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MYCOLOGICAL FLORA ON TREE FRUITS, CRUST, LEAVES AND POLLEN *SORBUS DOMESTICA* L.

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Abstract: Plant-microbial interactive relations with respect to determine mycoflora of the *Sorbus domestica* L. – fruits, crust, leaves and pollen were studied in 2 Slovak regions. On the fruit samples the genera *Alternaria*, *Botrytis*, *Cladosporium*, *Mucor* and *Penicillium* occurred, on the leaves the genera *Alternaria*, *Cladosporium* and *Penicillium* prevailed and on crust the genera *Alternaria*, *Cladosporium*, *Penicillium* and *Trichoderma* appeared to be dominant, respectively. Isolates from the *Sorbus domestica* L. fruits were present by 11 genera and 13 species of microscopic fungi and isolates from pollen such as *Alternaria*, *Botrytis*, *Cladosporium*, *Penicillium*, *Mycelia*, *Mucor* and *Trichoderma* appeared to be the most frequently occurring genera. On the basis of further taxonomic determination, from the genera *Aspergillus* were isolated and identified representatives of species *A. clavatus*, *A. fumigatus*, *A. niger*, *A. ochraceus*, *A. terreus*. It is necessary to point out that the isolated genera *Aspergillus* and *Penicillium* are considered as the most important producers of mycotoxins.

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INTRODUCTION

The fruits of *Sorbus domestica* L. are suitable for direct consumption and/or for processing (canned fruits or jam). In folk medicine, brandy produced via fermentation and distillation is also used. The morphological studies proved that fruits of a different colour (yellow, red, wine-red, brown) were different also in shape. The nutritional value of the fruit is excellent, and there are several easily utilizable monosacharids and organically bound metal ions. In some studies [16], it turned out that the cation content of sorb-apple is higher than in apples or pears. There are also 3-4 times more potassium and calcium. In the propagation experiments it was proved that there is a compatibility with quince.

All plant surfaces have a natural flora of microorganisms. The numbers of organisms on the surfaces of healthy, young plant leaves may be quite low, but the species which do occur are well adapted for this highly specialized environment. There were found mostly the representatives of moulds such as *Cladosporium* and the so-called black yeasts [4, 5].

Despite the high water activity of most fruits, the low pH leads to their spoilage predominantly caused by fungi, both yeasts and moulds, but especially the latter [1].

For the better understanding of ecological and practical interactions occurring on the fruit, crust, leaves and pollen of *Sorbus domestica* L., an analysis including the study of the diversity and exact species identification is inevitable. Our analyses were oriented on the survey of fungal community composition occurring on 4 different parts of *S. domestica* L. species from 2 different localities.

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MATERIALS AND METHODS

Sample collection. The samples were taken from 4 different parts of *Sorbus domestica* L. in the localities of Modra and Nové Mesto nad Váhom, Slovakia.

1. Fruit sample number: F1, F2, F3, F4, F8, F9
2. Leaves sample number: L1, L2, L3, L4, L5, L6, L7, L8, L9.
3. Crust sample number: C1, C2, C3, C4, C5, C6, C7, C8, C9.
4. Pollen sample number: P1, P2, P3.

Samples numbered 1, 2, 3, 4 were taken from the Modra locality and 5, 6, 7, 8, 9 from Nové Mesto nad Váhom.

Isolation and morphological characterization of fungi. For determination of fungal colony-forming units (CFU), 1 g of an homogenizated sample was soaked in 99 ml of sterile tap-water containing 0.02% Tween 80 and then shaken for 30 min. Dilutions (from 10^{-1}-10^{-5}) in sterile tap-water with 0.02% Tween 80 were prepared and 1 ml aliquots were inoculated on each of 3 plates of Czapek-Dox agar with streptomycin (to inhibit the bacterial growth). Petri dishes were inoculated using the spread-plate technique and incubated at 25°C. Total fungal CFU.g^{-1} counts in samples were determined after 5-10 days of incubation.

Malt agar and Czapek-Dox agar were used to isolate and identify individual genera and species. Incubation was carried out at 25°C for 5-10 days. After isolation, individual species were identified on the basis of their macro- and micromorphology in accordance with other scientific keys [3, 7, 14].

RESULTS AND DISCUSSION

In the isolation procedure with use of both selective media it was shown that on the fruit samples (Tab. 1) the

Table 1. Incidence of microscopic fungal species isolated from fruit samples.

Species of fungi	Fruit sample number						
	F1	F2	F3	F4	F7	F8	F9
Alternaria alternata (Fr.) Keissl.	-	+	+	+	+	+	+
Aspergillus fumigatus Fres.	-	-	-	+	-	-	-
Aspergillus niger v. Tiegh.	+	-	-	-	-	-	-
Aspergillus terreus Thom	-	-	-	-	-	+	-
Botrytis sp.	+	+	+	+	+	+	+
Cladosporium cladosporioides (Fres.) de Vries	+	+	+	+	+	+	-
Chryzosporium sp.	-	-	-	-	-	+	-
Fusarium sp.	-	-	+	-	+	+	+
Mucor sp.	-	-	+	+	+	+	+
Mycelia sterillia	-	-	-	-	+	+	-
Penicillium sp.	-	-	+	+	+	+	+
Talaromyces sp.	-	-	-	-	-	+	-
Trichoderma harzianum Rifai	-	-	+	-	+	+	-

Table 2. Incidence of microscopic fungal species isolated from leaf samples.

Species of fungi	Leaf sample number								
	L1	L2	L3	L4	L5	L6	L7	L8	L9
Alternaria alternata (Fr.) Keissl.	+	+	+	+	+	+	+	+	+
Aspergillus clavatus Desm.	-	-	-	-	-	-	-	+	-
Aspergillus niger v. Tiegh.	-	-	-	-	-	-	-	-	+
Botrytis sp.	-	+	-	-	-	-	-	+	-
Cladosporium cladosprioides (Fres.) de Vries	+	+	+	+	+	+	+	+	+
Fusarium sp.	-	+	-	-	+	-	-	-	-
Mucor sp.	-	-	-	-	-	-	-	+	+
Mycelia sterilia	+	-	+	-	+	-	+	+	+
Penicillium sp.	+	+	+	+	+	-	+	-	-
Rhizopus sp.	-	-	-	-	-	-	-	+	-
Rhizopus stolonifer (Ehrenb.: Fr) Vuill	-	-	-	-	+	-	-	-	-
Trichoderma sp.	-	-	-	+	-	+	-	-	-
Trichoderma harzianum Rifai	-	-	-	+	-	-	-	-	-

genera *Alternaria*, *Botrytis*, *Cladosporium*, *Mucor* and *Penicillium* occurred, on leaves (Tab. 2), the genera *Alternaria*, *Cladosporium* and *Penicillium* prevailed and on the crust (Tab. 3) the genera *Alternaria*, *Cladosporium*, *Penicillium* and *Trichoderma* appeared to be dominant, respectively.

Contamination of food products with micromycetes of the *Penicillium*, *Aspergillus*, *Fusarium*, *Alternaria*, *Paecilomyces*, *Trichotecium*, genera should receive particular attention. Some of the strains growing on a particular type of vegetable or fruit could synthesize and excrete different toxic secondary metabolites [9, 12].

Alternaria alternata (100%), *Cladosporium cladosporioides* (100%), *Mycelia sterilia* (67%) and *Penicilium* sp. (67%) were the most frequently isolated fungal species encountered on the surface of the leaf samples.

From the ecological point of view, it should be mentioned that microscopic fungi of the genera *Cladosporium* and *Alternaria*, which were found most often in examined samples, very frequently saprophyte to sweet products and fruit (Tab. 1) that flow to different parts of leaves, stalks of plants (Tables 2, 3). When these microscopic fungi have suitable surroundings, they can multiply on these substrates and cover them with sensorial visible black sediment coatings [10, 13].

Alternaria alternata (89%), *Cladosporium cladosporioides* (89%), *Mycelia sterilia* (89%), *Penicilium* sp. (89%) and *Trichoderma* sp. (67%) were the most frequently isolated fungal species encountered on the surface of the crust samples.

Penicillium species represent a widespread group of micromycetes in the environment. They can be found in soil, air, in living and dead bodies, they are also able to survive in poor substrates with very low nutritional and moisture level. Together with their capacity for antibiotic production, it should also be mentioned, that these fungi cause allergies, toxication, infections, and are possible producers of mycotoxins [11].

Related to pollen, 6 microscopic fungal species were isolated from pollen samples: *Alternaria alternata*, *Aspergillus flavus*, *Aspergillus niger*, *Cladosporium cladosporioides*, *Fusarium* sp., *Penicillium* sp. The most frequently species found in the pollen samples were *Penicillium* sp. (100%), *Aspergillus niger* (67%) and *Cladosporium cladosprioides* (67%) (Tab. 4).

Table 4. Incidence of microscopic fungal species isolated from samples of pollen.

Species of fungi	Pollen sample number		
	P1	P2	P3
Alternaria alternata (Fr.) Keissl.	-	-	+
Aspergillus flavus Link: Fr.	-	+	-
Aspergillus niger v. Tiegh.	-	+	+
Cladosporium cladosprioides (Fres.) de Vries	+	+	-
Fusarium sp.	-	-	+
Penicillium sp.	+	+	+

The most frequent colonies isolated from honey bee pollen were *Alternaria* sp. (49%) and *Cladosporium* sp. (28.30%). Less frequent, even rarely, were colonies of other microscopical fungi species such as *Aspergillus* sp., *Fusarium* sp., *Mucor racemosus*, *Mycelia sterilia*, *Penicillium* sp., *Rhizopus stolonifer*, and *Trichoderma* sp. [6].

The most frequently species on the tree fruit were *Botrytis* sp. (100%) *Alternaria alternata* (89%), *Cladosporium cladosporioides* (89%), *Mucor* sp. (78%) and *Penicillium* sp. (78%). Fresh fruits are prone to fungal contamination in the field, during harvest, transport, and marketing. It is important to identify fungal contaminants in fresh fruits because some moulds can grow and produce mycotoxins on these commodities, while certain yeasts and moulds can cause infections or allergies [15]. Early detection of contaminated raw materials is an important prevention tool to avoid their use in the food industry.

The most dominant endogenous contaminant on the fruits of *Sorbus domestica* L. according to [8] were *Cladosporium cladosporioides* followed by *Alternaria alternata* and *Penicillium expansum* Link with 88%, 63% and 54% frequency, respectively.

Table 3. Incidence of microscopic fungal species isolated from samples of crust.

Species of fungi	Crust sample number								
	C1	C2	C3	C4	C5	C6	C7	C8	C9
Alternaria alternata (Fr.) Keissl.	+	+	+	+	+	+	+	-	+
Aspergillus ochraceus Wilhelm	-	-	-	-	-	-	-	-	+
Botrytis sp.	-	+	-	+	+	-	-	-	+
Cladosporium cladosprioides (Fres.) de Vries	+	+	+	+	+	+	+	-	+
Fusarium sp.	+	+	-	-	-	-	-	-	-
Mucor sp.	+	+	-	-	+	-	+	-	-
Mycelia sterillia	+	+	+	+	+	+	+	-	+
Penicillium sp.	+	+	+	+	+	+	+	-	+
Rhizopus stolonifer (Ehrenb.: Fr.) Vuill.	-	+	-	-	-	+	-	-	-
Trichoderma sp.	+	+	+	+	+	-	+	-	-
Trichoderma harzianum Rifai	-	-	-	-	-	-	+	+	+

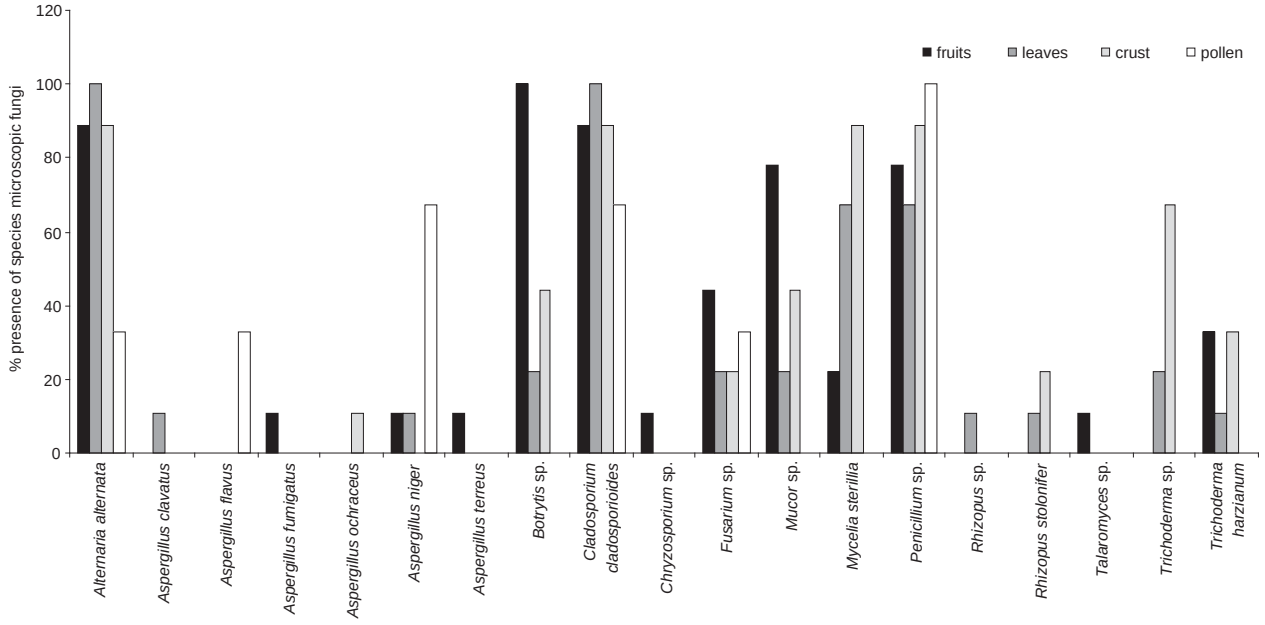

Figure 1. Representation of microscopic fungal species (%) on fruit, leaves, crust and pollen of *Sorbus domestica* L.

The genus *Aspergillus* was represented mainly by species of *Aspergillus clavatus*, *A. fumigatus*, *A. niger*, *A. ochraceus*, *A. terreus* (Fig. 1) in the mycoflora of *Sorbus domestica* L.

The mycoflora analysis of some dried fruits showed a wide range of fungal contamination – in 60 samples collected from different markets. Twenty-three species and one variety belonging to 15 genera were isolated from dried fruits (raisins, dates and figs) on 2 types of media. *Aspergillus niger*, *A. flavus*, *A. fumigatus*, *A. ochraceus*, *Penicillium chrysogenum* and *Rhizopus stolonifer* were the most common fungal species isolated on 1% glucose-Czapek's agar medium at 28ºC, while *Eurotium amstelodami*, *Zygosaccharomyces rouxii*, *A. niger* and *P. chrysogenum* were common on 40% sucrose-Czapek's agar [2].

CONCLUSION

The detection of fungal diversity on the *Sorbus domestica* L. brings new important data in the given special fruit, leaves, crust and pollen of *Sorbus domestica*. The list of detected fungi could also be used by occupational disease and allergy specialists, taking into account that several varieties of *Penicillium*, *Alternaria*, *Cladosporium* and others fungi are considered to be hazardous moulds. Tree fruits could be a source of significant fungal secondary metabolites, including mycotoxins, as is indicated by the presence of the specific fungal species.

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