

## DIFFERENT DRYING TECHNOLOGIES AND ALTERNATION OF MYCOBIOTS IN THE RAW MATERIAL OF *HYSSOPUS OFFICINALIS* L.

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**Abstract:** Contamination of medicinal plant mass with mycobiots is one of the negative factors deteriorating the quality of raw material. In order to evaluate the impact of the yield processing technologies upon the changes of mycobiots in raw material, the mycobiotic conditions of herb hyssop (*Hyssopus officinalis* L.) raw material were evaluated under various regimes of active ventilation and optimization of the drying parameters. The impact of ventilation intensity and temperature of drying agent upon the changes and abundance of mycobiota species in medicinal raw material was determined. Irrespective of the temperature of the airflow, the strongest suppressive effect upon the mycobiotic contamination in *Hyssopi herba* was produced by the 5,000 m<sup>3</sup>·(t·h)<sup>-1</sup> airflow. Analysis of the isolated fungi revealed the prevalence of *Penicillium*, *Aspergillus*, *Alternaria*, *Cladosporium*, *Mucor*, *Rhizopus* species in the raw material. In separate samples *Botrytis cinerea*, *Sclerotinia sclerotiorum*, *Aureobasidium pullulans*, *Chrysosporium merdarium*, *Cladorrhinum foecundissimum*, *Ulocladium consortiale*, *Trichoderma hamatum*, *T. harzianum*, *Gilmaniella humicola*, *Talaromyces flavus*, *Rhizomucor pusillus*, *Hansfordia ovalispora*, *Verticicladium trifidum*, *Trichosporiella cerebriformis* micromycetes were also rather abundant. Detection of the above-mentioned micromycetes in herb hyssop samples differed, and partially depended upon the medium used for their isolation.

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**Key words:** *Hyssopus officinalis* L., medicinal raw material, humidity, temperature, mycobiota, drying parameters.

### INTRODUCTION

In pharmacy, plant raw materials are important sources of new medicines and their substitutes. Natural medicines of plant origin have a wider therapeutic spectrum, milder action and less frequent side effects compared with synthetic substances. According to the data of the World Health Organization, about 70,000 plant species are currently used for medicinal purposes; about 1,000 species are used in the European pharmaceutical industry; in Lithuanian traditional and folk medicine about 460 plant species are used.

The most popular medicinal plants are *Matricaria recutita* L., *Thymus vulgaris* L., *Mentha piperita* L., *Calendula officinalis* L., *Echinacea purpurea* (L.) Moench., *Valeriana officinalis* L., *Melissa officinalis* L., *Leonurus cardiaca* L. In Lithuania, about 3,000 t of medicinal plant material is consumed annually; 29% of medicinal herbs are gathered in natural habitats, 6% are grown on industrial farms and 65% of the raw material is imported, mostly from Poland, Ukraine, Germany, Turkey, Egypt, and China [25, 42, 44].

Lately, the demand for *Hyssopus officinalis* L. in Lithuania has increased rapidly. *Hyssopus officinalis* L. is

a perennial aromatic semi-shrub of the *Lamiaceae* family, *Lamiidae* subclass, *Magnoliopsida* class, *Magnoliophyta* division [16, 19]. The plant originates from the Mediterranean region; it is widespread in southern Europe, European Russia, the Caucasus, Central Asia and the Altai region. Presently, the herb hyssop is cultivated in many countries. It grows well in sunny places, in weedless and fertile sandy loam, or loam soils. The plant can be cultivated for 10 or more years in the same place [28, 43].

The above-ground part of plants, i.e. *Hyssopi herba*, is used as medicinal raw material. The above-ground part is gathered twice during the vegetation, i.e. during plant flowering – end of June to the beginning of July, and at the end of September. The yield during the first year of cultivation is 21.3 cnt·ha<sup>-1</sup>, during the second year – 42.8 cnt·ha<sup>-1</sup>, in the course of the third year and later – 76 cnt·ha<sup>-1</sup> of the air-dry raw material. Crude and dried *Hyssopi herba* is aromatic, slightly bitter in flavour. According to medicinal references [43], herb hyssop is used to alleviate cough, in cases of asthma and bronchitis, as well as to stimulate digestion and appetite, regulate perspiration, treatment of wounds and bruises, as well as to relief rheumatics.

One of the factors influencing the quality of plant raw material is its contamination with microscopic fungi. Mycobiotic contamination of the medicinal raw material influences its storing period, quality, and reduction of active substances [7, 8, 38, 41]. The activity of mycobiots depends upon environmental conditions, especially humidity [30]. Various ways suppressing the micromycete activity and reducing the mycobiotic contamination of herbs are proposed: chemical disinfection [20], processing by ionized gas or vapours [7, 23, 53]. The post-harvest processing technologies and regimes worsening the sensual properties of the raw material, impairing the active substances accumulated in the raw material, and thus causing a hazard to people's health and environment [7] should be avoided. Preference is given to drying, during which the content and activity  $a_w$  [7, 9, 38, 47] of water accumulated in the raw material is reduced. The majority of micromycetes are non-viable when the water activity in medicinal raw material does not exceed  $a_w=0.8$  [22, 38], and when the raw material dries to 10–15% [43].

Technologies and parameters of the drying should be chosen considering the amount of raw material, morphologic and anatomical structure, chemical composition and stability of biologically active substances. Investigations on various ways of drying and their impact upon the quality of medicinal raw material were performed both in Lithuania and other countries. The results showed that microwave-drying reduced the process by 95–98% in comparison with convection dryers [8, 36]. In such cases, however, the majority of the investigated medicinal plants lose their natural colour as well as large quantities of active substances and chlorophyll. *Salvia officinalis* L., *Melissa officinalis* L., *Levandula angustifolia* Mill., *Hyssopus officinalis* L., *Rozmarinus officinalis* L. are particularly sensitive to intense

drying. Smaller losses of active substances and chlorophyll occur when less intense, i.e. convection drying technologies are used: convection dryers, active ventilation, natural drying or curing [2, 8, 9, 10]. The best results are achieved when active ventilation is used for drying of the medicinal raw material. Comparative investigations of different drying technologies revealed that in the raw material of *Levisticum officinale* Koch, *Hyssopus officinalis* L., *Levandula angustifolia* Mill., *Melissa officinalis* L., *Origanum vulgare* L. and *Salvia officinalis* L. dried using active ventilation the amount of essential oils is by 4.3% higher than in raw material dried using other convection technologies; the amount of chlorophyll is by 8.4% higher than in a heat drier, and by 11.1% higher than in naturally dried raw material [8]. Other authors also indicate the slighter negative impact of active ventilation upon medicinal raw material of *Roman chamomile*, *Petroselinum crispum* L. and *Satureja hortensis* [1, 24, 40, 51].

If drying of the medicinal raw material is delayed, the humidity regime, favourable for the development of mycobiots, forms. According to some references, *Hyssopi herba*, if dried improperly, can accumulate plenty of micromycetes that intensively produce and excrete mycotoxins hazardous for human health. Mycotoxins can combine with certain compounds present in *Hyssopi herba* and make it completely unusable [12, 13, 35, 52]. Therefore, it is essential to choose the regimes of active ventilation, especially air filtration speed, that ensure fast drying of the herb. Only then the active ventilation drying would be successful and the raw material would be of high quality [32, 38, 57].

The aim of the work was to evaluate the mycobiotic contamination of the herb hyssop (*Hyssopus officinalis* L.) raw material employing different regimes of drying with active ventilation. Another aim was to study the micromycete species diversity and its changes in order to reduce the contamination of raw material with micromycetes, and obtain the most valuable medicinal raw material.

## MATERIALS AND METHODS

The research was performed in 2006–2007. Medicinal raw material – *Hyssopi herba*, grown in the Kaunas Botanical Garden of Vytautas Magnus University was investigated. The herb hyssop was introduced in the collection of Medicinal, Spice and Melliferous plants of the Botanical Garden in 1980. In central Lithuania, the average temperature is +6.7°C ( $\Sigma T > 10^\circ\text{C} - 2,100-2,300$  hours,  $T^\circ \text{n (m)} - 2-26^\circ\text{C}$ ), yearly amount of precipitation – 500–750 mm [4], clayey plains (70–150 m above sea level) and Gleyic Luvisols – IDg soils prevail [29]. At the growing site the soil is turf, gleyic, medium loam, unfertilized, dried by applying closed drainage, long-fallow land before the experimental trial. The soil is characterized by a high content of phosphorus (188.0–617.0 mg·kg<sup>-1</sup>), medium content of potassium (62.0–166.0 mg·kg<sup>-1</sup>) and humus (4.4–10.0%), total nitrogen – 0.16–0.29%, pH – 6.9–7.2.

**Table 1.** Drying conditions of *Hyssopi herba*.

	I drying variant				II drying variant			
	Drying container				Drying container			
	1	2	3	4	1	2	3	4
<i>Hyssopi herba</i> amount, kg	5	5	5	5	4	4	4	4
Layer height, cm	96	90	94	91	81	91	87	88
Ventilation intensity, m <sup>3</sup> ·(t·h) <sup>-1</sup>	650	2110	4310	7330	1320	2000	4360	6860
Filtration rate, m·s <sup>-1</sup>	0.03	0.11	0.22	0.37	0.05	0.08	0.18	0.28
<i>Hyssopi herba</i> moisture content, %								
before drying	72.0±0.36				70.3±0.39			
after drying	43.9±0.26	12.7±0.14	13.2±0.31	13.0±0.57	13.0±0.88	13.2±0.34	12.2±0.30	13.0±0.34
Drying agent								
temperature, °C	22.9±0.12				32.3±0.03			
relative humidity, %	51.3±0.32				28.8±0.21			

*Hyssopi herba* cut twice during the first flowering, i.e. at the beginning of flowering and during the mass flowering, was used for the investigation of drying technologies and microbiological studies. The medicinal raw material was cut into 4 cm long pieces and dried to optimum dryness of 13% [24]. Medicinal raw material was dried in two different variants at the Lithuanian University of Agriculture (Tab. 1). *Hyssopi herba* cut at the beginning of flowering was dried with unheated ambient air of 22.9±0.12°C temperature and 51.3±0.32% relative humidity (I drying variant). The raw material cut during the mass flowering was dried with heated ambient air of 32.4±0.03°C temperature and 28.7±0.21% relative humidity (II drying variant).

The drying stand consisted of a ventilator, electric heater, airflow distribution collector and drying containers [32]. Simultaneously, *Hyssopi herba* was being dried in four 1.15 m high and 0.18 m wide containers using different ventilation intensity. Prior to drying, the ventilation intensity was adjusted by a valve at the bottom of each container. In the course of drying trials, temperature and relative humidity of the drying agent were registered every 10 min using the device ALMEMO 3290 with sensors FH A646–21; the procedure was performed at the ventilator intake opening, airflow distribution collector, bottom and top of the dried *Hyssopi herba* layer (10 cm distance from the bottom and top). The weight of the dried medicinal raw material was registered 2–3 times a day by weighing the drying containers.

*Hyssopi herba* was dried until the average moisture content of the medicinal raw material layer reduced to 13%. Then the air flow into the container was stopped, the dried raw material was turned over, mixed and an average sample for determination of mycobiotic contamination was taken. Microbiological investigations were performed at the Institute of Chemistry.

Abundance of mycobiots in medicinal raw material was determined by applying the quantitative method [56]. For colony identification, malt extract agar and Sabouraud's

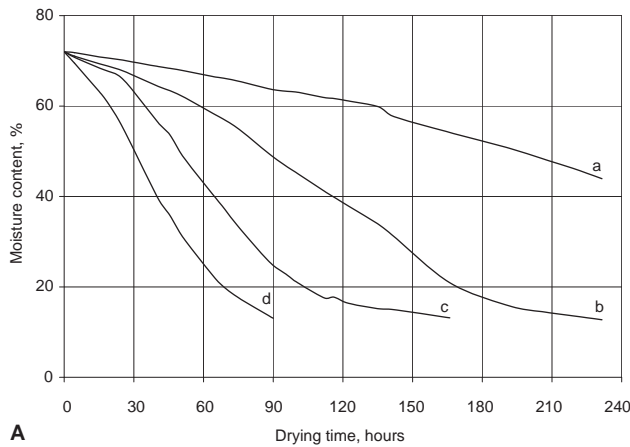
medium enriched with 0.5 g·l<sup>-1</sup> of chloramphenicol were used. Samples were incubated for 5 days in a thermostat at 26±2°C. The results were expressed as colony forming units per gram (ksv·g<sup>-1</sup>) of the medicinal raw material. Detection frequency of micromycete genera in a sample was calculated according to T. Mirczink [37].

The colonies of microscopic fungi were subcultured in order to obtain monocultures. In order to achieve this, each isolate was inoculated on three standard agar media: malt extract, Czapek medium and synthetic medium with maize extract. As the colonies formed, their cultural properties were described, indicating the growth rate, colony structure and appearance, colour of mycelium and reverse of the colony, other properties. Applying methods of light microscopy, the morphologic peculiarities of each fungal species were investigated during the process of conidiogenesis. Systematic position was determined according to various manuals [11, 14, 15, 21, 27, 31, 39, 45, 49]. Potential possibilities of the recorded micromycete isolates to produce and excrete toxic metabolites were estimated according to methods of primary screening proposed by Frisvad [17], Samson *et al.* [49] and abundant literature references [5, 6, 26, 33].

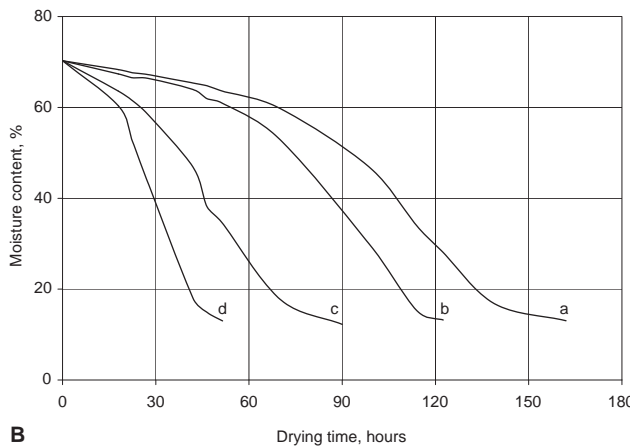
The research results were processed using the MS Office Excel program.

## RESULTS AND DISCUSSION

Relative humidity of the unheated air flow used for *Hyssopi herba* drying during the whole period of the research was lower than 60–65% [38]. Therefore the humidity sorption from flowing air and dampening of the medicinal raw material was avoided: moisture content of *Hyssopi herba* reduced consistently but slowly. We did not succeed in drying the raw material in the time recommended by literature references, i.e. 3–5 days [7, 38, 41, 43]. Only herbs ventilated by 7,330 m<sup>3</sup>·(t·h)<sup>-1</sup> airflow dried in 90 hours – less than 5 days (Fig. 1). At 4,310 m<sup>3</sup>·(t·h)<sup>-1</sup> and 2,110 m<sup>3</sup>·(t·h)<sup>-1</sup>



A



B

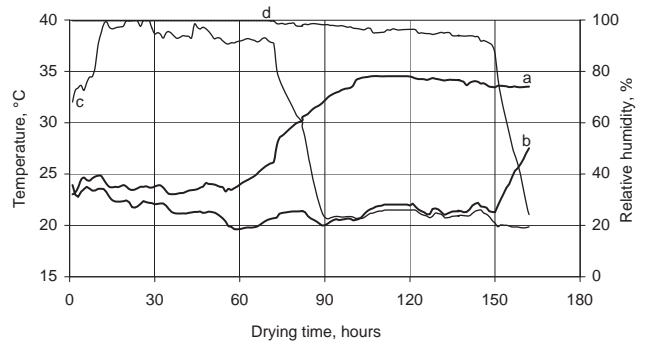
A – unheated ambient air:  
 a –  $650 \text{ m}^3 \cdot (\text{t}\cdot\text{h})^{-1}$ , b –  $2,110 \text{ m}^3 \cdot (\text{t}\cdot\text{h})^{-1}$ , c –  $4,310 \text{ m}^3 \cdot (\text{t}\cdot\text{h})^{-1}$ , d –  $7,330 \text{ m}^3 \cdot (\text{t}\cdot\text{h})^{-1}$   
 B – heated ambient air:  
 a –  $1,320 \text{ m}^3 \cdot (\text{t}\cdot\text{h})^{-1}$ , b –  $2,000 \text{ m}^3 \cdot (\text{t}\cdot\text{h})^{-1}$ , c –  $4,360 \text{ m}^3 \cdot (\text{t}\cdot\text{h})^{-1}$ , d –  $6,860 \text{ m}^3 \cdot (\text{t}\cdot\text{h})^{-1}$

**Figure 1.** Changes in *Hyssopi herba* moisture content while drying with different airflows.

airflows the drying took 168 and 228 hours, respectively. Drying of *Hyssopi herba* with  $650 \text{ m}^3 \cdot (\text{t}\cdot\text{h})^{-1}$  airflow was stopped as the herbal mass did not reach the 13% moisture content: during 228 hours of ventilation the moisture content of the raw material reduced only to 43.9%. Based on the tendency of the drying process, the 13% moisture content could be reached only in 380 hours.

*Hyssopi herba* ventilated by the airflow of  $9.4^\circ\text{C}$  higher temperature dried 1.95 times quicker. Drying of raw material with  $1,320 \text{ m}^3 \cdot (\text{t}\cdot\text{h})^{-1}$  airflow took 162 hours, it was by 39 hours, 76 hours and 110.5 hours longer than ventilating the herbs with  $2,000 \text{ m}^3 \cdot (\text{t}\cdot\text{h})^{-1}$ ,  $4,360 \text{ m}^3 \cdot (\text{t}\cdot\text{h})^{-1}$  and  $6,860 \text{ m}^3 \cdot (\text{t}\cdot\text{h})^{-1}$  heated ambient airflows, respectively.

It is maintained that a higher temperature of the drying agent increases the rate of moisture exchange between the dried raw material and drying agent [32, 57]. The data of the *Hyssopi herba* drying experiments reveal the improvement of the sorptive properties of the air: one cubic meter of  $22.9 \pm 0.12^\circ\text{C}$  and  $51.3 \pm 0.32\%$  relative humidity of ambient air can absorb 2.8 g of moisture, while heated  $32.4 \pm 0.03^\circ\text{C}$



a – temperature at bottom of layer, b – temperature at top of layer, c – relative humidity at bottom of layer, d – relative humidity at top of layer

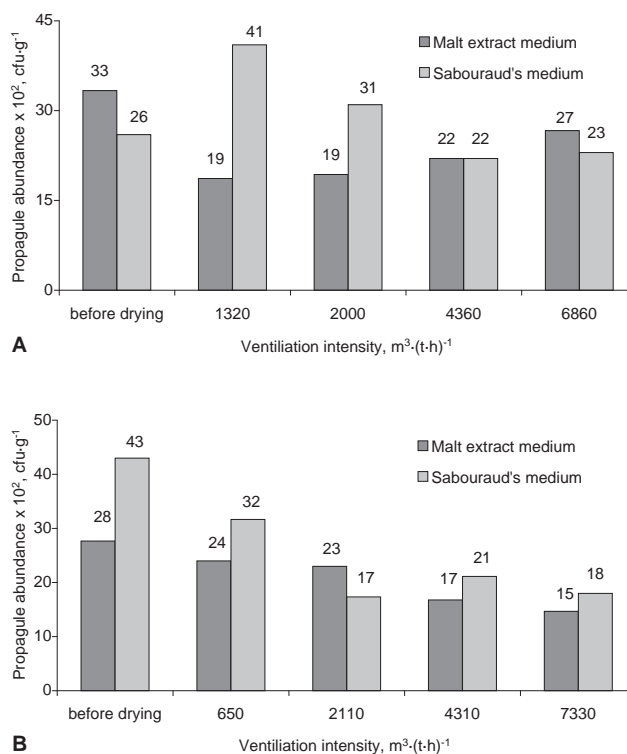
**Figure 2.** Temperature and relative humidity of the airflow in the *Hyssopi herba* layer when dried with heated ambient air, ventilation intensity  $1,320 \text{ m}^3 \cdot (\text{t}\cdot\text{h})^{-1}$ .

and  $28.7 \pm 0.21\%$  relative humidity ambient air can absorb 5.4 g of moisture. More stable drying conditions are also created: as ambient air airflow is heated by  $9.4^\circ\text{C}$ , the variance of temperature and relative humidity reduced from  $2.4^\circ\text{C}$  and  $17.2\%$  up to  $0.08^\circ\text{C}$  and  $6.5\%$ .

Additional heating of the drying agent, causing its temperature and especially relative humidity variation, can create favourable conditions for condensation and sorption processes in the layer of dried herbs. Under such conditions the herbs get wet, their drying is protracted and the period favourable for the development of micromycetes lasts longer. Heated ambient air is particularly unsafe for drying the medicinal raw material. Due to the high temperature of the drying agent, the bottom of the herb layer dries rapidly, and relative humidity of the flowing air increases up to 95% and more. Moving towards the top of the layer the airflow reaches the cooler upper herb layers. When ventilation with  $1,320 \text{ m}^3 \cdot (\text{t}\cdot\text{h})^{-1}$  heated airflow is used for 59 hours, the temperature of *Hyssopi herba* at the height of 0.7 m was on average by  $1.9 \pm 0.1^\circ\text{C}$  lower than at the height of 0.1 m (Fig. 2). Such a temperature difference is sufficient for condensate to form as the drying agent of  $96 \pm 0.4\%$  relative humidity flows through the upper layers. Formation of the condensate could not be avoided, even by increasing the airflow to  $2,000 \text{ m}^3 \cdot (\text{t}\cdot\text{h})^{-1}$ .

While ventilating with  $1,320 \text{ m}^3 \cdot (\text{t}\cdot\text{h})^{-1}$  and  $2,000 \text{ m}^3 \cdot (\text{t}\cdot\text{h})^{-1}$  airflows, at the top of the *Hyssopi herba* layer humid conditions with relative humidity higher than 90% persisted during 86% of the drying period. Almost half of this period was particularly favourable for the micromycete development because of the process of condensation. The condensate accumulated in the upper layers and on top of the raw material. Moisture covering the top of the herbs created favourable conditions for the micromycete development. At the top of the *Hyssopi herba* layer, drying started after 53 hours (at  $1,320 \text{ m}^3 \cdot (\text{t}\cdot\text{h})^{-1}$  airflow) and after 39 hours (at  $2,000 \text{ m}^3 \cdot (\text{t}\cdot\text{h})^{-1}$  airflow) of ventilation. Surface moisture evaporated from the herb surface, but the ambient relative humidity remained higher than 90% for a long time.



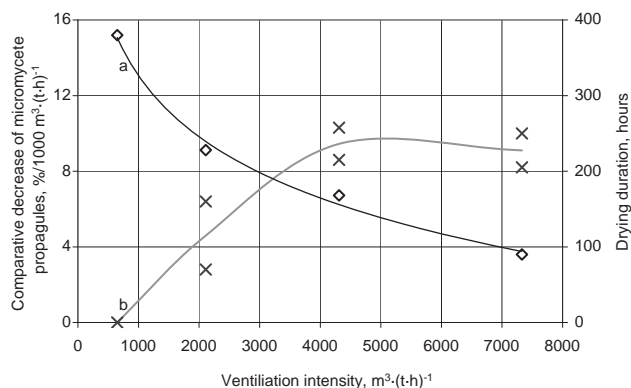


A – after drying with heated ambient air, B – after drying with unheated ambient air

**Figure 3.** Mycobiotic contamination of *Hyssopi herba* before and after drying.

These circumstances determined high mycobiotic contamination of the dried raw material (Fig. 3A). Micromycetes developed most intensively on the Sabouraud's medium. Mycobiotic contamination of the *Hyssopi herba* dried with 1,320 m<sup>3</sup>·(t·h)<sup>-1</sup> and 2,000 m<sup>3</sup>·(t·h)<sup>-1</sup> airflows increased by 1.6 and 1.2 times compared with crude raw material.

Temperature differences between the top and bottom of the *Hyssopi herba* layer, as well as the appearance of condensate, were avoided by increasing the ventilation intensity up to 4,360 m<sup>3</sup>·(t·h)<sup>-1</sup> and 6,860 m<sup>3</sup>·(t·h)<sup>-1</sup>. Medicinal raw material started drying almost simultaneously at the top and bottom of the layer. The period of intense drying also shortened. Humid environment at the top of the layer persisted for about 50% of the drying period: 42 hours (at 4,360 m<sup>3</sup>·(t·h)<sup>-1</sup> airflow) and 21 hours (at 6,860 m<sup>3</sup>·(t·h)<sup>-1</sup> airflow). From this moment the relative air humidity of the environment rapidly decreased. Investigations on mycobiotic contamination of the raw material revealed that after drying, the amount of micromycete propagules on malt extract medium was on an average by 25.8% lower than before drying, and on Sabouraud's medium – by 13.5% lower. The micromycete abundance on the Sabouraud's medium with peptone decreased. They were less abundant than on the malt extract medium. It should be mentioned that in the case of 6,860 m<sup>3</sup>·(t·h)<sup>-1</sup> airflow of heated ambient air the micromycete propagules in the raw material were more abundant than in case of ventilation with 4,360 m<sup>3</sup>·(t·h)<sup>-1</sup> airflow.



a – *Hyssopi herba* drying duration,

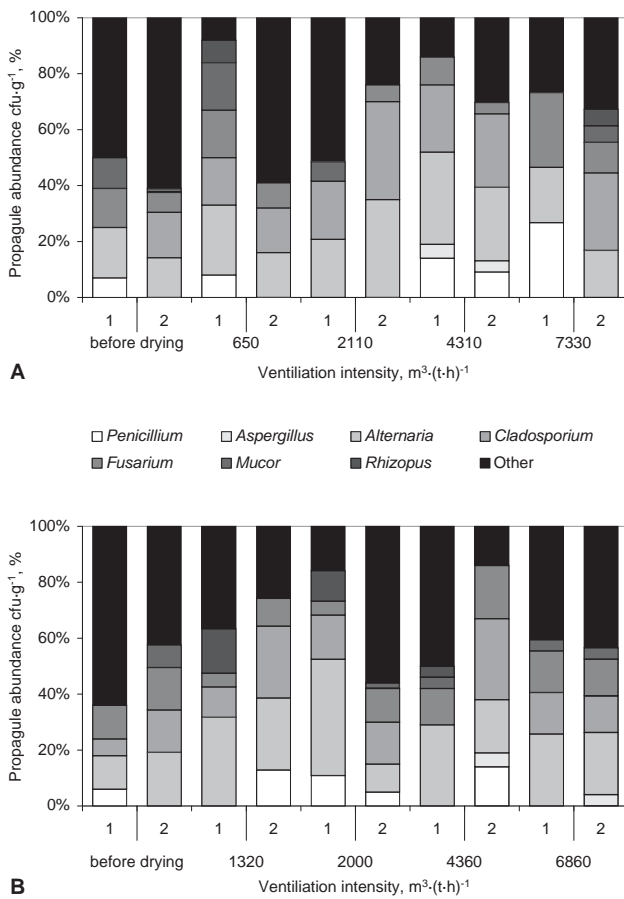
b – comparative suppressive effect of the 1000 m<sup>3</sup>·(t·h)<sup>-1</sup> airflow on micromycete abundance

**Figure 4.** Impact of ventilation intensity upon *Hyssopi herba* drying duration and mycobiotic contamination.

Very high ventilation intensity and rapid drying contributed to the formation of badly drying zones, trouble-spots, at the top of the layer. There, *Hyssopi herba* stuck, thus preventing smooth air flow in the herb layer. In the centre of trouble-spots conditions favourable for the development of mycobiotics persisted for a longer period. The formed source of mycobiotic contamination hastened higher micromycete abundance in the dried medicinal raw material.

While drying with unheated ambient air, additional *Hyssopi herba* dampening due to condensation and sorption processes was avoided. Mycobiotic contamination of medicinal raw material was determined by the drying intensity – rate of raw material drying at the top of the layer, where conditions favouring the micromycete development persist for the longest time [57]. Increase in ventilation intensity from 650 m<sup>3</sup>·(t·h)<sup>-1</sup> to 7,330 m<sup>3</sup>·(t·h)<sup>-1</sup> reduced the duration of *Hyssopi herba* drying to 13% moisture content from 380 to 90 hours, according to logarithmic dependence. The stronger the airflow, the sooner the drying process started at the top of the layer and the less pronounced was the activity of micromycetes. The lowest amount of micromycete propagules was recorded in herbs dried with 7,330 m<sup>3</sup>·(t·h)<sup>-1</sup> airflow, and the highest amount – in herbs dried with 650 m<sup>3</sup>·(t·h)<sup>-1</sup> airflow (Fig. 3B). It should be noted that airflow of different intensity produces an unequal suppressive effect upon micromycetes. The most evident comparative suppressive effect of the 1,000 m<sup>3</sup>·(t·h)<sup>-1</sup> airflow was revealed at ventilation intensity of about 5,000 m<sup>3</sup>·(t·h)<sup>-1</sup> (Fig. 4): the amount of mycobiotics in the medicinal raw material decreased by 48%, each 1,000 m<sup>3</sup>·(t·h)<sup>-1</sup> of airflow reduced the micromycete abundance by 9.6%.

The abundance of micromycetes in *Hyssopi herba* raw material, ventilated with ambient air, was predetermined by the drying process. When heated ambient air was used for drying, the formation of fungi was stimulated by condensate formed in the raw material. Systematic analysis of the isolated micromycetes revealed the dominance of the *Penicillium*, *Aspergillus*, *Alternaria*, *Cladosporium*,



A – dried with ambient air, B – dried with heated ambient air,  
1 – on malt extract medium, 2 – on Sabouraud's medium

**Figure 5.** Distribution of the micromycete genera isolated from *Hyssopi herba* according to different agar media.

*Mucor*, *Rhizopus* genera fungi in *Hyssopi herba* (Fig. 5). In separate samples, *Botrytis cinerea*, *Sclerotinia sclerotiorum*, *Aureobasidium pullulans*, *Chrysosporium merdarium*, *Cladorrhinum foecundissimum*, *Ulocladium consortiale*, *Ulocladium oudemansii*, *Trichoderma hamatum*, *T. harzianum*, *Gilmaniella humicola*, *Talaromyces flavus*, *Rhizomucor pusillus*, *Hansfordia ovalispora*, *Verticicladium trifidum*, *Trichosporiella cerebriiformis* were rather abundant. Detection of the above-mentioned micromycetes in herb samples was unequal and partly depended upon the medium used for their isolation. Some of them were recorded only on Sabouraud's medium containing peptone. This indicates their alleged pathogenicity to warm-blooded animals [6, 37, 45, 56].

Data on species composition of the micromycetes isolated from *Hyssopi herba* are presented in Table 2. In samples before drying, the yeast-like fungi *Candida albicans*, *Rhodotorula rubra*, *Aureobasidium pullulans*, characterized by pathogenicity, prevailed. *Botrytis cinerea*, *Sclerotinia sclerotiorum*, *Fusarium proliferatum*, *F. moniliforme* were abundant on malt extract medium. According to literature references [5, 33, 46, 54], ability to synthesize toxic

secondary metabolites is characteristic to some of these fungi; it is particularly hazardous in the case of medicinal and spice raw material.

In all variants, both before and after drying, micromycetes of the *Alternaria* genus were abundant on *Hyssopi herba*. *Alternaria alternata* dominated, though on some *Hyssopi herba* samples *Alternaria radicina* prevailed; *A. dianthi*, *A. tenuissima* were rather frequent, *A. plurisepitata* was occasionally recorded. In the course of drying, the abundance of these fungi did not reduce significantly. *A. alternata* was more sensitive to drying than *A. radicina*, *A. tenuissima* fungi. The recorded *Alternaria* fungi are able to produce active substances altenuenes, which suppress the development of *Bacillus mycoides*, *B. subtilis*, *Neisseria gonorrhoeae*, *Sarcina lutea*, *Staphylococcus aureus* bacteria [5, 6, 34, 54]. Other substances synthesized by these fungi, e.g. alternariol prevent the development of bacteria and have a toxic impact upon warm-blooded animals. It is also characteristic of other compounds synthesized by these fungi: altenuisol, alternariol, altenuisin, dehydroaltenuisin. It should also be mentioned that some *A. alternata* strains are able to synthesize ergosterol, which under the impact of UV irradiation activates functionality of vitamin D<sub>1</sub>. The method of chromatography on silica gel [54] revealed that the majority of the recorded *Alternaria* genus fungi are able to produce tenuazonic acid, characterized by antibacterial, antiviral activities and toxicity towards the warm-blooded animals, especially birds.

Micromycetes of the *Cladosporium* genus were widely spread. The majority of fungi ascribed to this genus are very resistant to external physical factors and various chemical substances; they easily adapt to various substrates [31]. During the research, these fungi were abundant in almost all samples. *C. herbarum* fungi dominated. *C. cladosporioides*, *C. sphaerospermum* were more frequent on *Hyssopi herba* dried with airflows of 650 m<sup>3</sup>·(t·h)<sup>-1</sup>, 2,110 m<sup>3</sup>·(t·h)<sup>-1</sup>, 4,310 m<sup>3</sup>·(t·h)<sup>-1</sup> unheated and 1,320 m<sup>3</sup>·(t·h)<sup>-1</sup>, 2,000 m<sup>3</sup>·(t·h)<sup>-1</sup> heated ambient air when ventilation of medicinal raw material was prolonged due to the inefficient drying process or the formed condensate. Biological peculiarities of the fungi that allow easy detachment of propagules from primary substrate and distribution with airflow and dust, as well as ability to remain viable under most unfavourable conditions, contributed to their abundance in the dried *Hyssopi herba*. Toxicity of the *Cladosporium* fungi towards other biots is still understudied. Metabolites excreted by these micromycetes could cause allergic and respiratory diseases, sometimes even toxicoses [11, 18, 30, 31, 50, 55].

Drying considerably suppressed the distribution of the *Penicillium* species. 16 species of this genus were isolated from *Hyssopi herba*: *P. atramentosum*, *P. brevicompactum*, *P. canescens*, *P. capsulatum*, *P. chrysogenum*, *P. clavigerum*, *P. commune*, *P. cyaneum*, *P. cyclopium*, *P. funiculosum*, *P. godlewskii*, *P. islandicum*, *P. janthinellum*, *P. puberulum*, *P. simplicissimum*, *P. verrucosum*.

**Table 2.** *Hyssopi herba* contamination with micromycetes.

Ventilation intensity, m <sup>3</sup> ·(t·h) <sup>-1</sup>	Micromycetes of prevailing species (distribution frequency >50%)	
	Malt extract medium	Sabouraud's medium
Drying with unheated ambient air (I drying variant)		
Before drying	<i>Alternaria alternata</i> (Fr.) Keissl. <i>Botrytis cinerea</i> Pers. et Fr. <i>Sclerotinia sclerotiorum</i> (Lib.) de Bary <i>Fusarium proliferatum</i> (Matsushima) Nirenberg <i>Fusarium moniliforme</i> J. Sheld.	<i>Alternaria alternata</i> (Fr.) Keissl. <i>Aureobasidium pullulans</i> (de Bary) G. Arnaud <i>Rhodotorula rubra</i> (Demme) Lodder <i>Candida albicans</i> (Robin) Berkhout. <i>Sclerotinia sclerotiorum</i> (Lib.) de Bary
650	<i>Mucor racemosus</i> Fresen. <i>Alternaria alternata</i> (Fr.) Keissl. <i>Mucor silvaticus</i> Hagem <i>Alternaria dianthi</i> F. Stevens & J.G. Hall	<i>Fusarium oxysporum</i> Schldtl. <i>Sclerotinia sclerotiorum</i> (Lib.) de Bary <i>Cladosporium herbarum</i> (Pers.) Link ex Gray <i>Alternaria radicina</i> Meier, Drechsler et E.D. Eddy
2110	<i>Botrytis cinerea</i> Pers. et Fr. <i>Mucor circinelloides</i> Tiegh. <i>Alternaria alternata</i> (Fr.) Keissl. <i>Cladosporium herbarum</i> (Pers.) Link ex Gray	<i>Cladosporium herbarum</i> (Pers.) Link ex Gray <i>Ulocladium consortiale</i> (Thüm.) E.G. Simmons <i>Chrysosporium merdarium</i> (Link ex Grev.) J.V. Carmich. <i>Alternaria alternata</i> (Fr.) Keissl.
4310	<i>Aureobasidium pullulans</i> (de Bary) G. Arnaud <i>Alternaria alternata</i> (Fr.) Keissl. <i>Alternaria dianthi</i> F. Stevens & J.G. Hall <i>Alternaria tenuissima</i> (Kunze ex Pers.) Wiltshire	<i>Alternaria radicina</i> Meier, Drechsler et E.D. Eddy <i>Alternaria alternata</i> (Fr.) Keissl. <i>Ulocladium consortiale</i> (Thüm.) E.G. Simmons <i>Cladosporium herbarum</i> (Pers.) Link ex Gray
7330	<i>Aureobasidium pullulans</i> (de Bary) G. Arnaud <i>Fusarium proliferatum</i> (Matsushima) Nirenberg <i>Penicillium verrucosum</i> Dierckx <i>Penicillium godlewskii</i> K.M. Zalessky	<i>Aureobasidium pullulans</i> (de Bary) G. Arnaud <i>Trichoderma hamatum</i> (Bonord.) Bainier <i>Alternaria alternata</i> (Fr.) Keissl. <i>Ulocladium oudemansii</i> E.G. Simmons
Drying with heated ambient air (II drying variant)		
Before drying	<i>Geomyces pannorum</i> (Link) Sigler & J.W. Carmich. <i>Cladosporium herbarum</i> (Pers.) Link ex Gray <i>Fusarium culmorum</i> (W. G. Sm.) Sacc. <i>Alternaria radicina</i> Meier, Drechsler et E.D. Eddy	<i>Botrytis cinerea</i> Pers. et Fr. <i>Fusarium oxysporum</i> Schldtl. <i>Chrysosporium merdarium</i> (Link ex Grev.) J.V. Carmich. <i>Alternaria radicina</i> Meier, Drechsler et E.D. Eddy
1320	<i>Alternaria radicina</i> Meier, Drechsler et E.D. Eddy <i>Alternaria alternata</i> (Fr.) Keissl. <i>Trichoderma harzianum</i> Rifai <i>Gilmaniella humicola</i> G.L. Barron	<i>Alternaria alternata</i> (Fr.) Keissl. <i>Alternaria radicina</i> Meier, Drechsler et E.D. Eddy <i>Alternaria pluriseptata</i> (P. Karst. & Har. ex Peck) Jørst <i>Ulocladium oudemansii</i> E.G. Simmons
2000	<i>Alternaria alternata</i> (Fr.) Keissl. <i>Alternaria radicina</i> Meier, Drechsler et E.D. Eddy <i>Chrysosporium merdarium</i> (Link ex Grev.) J.V. Carmich. <i>Talaromyces flavus</i> (Klöcker) Stolk & et Samson	<i>Hansfordia ovalispora</i> S. Hughes <i>Verticicladium trifidum</i> Preuss <i>Trichosporiella cerebriformis</i> (G.A. de Vries et Kleine-Natrop) W. Gams <i>Cladorrhinum foecundissimum</i> Sacc. & Marchal
4360	<i>Rhizomucor pusillus</i> (Lindt) Schipper <i>Fusarium graminearum</i> Schwabe <i>Fusarium sporotrichioides</i> Sherb. <i>Alternaria radicina</i> Meier, Drechsler et E.D. Eddy	<i>Botrytis cinerea</i> Pers. et Fr. <i>Penicillium janthinellum</i> Biourge <i>Alternaria alternata</i> (Fr.) Keissl. <i>Penicillium simplicissimum</i> (Oudem.) Thom
6860	<i>Alternaria radicina</i> Meier, Drechsler et E.D. Eddy <i>Ulocladium oudemansii</i> E.G. Simmons <i>Ulocladium consortiale</i> (Thüm.) E.G. Simmons <i>Sclerotinia sclerotiorum</i> (Lib.) de Bary	<i>Chrysosporium merdarium</i> (Link ex Grev.) J.V. Carmich. <i>Fusarium solani</i> (Mart.) Appel et Wollenw. <i>Alternaria alternata</i> (Fr.) Keissl. <i>Alternaria dianthi</i> F. Stevens & J.G. Hall

According to the literature references, the majority of the isolated *Penicillium* fungi are able to synthesize and excrete toxic metabolites hazardous to people and animals. Among the isolated fungi of this genus, *P. verrucosum* were widespread; these fungi are known to produce ochratoxin A, penicillic acid, citrinin, verruculogen, verrucin and other toxins [46, 48]. *P. cyclopium* recorded in *Hyssopi herba* are able to produce patulin, claviformin, clavitin, clavacin, mycoin, and xanthomegnin. *P. chrysogenum* are producers of cyclopiazonic acid, patulin, ochratoxin A, roquefortine C & D, *P. islandicum* – of rugulovasine A & B, emodin, islanditoxin, skyrin, *P. janthinellum* – of verruculogen, janthitrem B, E, F & G, penicillic acid [6, 26, 33, 48]. It should be noted that metabolites excreted by some fungi of the *Penicillium* genus could prevent the development of other microorganisms. Such properties are characteristic of *P. cyaneum*, producing cyanein, *P. chrysogenum* – penicillin, *P. funiculosum* – helanin [3, 31].

It is maintained that fungi of the *Penicillium* genus can grow in a dryer environment than *Alternaria* and *Fusarium* fungi. The minimum relative humidity of the environment suitable for the development of *Penicillium* fungi is 78–84% [25, 31]. During the research, the most abundant *Penicillium* propagules occurred on rapidly dried raw material, i.e. which used airflows of 7,330 m<sup>3</sup>·(t·h)<sup>-1</sup> unheated and 4,360 m<sup>3</sup>·(t·h)<sup>-1</sup> heated ambient air. Rapidly decreased ambient humidity retarded the development of other fungi. It stimulated the increase in the propagules abundance of *Penicillium* genus fungi on dried medicinal raw material.

Fungi of the *Fusarium* genus ascribed to *F. avenaceum*, *F. culmorum*, *F. graminearum*, *F. heterosporum*, *F. moniliforme*, *F. oxysporum*, *F. poae*, *F. proliferatum*, *F. sambucinum*, *F. solani*, *F. sporotrichioides* were abundant in the medicinal raw material. Some of them are pathogenic to plants, people and animals. The above-mentioned *Fusarium* fungi excrete such mycotoxins as T-2 toxin, T-2 tetraol, fusarubin, fusarenon, HT-2 toxin, zearalenon, solaniol, neosolaniol, fumanisine, various trichothecenes, moniliformin, and nivalenol [5, 6, 26, 31]. Distribution of the *Fusarium* genus fungi on medicinal raw material is unacceptable and hazardous to human health, and therefore, its suppression should be under constant control.

## CONCLUSIONS

1. Moisture content of *Hyssopi herba* at harvesting reaches 70%. The herb surface abounds in propagules of various microbes including micromycetes of the *Alternaria*, *Cladosporium*, *Fusarium*, *Penicillium*, *Chrysosporium*, *Mucor*, *Rhizopus*, *Aspergillus*, sometimes other genera, that start destruction processes after the herb stops functioning. The value of medicinal raw material decreases, the risk of the accumulation of hazardous compounds of mycobiota and complex origin occurs.

2. The primary parameter, determining rate and success of drying with active ventilation, is the used airflow; secondary

parameter is the air temperature. As the airflow intensity was increased from 650 m<sup>3</sup>·(t·h)<sup>-1</sup> up to 7,330 m<sup>3</sup>·(t·h)<sup>-1</sup>, the dried *Hyssopi herba* reached the 13% moisture content 4.2 times quicker. The 5,000 m<sup>3</sup>·(t·h)<sup>-1</sup> airflow most efficiently suppresses the mycobiota contamination of the raw material. As the drying agent was heated from 22.9±0.12°C up to 32.4±0.03°C, *Hyssopi herba* dried by 1.95 times quicker than in case of unheated ambient air.

3. While selecting the drying technology of *Hyssopi herba* and other medicinal and spice plants, it is essential to balance the airflow and its temperature in order to prevent the increase in the activity of micromycetes which could occur due to dampening of the raw material or prolonged drying process, and to avoid the possibilities of formation and accumulation of toxic compounds. It is particularly important when the drying agent is additionally heated because in cases of too low or too high heated airflow, it is more difficult to avoid condensation and sorption as well as formation of poorly drying zones.

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