambers at different storage duration (factor A) for 1, 2, 3 and 4 months in the dark and at 20–22°C (20 samples) and at 8–9°C (20 samples) ambient temperatures (factor B). The initial moisture content, mycological conditions and concentrations of mycotoxins in the remaining (6) samples of bee pollen were determined after 3 days of storage at different ambient temperatures (control treatment).

Isolation and identification of microscopic fungi. The standard plate dilution method was applied for culturing and isolating the microscopic fungi. For determination of the fungi, 10 g of the sample was soaked in 90 ml NaCl solution (8.5 g L^{-1}) and then shaken for 15 min. A portion of 1 ml of dilutions (from 10^{-1} to 10^{-4}) was poured into Petri plates, then the medium was added on top. All assays were performed in three replications. To isolate and identify individual genera and species, the potato dextrose (PDA) and Czapek-Dox (CDA) agars (Sigma-Aldrich, Germany) were used. The plates were incubated at 26 ± 2°C temperature in the dark for 5 days. For the isolation of yeast, the plates with cultures were maintained for 3 days. The total fungal count was identified and evaluated as colony-forming units per gram (cfu g^{-1}) of bee pollen. The morphological characteristics of isolated colonies and distinct fungal species were identified based on morphological identification keys of Samson et al. (2002) and Pitt and Hocking (2009).

Determination of mycotoxin concentration by the ELISA assay. The same samples as used for fungal isolation were analysed for mycotoxins. The mycotoxins deoxynivalenol (DON), zearalenone (ZEN), T-2 toxin (T-2), total (B_1 and B_2) aflatoxins (AFL) and ochratoxin A (OTA) were analysed by the enzyme-linked immunosorbent assay (ELISA) method (Horwitz, Latimer, 2005). The RIDASCREEN® quantitative test kits (R-Biopharm AG, Germany) approved by the AOAC Research Institute (certificate No. 9001) were used for the analysis Nos R5906, R5502, R4701 and R1312. Mycotoxin extraction and tests were performed according to manufacturer's recommendations.

Preparation of samples for mycotoxin estimation. Five grams of milled bee pollen sample was weighed. Extraction of samples was carried out in distilled water for DON, in methanol to water ratio (70:30 v/v) for ZEN and AFL, (50:50 v/v) for T-2 and in ECO extractor to water ratio (1:10 v/v) for OTA. The extraction was conducted in a rotary shaker for 3 minutes. The extract was filtered through Whatman No. 1 filter and 50 µl of the filtrate per well was used in the test.

The absorbance was determined using the microwell strip reader (Bio-tek Synergy HT, USA) at 450 nm. A calibration curve for the standards for each DON, ZEN, T-2, AFL and OTA dilution was plotted using a standard concentration against the percentage inhibition of the respective standard. For determination, each mycotoxin concentration was automatically calculated from the calibration curves, obtained by plotting absorbance intensity against the logarithm of analytic concentration. The measured absorbance was automatically converted to the mycotoxin concentration units (µg kg^{-1}). The analytical methods were validated by the kit's manufacturer with the sample matrices for bee pollen. The limit of detection (LOD) for DON is 18.5 µg kg^{-1}, ZEN – 17.0 µg kg^{-1}, T-2 – 5.0 µg kg^{-1}, AFL – 1.75 µg kg^{-1} and OTA – 0.5 µg kg^{-1}.

Statistical analysis. Research data were analysed using software *Statistica*, version 10 (StatSoft Inc., USA). The data were statistically evaluated for quantitative methods using a one-way and two-way ANOVA (Raudonius, 2017). Significant interaction between the investigated factors was established, and the means of the main effects are not presented. The correlation between individual values obtained for bee pollen storage duration at different ambient temperatures, total fungi count and ZEN and DON was determined using Pearson's correlation coefficient.

Results

Prior to storage, contamination of bee pollen with microscopic fungi was determined. Fungal content of the bee pollen ranged from 1×10 to 2.24×10^3 cfu g^{-1} (Table 1). Generally, the genera of *Alternaria* (29.0%), *Penicillium* (25.7%), *Cladosporium* (10.1%) and *Acremonium* (11%) were the most abundant in the bee pollen. Yeast and *Botrytis* genera in the bee pollen made up 7.7% and 6.3%, respectively. The detection rate of fungi from other genera was less than 6%.

Moisture content of the bee pollen prepared for storage comprised 8% (Table 2). Moisture content

Table 1. Levels of fungal contamination in bee pollen samples before storage

Fungi	Contamination level cfu g^{-1}
Acremonium spp.	1.2×10^3
Alternaria spp.	2.2×10^3
Aspergillus niger Tiegh.	–
A. flavus Link.	1×10
Botrytis spp.	1×10^2
Chaetomium spp.	1×10
Cladosporium spp.	1.1×10^3
Epiccocum spp.	1×10
Fusarium spp.	1.8×10^1
F. sporotrichioides Sherb.	1.5×10
F.graminearum Swabe	1×10^1
Mucor spp.	1×10^1
Paecilomyces	1×10^1
Penicillium spp.	2×10^3
P. verrucosum Dierckx	1.7×10
Rhizopus spp.	1×10^1
Trichoderma spp.	1.1×10
Yeasts	1.5×10^2

of the pollen stored at 20–22°C temperature was increasing throughout the storage period. The highest moisture content was found after 4 months of storage, it was by 0.44% significantly higher than in the control treatment. Moisture content of the pollen stored at 8–9°C temperature increased insignificantly between 0.12% and 0.14% in different storage periods.

In bee pollen stored at 8–9°C temperature, fungi started to develop more intensively after 2 months of storage (Table 3). The highest fungal contamination was found after 3 months of storage. The fungal content significantly increased to 48.4% compared to the control treatment. Fungi developed and proliferated more intensively at 20–22°C than at 8–9°C temperature. After 3 and 4 months their abundance significantly increased up to 97.4% and 100%, respectively.

Contamination level by toxin-producing fungi in the stored bee pollen varied from 1×10 to 3.5×10^3 cfu g^{-1} (Table 4).

Table 2. The moisture content (%) of bee pollen samples during storage

Storage duration (factor A)	Ambient temperature (factor B)	
	8–9°C	20–22°C
3 days (control)	8.00 d	8.00 d
1 month	8.12 c	8.13 c
2 months	8.13 c	8.25 b
3 months	8.12 c	8.42 a
4 months	8.14 c	8.44 a

Note. Means not sharing common letters are significantly different at $P < 0.05$.

Table 3. The total levels of fungal contamination (cfu g^{-1}) in bee pollen samples during storage

Storage duration (factor A)	Ambient temperature (factor B)	
	8–9°C	20–22°C
3 days (control)	1.8×10^3 d	1.9×10^3 cd
1 month	2.3×10^3 bc	2.5×10^3 b
2 months	2.4×10^3 b	3.7×10^3 a
3 months	2.9×10^3 b	3.8×10^3 a
4 months	2.8×10^3 b	3.9×10^3 a

Note. Means not sharing common letters are significantly different at $P < 0.05$.

Table 4. Levels of toxin-producing fungi (cfu g^{-1}) in bee pollen samples during storage

Fungi	Ambient temperature 8–9°C			
	1	2	3	4
Aspergillus niger Tiegh.	1×10	–	–	–
A. flavus Link.	1×10	1×10	–	–
Penicillium spp.	2.1×10^3	2.2×10^3	2.5×10^3	2.7×10^3
P. verrucosum Dierckx	1.4×10^1	1.6×10^1	1×10^2	1×10^2
Fusarium spp.	1.1×10^1	1.4×10^1	1.6×10^1	1.2×10^1
F. sporotrichioides Sherb.	1×10^1	1.1×10^1	1×10^1	1.2×10^1
F. graminearum Swabe	1×10^1	1.2×10^1	1.2×10^1	1.1×10^1
Ambient temperature 20–22°C				
A. niger Tiegh.	–	1×10	1×10	2×10
A. flavus Link.	1×10	1×10	2×10	2×10^1
Penicillium spp.	2.4×10^3	3.1×10^3	3×10^3	3.5×10^3
P. verrucosum Dierckx	1.5×10^1	1.2×10^2	1.7×10^2	1.6×10^2
Fusarium spp.	2×10^1	2.1×10^1	2.3×10^1	2.2×10^1
F. sporotrichioides Sherb.	1.2×10^1	1.1×10^1	1.2×10^1	1.2×10^1
F. graminearum Swabe	1.2×10^1	1.4×10^1	1.2×10^1	1.2×10^1

1, 2, 3, 4 – storage duration, months

It was found that during 1–4 months the amount of toxigenic fungi of *Penicillium* increased with increasing storage time and storage temperature. *Penicillium* accounted for 78.2% of the toxigenic fungi. At a storage temperature of 8–9°C, after 4 months their amount increased the most – by 10.7% compared to the control treatment. At the temperature from 20°C to 22°C, *Penicillium* fungi began to multiply intensively already in the first month of storage, and an increase of 5.3% was found compared to the control treatment. After 4 months of storage, the amount of these fungi increased by 18.2%. *P. verrucosum* also increased with increasing storage time. After 4 months of storage at 20–22°C temperature, the fungi content was 27.4% higher compared to the fungi content at 8–9°C during the same period.

The amount of fungi of the genus *Aspergillus* in bee pollen prepared for storage was very low – 4.3%. *A. niger* in the tested bee pollen samples was not detected. The temperature of 20–22°C was more favourable for *Aspergillus* fungi: after 4 months of storage their amount increased by 4.5%. The temperature of 20–22°C was more favourable for *A. flavus* than for *A. niger*: the number of *A. flavus* species after 4 months of storage increased from 2.9% to 4.0%.

Fusarium spp. contamination in the control treatment comprised 15.3%. The highest contamination by the genus was found after 4 months of storage: at 20–22°C temperature its amount increased by 3.1%. The bee pollen was dominated by two *Fusarium* species *F. sporotrichioides* and *F. graminearum*, and their contamination comprised 6.5–6.6% and 7.0–7.2%.

Contamination with mycotoxins AFL, OTA and T-2 in all bee pollen samples was found to be below the detection limit (Table 5). The collected bee pollen was

Table 5. The concentration of mycotoxins (μg kg^{-1}) in bee pollen samples (n = 46) during storage

Storage duration (factor A)	Ambient temperature (factor B) 8–9°C				
	ZEN	DON	T-2	AFL	OTA
3 days (control)	<LOD	<LOD	<LOD	<LOD	<LOD
1 month	<LOD	<LOD	<LOD	<LOD	<LOD
2 months	500 a	<LOD	<LOD	<LOD	<LOD
3 months	430 b	<LOD	<LOD	<LOD	<LOD
4 months	500 a	185 a	<LOD	<LOD	<LOD
	Ambient temperature 20–22°C				
3 days (control)	70 c	<LOD	<LOD	<LOD	<LOD
1 month	830 a	<LOD	<LOD	<LOD	<LOD
2 months	500 b	<LOD	<LOD	<LOD	<LOD
3 months	<LOD	175 a	<LOD	<LOD	<LOD
4 months	<LOD	<LOD	<LOD	<LOD	<LOD

Note. < – below at limit of detection (LOD); means not sharing common letters are significantly different at $P < 0.05$.

contaminated mostly with ZEN. At 8–9°C temperature after 2 and 4 months of storage its content comprised 500 μg kg^{-1}. The strong positive correlation ($r = 0.99$, $P < 0.05$) between the total count of fungi, storage time and level of ZEN was determined. At 20–22°C temperature, a significant increase of ZEN concentration was observed after 1 month of storage. It comprised 830 μg kg^{-1} and was the highest concentration of this mycotoxin found in all treatments. The strong positive correlation ($r = 0.99$, $P < 0.01$) between the total count of fungi, storage time and contamination level of ZEN was determined.

The highest DON concentration (175 μg kg^{-1}) was ascertained at 20–22°C temperature after 3 months of storage. Its quantities were significantly lower in both temperature regimes. No correlation was found between DON and the total amount of fungi in the two different temperature regimes with increasing storage period. No correlation was detected between the moisture content of bee pollen and mycotoxins ZEN and DON contamination levels.

Discussion

In this study, all the tested bee pollen samples were found to be contaminated by microscopic fungi. In the experiment, bee pollen contamination did not exceed the limits for the number of yeast and mould (<5 × 10^4 cfu g^{-1}) recommended by the scientists from the International Honey Commission (IHC) (De-Melo et al., 2015). The values of fungi (between <10–10^4 cfu g^{-1}) are in agreement with the results reported in the literature (Belhadj et al., 2012; Rocha, 2013).

The present survey revealed biodiversity in fungal species. Microbiological contamination of dried and ready-to-store pollen was found to be dominated by fungi belonging to the genera *Alternaria*, *Penicillium*, *Cladosporium* and *Acremonium*. These fungal genera also dominated in the bee pollen studied by Brindza et al. (2010), Kačániová et al. (2011) and Sinkevičienė and Amšiejus (2019). Contamination of bee pollen could result from bee activities, its natural habitat or human handling operations such as harvesting, drying and packaging (Beev et al., 2018).

It was found that contamination of bee pollen samples by *Alternaria* and *Penicillium* fungi was the highest. The results of our experiment are in line with those obtained by González et al. (2005), Kačániová et al. (2011) and Beev et al. (2018). Bee pollen also contained small amounts of *Aspergillus*, *Botrytis*, *Chaetomium*, *Epiccocum*, *Fusarium*, *Mucor*, *Paecilomyces*, *Rhizopus*, *Trichoderma* and yeast genera. Like in our experiment, Brindza et al. (2010) and Petrović et al. (2014) also identified a variety of these fungi in bee pollen.

Fungal growth and the possibility of mycotoxin biosynthesis can prevent drying of bee pollen (González et al., 2005). For storage, bee pollen was dried to 8% moisture content. There are no set bee pollen quality (including moisture content) control parameters in Lithuania. Some countries have established minimal water content requirements for dried bee pollen: Brazil – max. 4 g 100 g^{-1}, Switzerland and Poland – max. 6 g 100 g^{-1}, Uruguay – max. 8 g 100 g^{-1}, Bulgaria – max. 10 g 100 g^{-1} (Campos et al., 2008). Improper storage of bee pollen can increase its humidity, which can cause the development of mould (González et al., 2005). In our experiment, the moisture content of bee pollen varied insignificantly when stored at a low 8–9°C ambient temperature. However, when stored at 20–22°C ambient temperature, after 4 months moisture content of bee pollen increased to 0.44%. It can be assumed that the increase in moisture content may have been influenced by relative ambient humidity (>75%), caused by frequent opening of the samples and physiological processes happening in bee pollen.

Bee pollen as a dietary supplement can be used for 1 to 3 months (Bogdanov, 2014). In our experiment, the mycotoxicological status of bee pollen was examined for 4 months to determine the fungal changes in the bee pollen, if they were used for more than 3 months.

Among the bee pollen samples analysed, fungi of the genus *Penicillium* were the most abundant. Fungi of the genus *Penicillium* were also dominant in the bee pollen samples of studies conducted by Kačániová et al. (2011) and Beev et al. (2018). During our experiment, fungi of the genus *Penicillium* proliferated abundantly under both storage conditions at different temperatures. However, a more abundant increase in the fungal amount of the genus was observed in the bee pollen stored at 20–22°C temperature. The highest contamination was found after 4 months of storage. Meanwhile, the amount of *Penicillium* fungi at 8–9°C temperature increased insignificantly. *Penicillium* spp. spreading frequency in bee pollen is associated with low substrate humidity (González et al., 2005), also they are mesophilic fungi, whose maximum growth *in vitro* is obtained at 23°C temperature (Pitt, Hocking, 2009). *P. verrucosum* isolated in bee pollen is known as a producer of mycotoxin OTA (Cabañes et al., 2010). This species was also isolated in our experiment. Similar results were reported by Kačániová et al. (2011).

The spread of *Aspergillus* fungi was very insignificant in the bee pollen analysed. *A. niger* and *A. flavus* were detected in 26.4% of the bee pollen samples. In the study by González et al. (2005), *Aspergillus* was detected in 80% of the bee pollen samples. *A. flavus* was found in 23.3% of the bee pollen samples, which was different from the results of our experiment. According to the study of Holmquist et al. (1983), a greater effect on the growth of *A. flavus* and *A. parasiticus* has water activity than temperature: they grow in lower temperatures only under high water activity. In our experiment, the

temperature of 8–9°C did not promote the activity of these fungi, and after 1 month the amount of these fungi remained unchanged. A more intensive growth of both *Aspergillus* fungi was observed at 20–22°C temperature after 4 months of storage.

In our experiment, *Fusarium* spp. in bee pollen was among the most abundant after *Penicillium* spp. In the study of Brindza et al. (2010), *Fusarium* fungi was also most frequent. The amount of *Fusarium* spp. in the samples was small at 8–9°C and 20–22°C temperatures. Storage temperature and substrate humidity did not adversely affect *Fusarium* fungi over all storage months and neither encouraged their proliferation and nor increased their quantity. The main species detected were *F. sporotrichioides* and *F. graminearum*. These species were predominant also in the study by Kačániová et al. (2011).

Very little has been reported in literature on mycotoxins in bee pollen. The detection of AFL in the samples of bee pollen from the most diverse parts of the world is becoming a growing problem (Kačániová et al., 2011; Xue et al., 2014; Estevinho et al., 2018). Climatic changes extensively influence weather conditions in temperate areas (such as the majority of Europe), and the presence of AFL in these areas is becoming more frequent. AFL B_1 and OTA are mycotoxins commonly produced post-harvest (Kostić et al., 2019). In our experiment, AFL and OTA in all bee pollen samples were found to be below the limit of detection. This is an important result, because they are potentially pathogenic.

Fusarium species produce mycotoxins before or immediately after harvest, *Penicillium* and *Aspergillus* species are more commonly found as contaminants of commodities and foods during drying and subsequent storage (Pitt, Hocking, 2009). *Fusarium* spp. is the primary source of mycotoxins DON, ZEN and T-2 (Bennet, Klich, 2003). In bee pollen was identified *F. sporotrichioides*, which is known as the producer of ZEN and T-2 (Leslie et al., 2006), and *F. graminearum*, which produces ZEN and DON (Kačániová et al., 2011).

In bee pollen, toxins DON and ZEN are dominant (Rodríguez-Carrasco et al., 2013), which was confirmed by our experiment as well. However, as field fungi, *Fusarium* spp. produce mycotoxins pre-harvest (Pitt, Hocking, 2009). The detection of *Fusarium* toxins is only a retrospective indicator for the conditions in the field and is not directly related to the toxin content during storage. The mycotoxin ZEN was observed in pollen stored for 2–4 months at 8–9°C temperature, whereas in pollen stored for 1 month it was not detected. Meanwhile, bee pollen stored at 20–22°C temperature contained the highest amounts of ZEN after the first two months of storage. The mycotoxin DON was found in the bee pollen only after 3 months of storage. The distribution of mycotoxin concentration could be influenced by contamination of floral pollen grains on the plant or by bees (Nogueira et al., 2012).

In bee pollen samples, the amount of T-2 was below the limit of detection. This finding is in accordance with the results by Rodríguez-Carasco et al. (2013), when the presence of T-2 was checked in fifteen pollen samples from Spain, but the content of this mycotoxin was below the limit of detection of applied GC-MS (gas chromatography-mass spectrometry) method.

The study of bee pollen storage conditions supplemented very scarce research on bee pollen performed in Lithuania. The results showed how important it is to keep bee pollen in the right conditions. Storage conditions play an important role in mycotoxin control since they influence overall fungal development. Toxin-producing fungi can significantly affect the quality of bee pollen, which as well as food quality is very important (Shanakht et al., 2014). Therefore, further research in this field would make control of the incidence of these toxins in bee pollen easier and would contribute to food quality and safety.

Conclusions

1. The highest increase of fungal contamination (48.4%) in bee pollen was found at 8–9°C temperature after 3 months of storage. At 20–22°C temperature, after 3 and 4 months of storage fungal contamination significantly increased up to 97.4% and 100%, respectively.
2. The level of contamination by toxin-producing fungi in the stored bee pollen varied from 1×10 to 3.5×10^3 cfu g^{-1}. *Penicillium* spp. fungi dominated (accounted for 78.2%) during the whole 4-month storage period.
3. Contamination with mycotoxins AFL, OTA and T-2 in all bee pollen samples was found to be below the limit of detection. The highest ZEN concentrations were found at 8–9°C temperature after 2 and 4 months (500 μg kg^{-1}) and at 20–22°C after 1 month (830 μg kg^{-1}) of storage. The highest DON concentration (185 μg kg^{-1}) was ascertained at 8–9°C after 4 months of storage.

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Sandėliavimo sąlygų įtaka žiedadulkių užterštumui mikroskopinių grybų gaminamais toksinais

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Santrauka

Tyrimo tikslas – nustatyti įvairios kilmės žiedadulkių sandėliavimo laikotarpių skirtingomis aplinkos temperatūromis įtaką jų užterštumui mikroskopiniais grybais ir jų gaminamais mikotoksinais. Mikroskopiniai grybai izoliuoti skiedimo metodu (kolonijas sudarantys vienetai, ksv g^{-1}). Imunofermentinės analizės (ELISA) metodu nustatyta aflatoksino (AFL), ochratoksino A (OTA), deoksinivalenolio (DON), zearalenono (ZEN) ir T-2 toksino (T-2) kiekiai. Sandėliavimo metu išskirti toksinus produkuojantys grybai priklausė *Penicillium*, *Fusarium* ir *Aspergillus* gentims; užterštumas jais kito nuo 1×10 iki $3{,}5 \times 10^3$ ksv g^{-1}. *Penicillium*, *Fusarium* ir *Aspergillus* grybų kiekis buvo mažiausias žiedadulkėse, sandėliuotose 1 mėnesį 8–9° C temperatūroje – jis kito nuo 1×10 iki $2{,}1 \times 10^3$ ksv g^{-1}. Didžiausias užterštumas grybais nustatytas po 4 mėnesių sandėliavimo 20–22° C temperatūroje – jis buvo nuo 2×10 iki $3{,}5 \times 10^3$ ksv g^{-1}. Žiedadulkių užterštumas AFL, OTA ir T-2 mikotoksinais visuose žiedadulkių mėginiuose buvo mažesnis už aptikimo ribą. Mikotoksinų ZEN ir DON po žiedadulkių sandėliavimo 1 mėnesį 8–9° C temperatūroje nenustatyta. Didžiausia DON koncentracija (185 µg kg^{-1}) nustatyta po žiedadulkių sandėliavimo 4 mėnesius 8–9°C temperatūroje, didžiausia ZEN koncentracija (830 µg kg^{-1}) – po sandėliavimo 1 mėnesį 20–22° C temperatūroje.

Reikšminiai žodžiai: bičių žiedadulkės, grybai, užterštumas, mikotoksinai, sandėliavimas.