

Małgorzata KOSTECKA¹ and Monika BOJANOWSKA^{1*}

MOLD CONTAMINATION OF COMMERCIALY AVAILABLE HERBAL PRODUCTS AND DIETARY SUPPLEMENTS OF PLANT ORIGIN

ZAGROŻENIE ZANIECZYSZCZENIA GRZYBAMI PLEŚNIOWYMI ZIÓŁ ORAZ SUPLEMENTÓW DIETY POCHODZENIA ROŚLINNEGO DOSTĘPNYCH NA RYNKU

Abstract: Recent years have witnessed a rapid development of the dietary supplement market and a revived interest in herbal products used for therapeutic purposes. Herbs and medicinal plants have been used in traditional medicine for centuries. The concentrations of active substances in industrially processed plant extracts are many times lower than those found in the original plant material. For this reason, long-term of use herbal preparations is safe, and it does not require medical consultation. Herbs and medicinal plants have a complex composition, and recent research indicates that they may produce adverse reactions with food ingredients or pharmaceuticals. Molds, mainly fungi of the genera *Fusarium*, *Penicillium* and *Aspergillus* which colonize growing plants, are thermophilous organisms that can survive drying and heat processing, and they can find their way to the final product. Those fungi produce mycotoxins, metabolites which are toxic for humans.

In our study, fungi of the genera *Aspergillus* and *Fusarium* were determined in commercially available fresh herbs, herbal teas and herbal dietary supplements in the form of tablets.

Keywords: molds, herbs, dietary supplements, *Aspergillus*, *Fusarium*

Introduction

Herbs constitute a large group of plants containing active substances which may deliver health benefits for consumers. In addition to herbs, this category of plants also includes trees and shrubs which have been used as spices and medicinal products since antiquity. The therapeutic properties of herbs are determined by their chemical composition, mainly the presence of alkaloids, anthra-compounds, azulenes, phenols, flavonoids, tannins, cardiac glycosides, glucosinolates, pectins, saponins and mucila-

¹ Department of Chemistry, University of Life Science, ul. Akademicka 15, 20–950 Lublin, Poland, phone: +48 81 445 68 46.

* Corresponding author: monika.bojanowska@up.lublin.pl

ginous compounds [1, 2]. Despite the predominance of chemical medications on the contemporary market, there has been a revived interest in medicinal plants which are easily available and pose an alternative to synthetic drugs. There are two categories of medicinal herbs:

- herbal medicines – herb plants (mint leaves, lemon balm leaves, dandelion root) and herbal mixes;

- herbal formulations – herbs in the form of tablets, dragées, capsules and liquids (dry extracts, condensed extracts, infusions, essential oils) [3].

Fresh and dried herbs are also used to bring out and enhance the natural flavor and aroma of food [4]. Owing to their medicinal properties, herbs ease digestion, increase nutrient availability and boost natural immunity. Herbs fortify hair, nails and skin, and they are widely used in the cosmetics industry.

Poland has been a significant supplier and exporter of herbs for decades. Campaigns promoting natural foods and cosmetics have turned the consumers' attention to products containing herbs and spices [5]. Herbal raw materials are subject to strict control which contributes to the quality of the final product and promotes the development of new formulations. Herbs are used as fresh and dried plants (whole or ground plant fragments, mixes containing several plants), essential oils, extracts and microcapsules [6]. Dried herbs are highly popular due to their long shelf-life. Dried herbs and spices can, however, be colonized by fungi which produce harmful mycotoxins [7].

Raw herbs can act as hosts for microorganisms which colonize plants, soil, water and air. Plant communities are popularly colonized by molds of the genera *Alternaria*, *Fusarium*, *Cladosporium*, *Penicillium* and, less frequently, *Aspergillus*, *Trichoderma*, *Mucor* and *Rhizopus*. The size of fungal populations in fresh plant matter may exceed 10^6 CFU/g. Microbiological soil pollutants may also be introduced during the harvesting, transport and storage of plants when the number and abundance of mold communities, in particular of the genera *Penicillium*, *Fusarium*, *Alternaria*, *Cladosporium* and *Verticillium*, grows rapidly [8, 9]. Molds representing the genera *Alternaria*, *Aspergillus*, *Cladosporium* and *Penicillium* are transferred from the air to herbal raw materials [10, 11]. The composition of the microflora from this source is determined mainly by the degree of urban development in the herb growing area, and it increases considerably in the vicinity of cities.

Dried herbs may undergo microbiological contamination at nearly every stage of production, beginning from harvesting and drying to packaging which should protect the final product from adverse environmental impacts [5, 11, 12]. Molds colonizing raw herbs produce mycotoxins, secondary metabolites which pose a health hazard for humans and animals. Those low-molecular weight compounds are weakly polar, and they are not damaged by pasteurization or high temperature. They are degraded in an alkaline environment and under exposure to UV radiation [13]. The most prevalent mycotoxins include aflatoxins, ochratoxin A, patulin, trichothecenes, sterigmatocystin and vomitoxin. Subject to fungal species and their habitat conditions, the produced mycotoxins may deliver mutagenic, teratogenic, neurotoxic and nephrotoxic effects [14–16]. Herbal plants are most often colonized by the following mold genera and species: *Alternaria* sp., *Aspergillus* sp. (*A. amstelodami*, *A. sydowii*), *Chaetomium* sp.,

Cladosporium sp., *Fusarium* sp., *Mucor* sp., *Penicillium* sp., *Rhizopus* sp., *Trichoderma* sp. and *Verticillium* sp. The size of fungal populations is estimated at 10^1 – 10^6 CFU/g [5, 8, 17].

Herbal ingredients are found in foods, spice mixes and dietary supplements, and their microbiological purity is a highly important criterion while evaluating their processing suitability. Herbs, in particularly dried plants, are often carriers of microorganisms which are introduced into the final product. Evaluations of the medicinal properties and sensory attributes of herbs should, therefore, be accompanied by microbial and fungal analyses.

The objective of this study was to evaluate the degree of mold contamination in commercially available fresh herbs, herbal teas and herbal dietary supplements in the form of tablets.

Materials and methods

The experimental material comprised fresh basil, lemon balm and mint, as well as dried herbs, herbal teas and dietary supplements. Herbs were harvested from an organic farm, a farm where plants were sprayed with Topsin M 500SC fungicide, and pots:

- Basil I – pot, Santa Maria (Swedoponic, Polska),
- Basil II – pot, “Pokarm życia” horticultural-breeding farm, Poland,
- Basil III – treated with fungicide,
- Basil IV – organic farm,
- Lemon balm I – pot, Santa Maria (Swedoponic, Polska),
- Lemon balm II – pot, “Pokarm życia” horticultural-breeding farm, Poland,
- Lemon balm III – treated with fungicide,
- Lemon balm IV – organic farm,
- Mint I – pot, “Pokarm życia” horticultural-breeding farm, Poland,
- Mint II – organic farm.

Dried herbs and herbal teas were purchased in grocery stores and the Tesco supermarket, and dietary supplements (herbal tablets) were acquired from pharmacies in the city of Lublin.

Mycological evaluation

Fresh herbs: Mycological analyses were performed on 7–8 leaves from mature shoots without flower buds. The leaves were washed, dried and chopped. Two samples of 5 g each were placed on Petri dishes lined with moistened filter paper and incubated at 23 °C. After 7 days, fungal cultures were transferred to potato dextrose agar (PDA) with the addition of chloramphenicol (100 mg/dm^3) and incubated at 28 °C for 5 days.

Dried herbs and herbal teas: Three samples of 5 g each were placed on Petri dishes lined with moistened filter paper. The procedure was identical to that applied to fresh herbs.

Herbal tablets: Five tablets were randomly selected from the package. They were ground in a ceramic mortar, and three samples of 5 g each were incubated on moistened filter paper at 23 °C. After 7 days, fungal cultures were transferred to a PDA medium.

Fungal strains were identified based on mycological keys [18, 19].

Results

Molds are common in the environment, they are characterized by low sensitivity to external factors and the ease of propagation. Molds develop at a temperature of 18 to 32 °C, and they can survive in a wide temperature range of 6 to 60 °C (in particular in aerobic habitats, but also under anaerobic conditions) and at pH of 2–8.5 [20]. Molds remain stable in environments with relative humidity of 20 to 75 %, and they are thermophilous and thermotolerant organisms [21]. Food contamination with molds, their spores and toxic metabolites is difficult to control, and it poses a significant health risk for consumers.

Herbal teas and tables have a very low moisture content in the range of 7–12 %, therefore, they do not offer a supporting habitat for microbial growth. Most molds produce spores which can survive in highly unsupportive environments. Spores are resistant to drying temperatures (the optimal temperature for drying herbal products is 35–50 °C) and low humidity levels. They remain dormant under the above conditions, but when the moisture content of plant material increases to 30 % due to inadequate storage, spores begin to germinate and grow. Molds can develop in much drier habitats than bacteria, therefore dried plants and herbs are more likely to be colonized by molds than bacteria.

Most fungi isolated from herbs and herbal teas belong to the genera *Fusarium* and *Aspergillus*, pathogens which are commonly found in the environment. Those molds are generally harmless, but selected species produce toxic metabolites. Food products offer a highly supportive habitat for microbial growth, and molds colonizing food ingredients are often a source of secondary contamination. Molds observed in the examined material (Table 1) were members of 7 genera, and the presence of *Aspergillus* and *Fusarium* was observed in all studied herbs. Representatives of the genera *Alternaria* and *Penicillium*, followed by *Mucor*, *Rhizopus* and *Uclodium* sp. were less frequently noted. Molds of the genera *Cladosporium* and *Trichoderma* were not determined. Similar results were reported by Wojcik-Stopczynska et al [22, 23] in whose study, herbs were most frequently colonized by fungal genera of *Alternaria*, *Botrytis*, *Penicillium*, *Fusarium* and *Aspergillus*. Our results corroborate the findings of Halt and Klapac [24] who evaluated the contamination of herbal teas with microbial pathogens. In the cited study, a prevalence of the genera *Penicillium*, *Aspergillus*, *Cladosporium* and *Alternaria* was determined in experimental material, and none of the analyzed samples were free of molds.

Rizzo et al [25] found 56 strains of toxin-producing fungi in dried herbaceous plants grown in Argentina. More than half of the samples contained strains of the genus *Aspergillus*, and 16 % of the samples were contaminated by the genus *Fusarium*. The above authors observed that the number of toxin-producing fungi varied depending on

Table 1

Mold strains isolated from fresh herbs

Strain	Basil I	Basil II	Basil III	Basil IV	Lemon balm I	Lemon balm II	Lemon balm III	Lemon balm IV	Mint I	Mint II
<i>Aspergillus fumigatus</i>	+	+	+	+	+	+	+	+		
<i>Aspergillus niger</i>	+	+	+	+	+	+	+	+	+	+
<i>Penicillium</i> spp.	+	+	+	+						
<i>Aspergillus flavus</i>	+	+	+	+	+	+	+	+		
<i>Fusarium avanaceum</i>	+	+	+	+	+	+	+	+	+	+
<i>Fusarium culmorum</i>	+	+	+	+	+	+	+	+	+	+
<i>Fusarium oxysporum</i>	+			+	+	+	+			
<i>Fusarium solani</i>		+		+	+	+				
<i>Alternaria alternata</i>	+	+	+	+			+		+	+
<i>Mucor</i>				+						
<i>Rhizopus</i>	+		+							
<i>Uclodium</i> sp.									+	+

the herb (*Melissa officinalis* L and *Mentha piperita* L) growing area, and none of the examined samples were free of contaminants. In our experiment, the predominant pathogens were *Aspergillus niger* and *Fusarium* strains, *F. avanaceum* and *F. culmorum* (Fig. 1).

The above species colonized all of the examined herbs, which is a serious cause for concern because they produce toxic metabolites. At higher concentrations, aflatoxins and *Fusarium* toxins, in particular zearalenone and deoxynivalenol, can lead to intestinal inflammations, genetic code damage and liver cell damage [26]. In the long-term, the studied pathogens exert teratogenic effects. *Aspergillus flavus* and *Aspergillus niger* were also prevalent molds (23.6 %) in a study by Bugno et al. [27] who examined herbs from various regions of Brazil. Similar results were reported by Mandeel [28] who isolated *A. flavus* from all evaluated mixes of more than 50 plants and herbs.

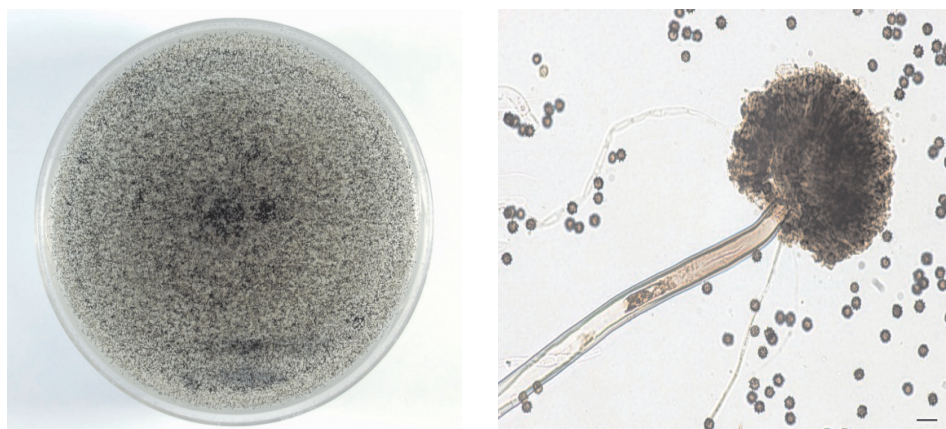


Fig. 1. *Aspergillus niger* isolated from basil, mint and lemon balm

In this experiment, the highest resistance against pathogens was observed in mint plants grown both in pots and in an organic farm. The above can be attributed to the presence of active substances in mint essential oil and various polyphenol compounds. Other authors have observed that mint extracts have bactericidal and fungicidal effects, and they inhibit the growth of fungi of the genera *Aspergillus*, *Penicillium* and *Fusarium* [29–31]. For this reason, fresh and dried mint as well as mint teas are least susceptible to colonization by toxin-producing fungi.

Basil is at the greatest risk of mold contamination. No significant differences in the number of pathogenic molds were observed between basil treatments. Basil grown in an organic farm was colonized by 10 mold strains, and plants from the remaining treatments – by 8 and 9 strains. The above indicates that the applied fungicides are ineffective and that molds have developed a resistance to active substances with fungicidal properties that have been in use for at least 10 years.

The bactericidal and fungicidal properties of active substances contained in basil essential oil have been studied in various research centers. Oxenham et al [32] demonstrated that basil essential oil inhibits the growth of *Botrytis fabae*. Koba et al [33] and Hussain et al [34] confirmed the bactericidal effects of basil oil on Gram-positive and Gram-negative bacteria, whereas basil extracts were relatively ineffective in combating fungi of the genus *Aspergillus* (MIC higher than 500 mg/dm³). Kocic-Tanackov et al found that ethanol and methanol extracts of basil exhibited fungicidal activity against laboratory-grown and naturally occurring fungal pathogens, but in their study, only two strains of each species were analyzed [35].

The results of analyses of dried herbs and herbal teas are presented in Table 2.

Similarly to fresh herbs, the prevalent pathogens colonizing dried herbs and herbal teas were *Aspergillus niger*, *F. avenaceum* and *F. culmorum*. Sage was most susceptible to contamination, whereas mint was most resistant to molds regardless of the brand and type of packaging. Zimowska demonstrated that sage is highly susceptible to colonization by various species of pathogenic fungi, and the majority of isolated strains belonged to the genus *Fusarium*, *Rhizoctonia solani* and *Phoma exigua* [36].

Table 2

Mold strains isolated from dried herbs and herbal teas

Strain	Sage	Sage leaf	Mint	Mint	Mint leaf	Lemon balm	Lemon balm	Lemon balm leaf
<i>Aspergillus fumigatus</i>	+	+				+	+	-
	+	+				-	+	+
	+	-				+	+	+
<i>Aspergillus niger</i>	+	+	+	+	+	+	+	+
	+	-	+	+	+	+	+	+
	+	+	+	+	+	+	+	+
<i>Aspergillus flavus</i>	+	-				-	+	
	+	+				-	+	
	+	+				+	+	
<i>Penicillium</i> spp.	+	+						
	-	+						
	-	-						
<i>Fusarium avenaceum</i>	+	+		+	+	+	+	+
	+	+		+	-	+	+	+
	+	+		+	-	+	+	+
<i>Fusarium culmorum</i>	+	+	+	+	+	+	+	+
	+	-	+	+	-	-	+	+
	+	+	-	+	-	+	+	+
<i>Fusarium oxysporum</i>	+	+				+	+	+
	-	-				+	+	+
	+	+				+	+	-
<i>Fusarium solani</i>	+	+				+	+	
	-	-				+	+	
	-	+				+	+	
<i>Alternaria alternate</i>	+	+	-	+			+	
	+	+	-	+			-	
	+	+	+	+			+	
<i>Rhizopus</i>		+						
		+						
		+						

Lemon balm was relatively resistant to fungal strains, and pathogenic colonization levels were particularly low in lemon balm leaves. The above could be attributed to the high quality of raw material and optimal storage conditions at the supplier's facility. The results of our study suggest that storage conditions (optimal temperature, stable humidity levels) and the quality of the production process largely affect contamination of the final product. Pokrzywa et al. analyzed mycotoxin contamination of foodstuffs, including dried herbs and spices. The presence of aflatoxins was determined due to contamination with fungi of the genus *Aspergillus* [16].

In view of the growing popularity of herbal remedies in Poland, dried herbal tablets have been introduced on the market for greater convenience. Tablets are easy to ingest,

and they eliminate the brewing process. The risk that spores will develop into active molds in herbal tablets is reduced, but only if high quality raw materials are used. In our study, lemon balm, mint, sage and lovage tablets were analyzed (Table 3).

Table 3

Mold strains isolated from herbal tablets

Strain	Mint	Lemon balm	Sage	Lovage
<i>Aspergillus fumigatus</i>	+	+	+	+
<i>Aspergillus niger</i>	+	+	+	+
<i>Penicillium</i> spp.			+	+
<i>Aspergillus flavus</i>			+	+
<i>Fusarium avanaceum</i>		+	+	+
<i>Fusarium culmorum</i>		+	+	+
<i>Fusarium oxysporum</i>		+	+	+
<i>Fusarium solani</i>			+	+
<i>Alternaria alternata</i>			+	+

The results indicate that mint was most resistant to fungal colonization due to the bactericidal and fungicidal properties of its active substances. Only the most prevalent strains of the genus *Aspergillus* were found in mint. Sage and lovage were colonized by 9 strains of pathogenic fungi.

Our findings demonstrate that plant material not contaminated by molds and their toxic metabolites is very difficult to acquire. The microbiological purity of fresh and dried herbs, herbal and black teas has been investigated by numerous authors, and the presence of mold fungi was reported in all studies. Mycotoxin levels determined in various research centers indicate that safe toxin levels are exceeded in only a small percentage of foodstuffs. However, the presence of toxic metabolites in food and the environment poses a health risk for consumers.

The results of our study indicate that fungal contamination of herbal products is a common problem. This issue can be addressed by changing farming practices, using advanced fungicides that inhibit the growth of various pathogens and storing herbal products in environments characterized by a constant temperature and humidity.

Conclusions

1. The analyzed fresh and dried herbs as well as herbal tablets were contaminated with toxin-producing fungi.
2. The predominant pathogens were fungi of the genera *Aspergillus* and *Fusarium* which colonized the majority of the analyzed samples.
3. Mint was characterized by the highest resistance to mold contamination, which can be attributed to the bactericidal and fungicidal properties of the active substances found in mint essential oil.

4. Foodstuff contamination with molds which produce mycotoxins harmful for humans and animals poses a serious epidemiological risk.

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Katedra Chemii, Wydział Nauk o Żywności i Biotechnologii
Uniwersytet Przyrodniczy w Lublinie

Abstrakt: W ostatnich latach nastąpił szybki rozwój rynku suplementów diety i powrót do preparatów ziołowych stosowanych w profilaktyce i w lecznictwie. Ma to swoje uzasadnienie tym, że zioła i rośliny lecznicze były od lat stosowane z powodzeniem w medycynie tradycyjnej. Używane w przemyśle farmaceutycznym wyciągi z roślin zawierają w swoim składzie stężenia substancji aktywnych wielokrotnie niższe od tych zawartych w samych roślinach. Gwarantuje to bezpieczeństwo długotrwałego stosowania bez konsultacji z lekarzem. Jednak zioła i inne rośliny o właściwościach leczniczych mają bardzo złożony skład i coraz częściej okazuje się, że mogą łatwo reagować w sposób niekorzystny ze składnikami żywności czy innymi farmaceutykami. Dodatkowo dużym zagrożeniem okazują się grzyby pleśniowe, głównie z rodzaju *Fusarium*, *Penicillium* i *Aspergillus*, które kolonizują rośliny w stanie naturalnego wzrostu, a w procesie suszenia i przetwarzania ze względu na swoją termofilność przechodzą do produktu gotowego. Są one szczególnie niebezpieczne, ponieważ mają zdolność wytwarzania toksycznych dla człowieka produktów przemiany materii, miktotoksyn.

Jak wynika z przeprowadzonych badań własnych, grzyby z rodzaju *Aspergillus* i *Fusarium* występują nie tylko na świeżych ziołach dostępnych w sprzedaży, ale również w herbatkach ziołowych oraz w ziołowych suplementach diety w postaci tabletek.

Słowa kluczowe: pleśń, zioła, suplementy diety, *Aspergillus*, *Fusarium*

