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Mycobiota in bee pollen collected by different types of traps

Jolanta SINKEVIČIENĖ, Algirdas AMŠIEJUS

Vytautas Magnus University, Agriculture Academy Studentų 11, Akademija, Kaunas distr., Lithuania E-mail: jolanta.sinkeviciene@vdu.lt

Abstract

The aim of this study was to determine the impact of different types of bee pollen traps on the microbiological contamination of pollen influenced by the duration of pollen retention in traps. A total of 96 bee pollen samples were analyzed. The total count of microorganisms in the bee pollen during the study period varied from 4.5 to 19.3 \times 10³ cfu g⁻¹. Moisture content in the pollen was optimal for the growth and proliferation of microorganisms. In the pollen, the following most common 7 genera of fungi were found: *Penicillium, Fusarium, Alternaria, Acremonium, Cladosporium, Mucor* and *Rhizopus*. A study of the mycobiota of pollen collected from four different traps showed that the total number of microorganisms varied depending on the type of trap and pollen retention period. The pollen collected after 9 hours retention in traps was less contaminated with the microorganisms than that collected after 24 and 48 hours. The count of mycobiota was the lowest (4.5 \times 10³ cfu g⁻¹) in the pollen sampled after 9 hours retention from high-bottom traps compared to other types of traps. The highest contamination (19.3 \times 10³ cfu g⁻¹) of fresh bee pollen was found after 48 hours in traps with thermo chambers. Fungal contamination was the higest in the bee pollen collected by traps mounted at hive entrance after 9 and 48 hours, respectively 124 and 150 units. The *Penicillium* fungi dominated the pollen in traps with thermo chambers, *Fusarium* – in low-bottom, *Alternaria* – in the pollen of traps mounted at beehive entrance.

Key words: bee pollen, contamination, fungi, microorganisms, traps.

Introduction

Bee pollen is considered to be one of the most complete food items in nature (Petrovic et al., 2014). Pollen contains about 200 different substances, including proteins, carbohydrates, flavonoids, enzymes, trace elements, and numerous amino acids and vitamins (Deveza et al., 2015; Kieliszek et al., 2018).

An important issue concerning the criteria of pollen quality is its purity and microbiological safety (Deveza et al., 2015). It seems that the most critical step is the removal of pollen from traps. Long-term pollen retention in traps can cause an increase in humidity, which favours the growth of microorganisms (González et al., 2005; Estevinho et al., 2011). Microscopic fungi grow when water activity ranges from 0.61 to 1, but can sometimes grow on a very dry surface (Whitefield, 1998; Jay, 2007). Bee pollen is reported as a substrate that stimulates the production of mycotoxins (Medina et al., 2004), if a beekeeper does not provide adequate and prompt drying (Garcia-Villanova et al., 2004). The main mycotoxin-producing genera are Aspergillus, Fusarium and Penicillium, which can cause acute or chronic poisoning - mycotoxicosis (Estevinho et al., 2011; Petrovic et al., 2014). Aspergillus and Penicillium in the

trapped pollen present a potential risk for human health (Nardoni et al., 2016). *Alternaria* and *Cladosporium* are considered to be hazardous moulds (Kačaniova et al., 2011). Microscopic fungi are very common in nature and have spores resistant to various environmental factors. They can both contaminate pollen and be present in honey together with pollen (Popa et al., 2009). A number of studies (Brindza et al., 2010; Kačaniova et al., 2011; Petrovic et al., 2014) have revealed significant contamination levels of bee pollen by microorganisms. Therefore, it is very important to find out the presence and quantity of microscopic fungi that dominate pollen, as some of these fungi are potential mycotoxin producers, while others, although not producing toxic metabolites can cause allergic reactions (Estevinho et al., 2011).

Pollen collection is a very thorough job that needs to be done every day. It seems that the most important stage is pollen removal from traps (Gonzáles et al., 2005). If pollen is not removed from traps for a day, this poses a risk of fungal contamination, because there is a lack of research not only regarding the initial fungal contamination of pollen, which can be influenced by different pollen traps, but also regarding the safest

duration of pollen storage in them. As is well known to the authors of this study, few researchers have investigated the microbial contamination of a dried pollen substrate.

Therefore, due to the lack of information, the aim of this study was to determine the impact of different pollen traps on the fungal contamination depending on the duration of pollen retention in traps.

Materials and methods

Bee pollen samples. In June–July 2018, a total of 96 pollen samples were colected from beekeepers in Central Lithuania. Bees had collected pollen from different plant species.

Types of traps. The pollen was collected using four types of traps (factor A): 1) high-bottom trap, mounted inside hives, 40 cm above the bottom; 2) low-bottom trap, mounted inside hives, 15 cm above the bottom; 3) front trap at the hive entrance, mounted in front of the entrance on the outside of a hive; and 4) front trap at the hive entrance with a thermo chamber, mounted in front of the entrance on the outside of a hive.

Pollen retention periods. The pollen from the same traps was removed at different retention times (factor B): after 9 hours (32 samples), after 24 hours (32 samples) and after 48 hours (32 samples). Having removed the pollen on the same day, moisture content (%) of fresh bee pollen was determined and after 12 hours microbiological studies were carried out. The samples were placed in sterile plastic bags, vacuumed, packed and stored at -15°C temperature prior to microbiological analysis.

Determination of moisture content. Moisture content was determined by drying 5 g of a sample in an oven at 105°C temperature until constant weight.

Isolation and morphological characterization of microscopic fungi. The dilution plate technique was used

for isoliation of fungi from the samples. Microscopic fungi were isolated by adding 10 g of ground pollen to 90 ml of physiological saline (NaCl) 8.5 g L⁻¹ and shaken in a shaker for 15 min. A series of dilutions was prepared from the resulting suspension. Suspensions of 1 ml of 10^{-2} , 10^{-3} and 10^{-4} were poured into Petri dishes, before the potato dextrose agar (PDA) medium was added on top. All assays were performed in triplicate. The Petri dishes with cultures were incubated in a thermostat at $26 \pm 2^{\circ}$ C for 5–7 days, and for the isolation of yeast the plates with cultures were maintained for 2–4 days. Grown fungi were counted and evaluated as colony-forming units per gram (cfu g⁻¹) in bee pollen. The distinct fungal species were identified based on morphological identification keys of Pitt and Hocking (2009).

Statistical analysis of data was performed by the analysis of variance (ANOVA), software Statistica (StatSoft Inc., USA). Research data were statistically evaluated by one-way and two-way ANOVA of quantitative methods of evidence (Raudonius, 2017). Significant interaction between the investigated factors was established and means of main effects are not presented.

Results

After 9 hours, the moisture content in the pollen of the tested samples, ranged from 16.21% to 37.70% (Table 1). The highest moisture content in bee pollen was found in low-bottom traps. After 24 hours, the moisture content in the pollen ranged from 17.06% to 25.58%, and the highest moisture content was found in high-bottom traps (25.58%). After 48 hours, the moisture content ranged from 14.60% to 18.35%. The highest moisture content in pollen was recorded in traps mounted at beehive entrance equipped with thermo chambers.

Table 1. Moisture content (%) of the bee pollen collected by different traps

Type of trap	Pollen retention period (factor B)					
(factor A)	9 hours 24 hours		48 hours			
High-bottom	18.27 d	25.58 b	14.60 e			
Low-bottom	37.70 a	21.42 c	16.86 de			
At hive entrance	16.21 de	17.06 de	15.11 e			
At hive entrance with a thermo chamber	18.34 d	21.92 с	18.35 d			

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

The microbiological analysis of pollen condition showed that it varied depending on the type of traps and the duration of pollen retention in them. The total number of microorganisms in pollen during the study period ranged from 4.5 to 19.3×10^3 cfu g⁻¹ (Table 2).

The pollen removed from traps after 9 hours had the lowest microbiological contamination compared to other hours of the study. The total amount of microorganisms in the samples fluctuated from 4.5×10^3 to 9.5×10^3 cfu g⁻¹ with the average moisture content of

Table 2. Microbial contamination (cfu g-1) of the bee pollen collected by different traps

Type of trap	Pollen retention period (factor B)						
(factor A)	9 hours	24 hours	48 hours				
High-bottom	$4.5 \times 10^{3} \text{ g}$	$4.9 \times 10^{3} \text{ fg}$	$5.0 \times 10^{3} \text{ f}$				
Low-bottom	$7.0 \times 10^{3} e$	$8.4 \times 10^3 d$	$13.2 \times 10^{3} \text{ b}$				
At hive entrance	$5.7 \times 10^{3} \text{ f}$	$6.6 \times 10^{3} \text{ e}$	$6.7 \times 10^{3} \text{ e}$				
At hive entrance with a thermo chamber	$9.5 \times 10^3 \text{ c}$	$9.6 \times 10^3 \text{ c}$	$19.3 \times 10^{3} \text{ a}$				

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

22.6%. Comparison of the types of traps indicated that the lowest number of microorganisms after 9 hours in the high-bottom traps comprised 4.5×10^3 cfu g^{-1} . The pollen collected from traps mounted at the hive entrance, as compared to the low-bottom ones and traps with thermo chambers, was also found to have lower microbial contamination -5.7×10^3 cfu g^{-1} . The highest amount of microorganisms was found in the traps with thermo chambers.

The pollen removed from high-bottom traps after 24 hours showed the lowest level of microorganism contamination -4.9×10^3 cfu g⁻¹. The total number of microorganisms in the pollen collected by low-bottom traps was not the highest; it comprised 8.4×10^3 cfu g⁻¹; however, having compared this amount of microorganisms with that of the pollen removed on the same day, the highest increase in the amount of microorganisms was determined. The pollen collected by traps with thermo chambers had the highest number of microorganisms; however, it was not so high as compared to the pollen removed on the same day.

The highest amount of microorganisms was detected in the pollen stored in behives for 48 hours. The total number of microorganisms in the pollen collected by different traps ranged from 5.0×10^3 to $19.3 \times$

10³ cfug⁻¹. The average moisture content of the samples was 16.23%. The pollen collected in high-bottom traps did not favour the development of microorganisms. The pollen stored for 48 hours in these traps had the lowest levels of microorganism contamination. The contamination of pollen collected in traps mounted at the hive entrance also increased insignificantly as compared to the pollen retained in traps for 24 hours. The pollen collected by traps with thermo chambers was the most contaminated with microorganisms. In this type of traps, the most significant increase in microorganism contamination was detected after 48 hours of retention in hives with the contamination reaching $19.3 \times 10^3 \, \text{cfu g}^{\text{-1}}$. The proliferation of microorganisms was also more favoured in low-bottom traps. Compared to pollen stored in traps for 24 hours, contamination after 48 hours increased from 8.4×10^3 to 13.2×10^3 cfu g⁻¹.

The impact of different pollen traps on the fungal contamination depending on the duration of pollen retention in traps is shown in Table 3. The lowest fungal contamination (18.5%) was found in high-bottom traps. The pollen removed from traps mounted at the hive entrance after 9 and 48 hours had the highest fungal contamination, 30% and 29%, respectively.

Table 3. Fungal contamination (%) of the bee pollen collected by different traps

Type of trap	Pollen retention period (factor B)					
(factor A)	9 hours	24 hours				
High-bottom	14.5 i	21.4 f	19.5 g			
Low-bottom	17.2 h	24.2 e	25.0 de			
At hive entrance	30.0 a	26.8 c	29.0 ab			
At hive entrance with a thermo chamber	23.7 e	27.5 bc	26.5 cd			

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

During the studies, the most prevalent genera of fungi identified in bee pollen were *Penicillium*, *Fusarium*, *Alternaria*, *Acremonium*, *Cladosporium*, *Mucor* and *Rhizopus* (Tables 4–6). In addition to the dominant genera of fungi, the genera with a detection rate of 6% were isolated, namely the fungi of the *Chaetomium*, *Paecilomyces*, *Aspergillus* and yeast genera.

When analyzing the results of the pollen collected on the same day, it was found that of all the identified genera of fungi, the most common fungus in bee pollen was that of *Penicillium* genus (65%). This genus in traps at the hive entrance and in traps with thermo chambers was the most widespread and accounted for 36% (Table 4). The *Penicillium* genus was less prevalent in the pollen collected in high-bottom and low-bottom traps – up to 13.8%. The *Fusarium* fungi, as compared to the other identified genera, were also abundant – 10.3%. This genus was the most frequent

in the pollen collected by low-bottom traps and traps mounted at the hive entrance, accounting for 19.7% and 11.3%, respectively. The pollen collected by low-bottom traps and traps with thermo chambers was found to have the lowest *Fusarium* sp. amounts of 5.0% and 5.8%, respectively. The *Alternaria* genus was detected in 8.1% of the pollen. Comparing the types of traps, the highest amount of *Alternaria* sp. fungus was found in the pollen collected by traps mounted at the hive entrance, while the lowest amount was found in the pollen collected by traps with thermo chambers. The pollen contained some less common species of fungi: *Acremonium* and *Cladosporium* (both genera) accounted for 4.7%, *Mucor* and *Rhizopus* – for 3.9% and 3.6%, respectively.

After storing pollen for 24 hours in different traps, it was found that fungi in them had spread much more in comparison to the pollen removed after 9 hours (Table 3). If the total number of fungal colonies on the

Table 4. The number of microscopic fungi in the bee pollen after 9 hours' retention

Type of trap	Fungal genera cfu g ⁻¹						
	Penicillium	Fusarium	Alternaria	Cladosporium	Mucor	Rhizopus	Acremonium
High-bottom	32 b	3 b	7 b	7 a	5 a	0 b	6 a
Low-bottom	33 b	14 a	7 b	7 a	4 ab	6 a	0 b
At hive entrance	83 a	14 a	11 a	1 b	3 bc	6 a	6 a
At hive entrance with a thermo chamber	83 a	6 b	4 c	2 b	2 c	1 b	5 a

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

same day comprised 358 units (21.4%), the number in different traps after 24 hours increased by 104 units (24.9%).

The most favourable conditions were for the proliferation and spreading of the *Penicillium* genus with a prevalence of 57.8% (Table 5). The number of colonies found in the traps at the hive entrance and at entrance with a thermo chamber was 81 and 91, respectively. The number of the *Fusarium* fungi in the pollen retained in hives for two days amounted to 69 units. A more significant increase was found in the pollen stored in low-bottom

traps – by 13 units, the least – in traps at the entrance to hives – by 1 unit. The *Cladosporium* fungi accounted for 8.2% of the total number of identified fungal genera. The most noticeable increase was recorded in high-bottom traps – by 15 genera. The *Mucor* and *Rhizopus* sp. was determined in the pollen – the total number of colonies amounted to 31 and 23 units, respectively. The most favourable conditions for the proliferation of the *Mucor* fungi were in low-bottom traps, traps at hive entrance and traps with thermo chambers.

Table 5. The number of microscopic fungi in the bee pollen after 24 hours' retention

Type of trap	Fungal genera cfu g ⁻¹						
	Penicillium	Fusarium	Alternaria	Cladosporium	Mucor	Rhizopus	Acremonium
High-bottom	50 с	14 bc	2 c	22 a	3 с	3 c	5 b
Low-bottom	45 d	27 a	9 a	11 b	11 a	8 a	1 c
At hive entrance	81 b	15 b	7 b	3 c	9 b	5 b	4 a
At hive entrance with a thermo chamber	91 a	13 с	2c	2 d	8 b	7 a	4 a

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

The pollen stored for 48 hours contained the highest total amount of the *Penicillium* sp., which accounted for 52% (Table 6). The total amounts of the *Cladosporium* sp., *Rhizopus* and *Acremonium* sp. did not increase.

The amounts of the *Mucor* and *Alternaria* fungi increased in all types of traps. The pollen removed after three days contained 13% of *Mucor* on average. More favourable conditions for the spread of fungi occurred in traps at the hive entrance and in low-bottom traps.

Table 6. The number of microscopic fungi in the bee pollen after 48 hours' retention

Type of trap	Fungal genera cfu g ⁻¹						
	Penicillium	Fusarium	Alternaria	Cladosporium	Mucor	Rhizopus	Acremonium
High-bottom	50 c	12 c	17 c	9 b	11 c	2 a	0 c
Low-bottom	53 с	22 a	21 b	11 a	20 b	0 c	2 a
At hive entrance	77 b	21 a	24 a	4 c	23 a	0 c	1 b
At hive entrance with a thermo chamber	89 a	15 b	9 d	10 ab	11 c	1 b	2 a

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

The fungi of the *Alternaria* genus produced 71 units after 48 hours' retention in hives. The most abundant fungi of this genus were found in traps at the hive entrance as well as in high- and low-bottom traps.

Discussion

Pollen is highly hygroscopic. It can be exposed to high relative humidity environments which is typical of some beekeeping areas during flowering seasons (Gonzáles et al., 2005). Moisture content in the pollen varied from day to day in the study. On the first day of pollen collection and removal its moisture content was 22.6%. Other researchers also reported similar results: 14.6-37.7% (Nardoni et al., 2016) and 21-30% (Bobis et al., 2010). Moisture content of the pollen in traps after 24 hours' storage decreased by 1.1%, after 48 hours – by 6.4%. During the study period, the pollen with the highest moisture content was collected from low-bottom traps; the moisture content was 25.3%. Low-bottom traps were installed inside hives and the pollen in them was less ventilated, thus it had the highest moisture content, whereas the pollen at the hive entrance had the lowest moisture content 16.1%.

The lowest number of microorganisms $(4.5-9.5 \times 10^{-3})$ was recorded in the pollen removed from traps on the same day (after 9 hours), while the maximum

contamination was found in the pollen stored in traps for three days (after 48 hours), ranging from 5.0 to 19.3×10^3 cfu g⁻¹.

Penicillium, Fusarium and Alternaria sp. fungi dominated in the pollen; similar results were obtained with fresh pollen by Nardoni et al. (2016) and with dried pollen by Kačaniova et al. (2011). According to the mentioned authors, the recovery of fungi such as Penicillium sp. in trapped pollen presents a potential risk for human health and attention should be paid to all stages of the post-harvest process.

Fungal contamination in traps varied depending on their type. The lowest contamination of pollen by microbiota was found in high-bottom traps in the samples collected during all days of the study, while the highest one was in the traps with thermo chambers.

The pollen collected by high-bottom traps showed the lowest level of contamination by microorganisms and depending on different storage periods in hives, ranged from 4.5 to 5.0×10^3 cfu g⁻¹. Traps of this type are installed high from the bottom of the hive, where pollen is better ventilated; there is less dust, which could adversely affect the development of both bacteria and fungi. A comparatively small number of colonies of microorganisms were found in the pollen collected in traps mounted at the hive entrance.

The highest number of microorganism colonies $(9.5-19.3 \times 10^3 \text{ cfu g}^{-1})$ was found in traps with thermo

chambers which used solar energy to further dry out pollen. Toxic fungi are divided into field and storage ones (Logrieco, Visconti, 2004). With reduced pollen moisture content, favourable conditions were created for the spreading of both field and storage fungi. The highest amount of the Penicillium sp. fungi was found in this type of traps, as compared to other types, on all days of the study. Penicillium sp. in the pollen removed from these traps on the same day after 24 and 48 hours accounted for 80.6, 71.7 and 65 %, respectively. Compared to the total number of identified genera of fungi, contamination of the pollen by this fungus was also the highest. The studies conducted by Gonzáles et al. (2005) and Finola et al. (2007) also confirmed that the fungi of the genus Penicillium were dominant in pollen. Although the Penicillium genus is one of the most commonly encountered in stored products, it can often be found as the field fungus under favourable conditions for its spread. Moisture content of the substrate (Lacey, Magan, 1991) has a significant influence on its spread. Leistner (1984) reported a study dealing with toxinogenic Penicillium sp. in food. Penicillium sp. isolates determined in food-related habitats should be considered as potential mycotoxin producers according to this author.

The Fusarium fungi were found most frequently in pollen collected by low bottom traps during all the days of the study. The spread of the Fusarium sp. fungi requires a substrate containing about 60% of the available water (Llorens et al., 2004). Traps installed at the bottom of hives could have influenced the abundance of this genus. Fusarium is the main toxin producing genus of the field fungi (Logrieco, Visconti, 2004). Fusarium sp., unlike Penicillium sp. whose presence requires attention in all stages of pollen storage and processing, is not significant at these stages (Nardoni et al., 2016). However, improperly dried and stored for prolonged period pollen may provide favourable conditions for the growth of Fusarium sp. and the synthesis of mycotoxins. A similar trend is observed in this study.

Alternaria sp. prevalence in the pollen was not high. This genus was the most abundant in traps at the hive entrance and in low-bottom traps. Kačaniova et al. (2011) found that Alternaria sp. is a common genus found in honey. The Cladosporium genus in pollen did not spread extensively, although in Nardoni et al. (2016) studies Cladosporium sp. was the most frequently recovered mold.

The lowest amounts of the *Mucor* sp. fungi were found in the pollen removed on the same day. Fungal contamination of the pollen stored in traps for 24 and 48 hours was the highest in traps at the hive entrance and in low-bottom traps. These results were opposite to the research data by Kačaniova et al. (2011), where the genus *Mucor* was recorded as one of the most frequent isolates.

This study was focused on the determination of fresh pollen contamination by fungi depending on the type of traps and pollen retention duration in them. The studies showed that the amount of fungi in the pollen was dependent on the type of traps.

The fungal composition isolated from bee pollen showed that the *Penicillium*, *Alternaria* and *Fusarium* are the most common genera. These genera are capable of producing toxins, which can adversely affect the quality of this exceptional and therapeutic product under favourable conditions (Kačaniova et al., 2011; Rodríguez-Carrasco et al., 2013). One of the primary means of reducing contamination by microorganisms is the choice of the appropriate type of pollen traps.

Conclusions

- 1. The lowest contamination $(6.7 \times 10^3 \text{ cfu g}^{-1})$ of fresh bee pollen by microorganisms was determined after 9 hours of its removal from traps.
- 2. The study showed that pollen collected in high-bottom traps had the lowest levels $(4.8 \times 10^3 \text{ cfu g}^{-1})$ of contamination by microorganisms, while the highest contamination $(12.8 \times 10^3 \text{ cfu g}^{-1})$ was in the traps with thermo chambers.
- 3. Fungal contamination was the higest in the bee pollen collected by traps mounted at the hives entrance after 9 and 48 hours 124 and 150 units, respectively.
- 4. Moisture content of fresh pollen was favourable for the growth and spread of microorganisms. The highest moisture content was in the pollen collected from low-bottom traps, whereas pollen at the entrance to hives was the driest. Therefore, in order to prevent the activity of microorganisms, pollen removed from traps must be dried as soon as possible.

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Skirtingo tipo rinktuvais surinktų bičių žiedadulkių mikobiota

J. Sinkevičienė, A. Amšiejus

Vytauto Didžiojo universiteto Žemės ūkio akademija

Santrauka

Nešimo dieną žiedadulkių neišėmus iš rinktuvų, kyla jų mikrobiologinės taršos grėsmė. Trūksta tyrimų ne tik apie pradinę šviežių žiedadulkių mikrobiologinę taršą, kuriai gali turėti įtakos skirtingų tipų žiedadulkių rinktuvai, bet ir apie žiedadulkių saugiausią laikymo trukmę rinktuvuose.

Tyrimo tikslas – nustatyti skirtingų rinktuvų įtaką žiedadulkių mikrobiologinei taršai, priklausomai nuo jų laikymo rinktuvuose trukmės. 2018 m. ištirti 96 bičių žiedadulkių mėginiai. Mikroorganizmų pradų kiekis žiedadulkėse tyrimo laikotarpiu kito nuo 4,5 iki 19,3 × 10³ ksv (kolonijas sudarančių vienetų) g¹. Žiedadulkėse nustatytas drėgmės kiekis buvo optimalus mikroskopiniams grybams augti ir daugintis. Žiedadulkėse buvo nustatytos dažniausiai vyravusios 7 grybų gentys: *Penicillium, Fusarium, Alternaria, Acremonium, Cladosporium, Mucor* ir *Rhizopus*. Ištyrus keturiais skirtingais rinktuvais surinktų žiedadulkių mikrobiotą nustatyta, kad bendras mikroorganizmų kiekis kito priklausomai nuo rinktuvo tipo ir žiedadulkių buvimo avilyje trukmės. Žiedadulkių rinkimo dienos pabaigoje (po 9 val.) iš rinktuvų išimtos žiedadulkės pasižymėjo mažiausia mikrobiologine tarša. Mikroorganizmų pradų kiekis buvo mažiausias (4,5 × 10³ ksv g¹) aukšto dugno rinktuvuose. Visuose tyrimo variantuose didžiausia žiedadulkių mikrobiotos tarša nustatyta 48 valandas laikytose žiedadulkėse. Per tris tyrimo dienas surinktuose mėginiuose žiedadulkių mažiausia tarša (4,8 × 10³ ksv g¹) mikroorganizmais nustatyta aukšto dugno rinktuvuose, didžiausia (12,8 × 10³ ksv g¹) – rinktuvuose su termokameromis. Mikroskopinių grybų didžiausi kiekiai nustatyti žiedadulkėse, surinktose prieš avilio laką įrengtuose rinktuvais su termokamera, *Fusarium* – žemo dugno, *Alternaria* – prieš avilio laką įrengtais rinktuvais surinktose žiedadulkėse.

Reikšminiai žodžiai: bičių žiedadulkės, grybai, mikroorganizmai, tarša, žiedadulkių rinktuvai.