



# Mycobiota and mycotoxins in various kinds of vegetables and fruits as potential health risk factors for consumers – summary of a multiyear study

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## Abstract

Between 2015–2020, the concentrations and diversity of filamentous fungi as well as the levels of mycotoxins in 4 classes of vegetables and fruits were studied in samples from farms located in eastern Poland. The short communication summarizes the results with the use of statistical analysis and indicates the potential health hazards associated with the consumption of the produce contaminated with fungi and/or mycotoxins. The concentrations of filamentous fungi in all examined samples were moderate (2.813 – 4.146 log<sub>10</sub> CFU g<sup>-1</sup>). The highest values were noted in root vegetables, whereas the lowest values in fruit vegetables. The mycobiota revealed a marked biodiversity (67 species and 33 genera). *Penicillium* and *Fusarium* prevailed in the mycobiota of root vegetables, whereas *Cladosporium* and *Alternaria* in the remaining classes. Most of identified species (88.1%) were described as potentially pathogenic. The importance of mycotoxins as a potential health risk factor for vegetable consumers, was confirmed by the detection of a high prevalence (>55%) of total aflatoxin (AFT). The level of AFT in berry fruits was significantly higher than in other classes. The high prevalence (58.5%) of deoxynivalenol was noted in raspberries.

**Conclusion.** Filamentous fungi present in the examined vegetables and fruits may pose a potential health risk for consumers due to a high prevalence of potentially pathogenic species, mostly those producing mycotoxins.

## Key words

fruits, vegetables, filamentous fungi, aflatoxin, mycotoxin, deoxynivalenol

## INTRODUCTION

The great increase in the health-promoting vegetable and fruit consumption throughout this century has aroused interest in the microbiological safety of these products [1]. So far, most studies have focused on viruses, bacteria and protozoans that may cause outbreaks of acute gastrointestinal disease following consumption of infected produce, but much less attention has been paid to fungi [2]. Nevertheless, although fungi usually do not cause an acute disease, they represent a potential cause of chronic diseases following consumption of produce. This could be due mostly to mycotoxins, toxic secondary metabolites produced by many species of fungi, less often to allergic reactions or opportunistic infections [3, 4]. Considering these facts, a multiyear study was performed between 2015–2020 on the levels and diversity of filamentous fungi, and the levels of selected mycotoxins in various kinds of vegetables and fruits, consecutively: root (carrot and red beetroot) [5], leafy (lettuce and spinach) and fruit (tomato and red pepper) vegetables [6], and berry fruits (strawberry and raspberry) [7].

## OBJECTIVE

The aim of the present brief communication is to briefly summarize the results, compare the mycological results obtained in the above-mentioned 4 classes of vegetables and fruits with the use of statistical analysis, and to indicate the major potential health hazards associated with the consumption of the produce contaminated with fungi and/or mycotoxins.

## MATERIALS AND METHOD

The exact description of the samples and methods used has been published in earlier publications [5, 6, 7]. Briefly, a total of 418 samples of the fresh, symptomless vegetables and fruits were collected during 6 years (2015–2020) on farms located in the Lublin Province of eastern Poland. They included samples of root (carrots and beetroots), leafy (lettuce and spinach), and fruit (tomatoes and red pepper) vegetables, as well as berry fruits (strawberry and red raspberry). The concentration and species composition of filamentous fungi in homogenized samples was determined by culture, using the method of plate dilutions on malt agar. The concentration of mycotoxins, including total aflatoxin (AFT), aflatoxin B1 (AFB1) and deoxynivalenol (DON) were determined in all

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samples, except for root vegetables, by the immunoenzymatic ELISA method. The differences between the concentrations of fungi and mycotoxins in individual groups of vegetables and fruits were assessed by the Mann-Whitney non-parametric test.

## RESULTS

As shown in Table 1, presenting the concentrations of filamentous fungi in various classes of Polish vegetables and fruits, with the statistical differences between the classes, the concentrations of fungi ranged between 2.813–4.146 log<sub>10</sub> CFU g<sup>-1</sup>. The highest values were noted in the root vegetables which proved to be significantly higher compared to all other tested classes (P=0.000000). On the contrary, the lowest values were noted in the fruit vegetables and were significantly lower compared to all other studied classes (P=0.000000). No significant difference was found between the concentrations of fungi in leafy vegetables and berry fruits.

The data presented in Table 2 indicate that mycobiota isolated from the examined vegetables and fruits varied considerably. As many as 67 species belonging to 33 genera

were identified. Among them, 12 strains were identified to genus level only (Tab. 2). In root vegetables, the most common genera were *Penicillium* and *Fusarium*, whereas in the remaining 3 classes of vegetables and fruits, the genera of *Cladosporium* and *Alternaria* distinctly prevailed. *Penicillium* appeared to be a genus comprising the highest number of identified species (18), followed by *Aspergillus* (8) and *Fusarium* (7) (Tab. 2). The vast majority of identified species (59 out of 67; 88.1%) has already been described as potentially pathogenic [5, 6, 7]. Of these 59 species, 17 have been described as producers of mycotoxins that may cause a disorder described as ‘mycotoxicosis’, 13 as causative agents of infections, mostly opportunistic mycoses, 9 as causing mycotoxicoses and infection, 7 as causing mycotoxicoses and allergy, 6 as causing mycotoxicoses, infection and allergy, 5 as causing allergy only, and 2 as causing infection and allergy. From this summary, it is distinctly clear that mycotoxins produced by filamentous fungi present in vegetables and fruits pose a potential health risk for consumers, either alone or in conjunction with infectious and/or allergenic properties of these microorganisms (altogether 41 out of 59 species, i.e. 69.5%, were able to potentially act in that way).

Table 3 shows the concentrations and prevalence (percent

**Table 1.** Concentrations of filamentous fungi in various classes of Polish vegetables and fruits, and statistical differences between the classes

Class of vegetables or fruits	Root vegetables (carrot + beetroot) N=80	Leafy vegetables (lettuce + spinach) N=90	Fruit vegetables (tomato + red pepper) N=90	Berry fruits (strawberry + raspberry) N=158
Concentration of filamentous fungi (log <sub>10</sub> CFU g <sup>-1</sup> ) Median (range)	4.146 (3.000 – 6.190)	3.190 (0.000 – 4.462)	2.813 (0.000 – 3.778)	3.255 (2.000 – 4.430)
Statistical differences between concentrations of filamentous fungi in the individual classes of vegetables or fruits (Mann-Whitney test)	Root vs. leafy vegetables Z = 7.237 P = 0.000000 Difference highly significant	Root vs. fruit vegetables Z = 10.361 P = 0.000000 Difference highly significant	Root vegetables vs. berries Z = 9.542 P = 0.000000 Difference highly significant	
	Leafy vs. fruit vegetables Z = 6.930 P = 0.000000 Difference highly significant	Leafy vegetables vs. berries Z = -0.846 P = 0.397 Difference not significant	Fruit vegetables vs. berries Z = -8.431 P = 0.000000 Difference highly significant	

**Table 2.** Diversity and potential pathogenicity of filamentous fungi present in vegetables and fruits

	Root vegetables (carrot + beetroot) N=80*	Leafy vegetables (lettuce + spinach) N=90	Fruit vegetables (tomato + red pepper) N=90	Berry fruit (raspberry + strawberry) N=158	Total N=418
Number of isolated species	29	30	25	36	67
Number of isolated genera	15	18	13	23	33*
Prevailing genera (percent of total isolates)	<i>Penicillium</i> (39.5%) <i>Fusarium</i> (14.1%)	<i>Alternaria</i> (24.3%) <i>Cladosporium</i> (21.6%) <i>Fusarium</i> (10.4%)	<i>Cladosporium</i> (39.1%) <i>Alternaria</i> (16.9%)	<i>Cladosporium</i> (60.2%)	<i>Cladosporium</i> (33.7%) <i>Penicillium</i> (11.5%) <i>Alternaria</i> (7.8%) <i>Fusarium</i> (5.9%)
Genera with the highest number of identified species <sup>‡</sup>	<i>Penicillium</i> : 11 <i>Fusarium</i> : 4	<i>Penicillium</i> : 5 <i>Alternaria</i> : 4 <i>Aspergillus</i> : 4	<i>Penicillium</i> : 5 <i>Fusarium</i> : 4	<i>Penicillium</i> : 6 <i>Fusarium</i> : 6 <i>Aspergillus</i> : 4	<i>Penicillium</i> : 18 <i>Aspergillus</i> : 8 <i>Fusarium</i> : 7 <i>Alternaria</i> : 5 <i>Cladosporium</i> : 4
Potentially pathogenic species (number, percent of total species identified)	22 (75.9%)	28 (93.3%)	24 (96.0%)	34 (94.4%)	59 (88.1%)
Potential pathogenicity <sup>‡</sup>	M: 8, I: 6, A: 3 M+I: 4, M+A: 1 I+A: 0, M+I+A: 0	M: 5, I: 4, A: 1 M+I: 5, M+A: 4 I+A: 3, M+I+A: 6	M: 5, I: 3, A: 1 M+I: 5, M+A: 2 I+A: 3, M+I+A: 5	M: 6, I: 7, A: 0 M+I: 10, M+A: 3 I+A: 5, M+I+A: 3	M: 17, I: 13, A: 5 M+I: 9, M+A: 7 I+A: 2, M+I+A: 6

\* Results for rhizosphere soil published in a former article [5] have not been included; <sup>‡</sup> 12 strains have been identified to genus level only; <sup>§</sup> genera with the number of species ≥4 were shown; <sup>‡</sup> conditions that could be potentially evoked by fungal species found on specific vegetables or fruits are denoted as follows: M – solely mycotoxicoses, I – solely infections (mycoses), A – allergy, M+I – mycotoxicoses + infections, M+A – mycotoxicoses + allergy, I+A – infection + allergy, M+I+A – mycotoxicoses + infection + allergy

**Table 3.** Concentrations of mycotoxins in various classes of Polish vegetables and fruits, and statistical differences between the classes

Class of vegetables or fruits	Leafy vegetables (lettuce + spinach) N=90	Fruit vegetables (tomato + red pepper) N=90	Berry fruits (strawberry + raspberry) N=78
Concentration of total aflatoxins ( $\mu\text{g kg}^{-1}$ ) Median (range)	1.221 (<LOD – 7.848) Prevalence = 70.0%	0.393 (<LOD – 3.295) Prevalence = 58.9%	1.981 (<LOD – 3.411) Prevalence = 71.8 %
Statistical differences between concentrations of total aflatoxins in the individual classes of vegetables or fruits (Mann-Whitney test)	Leafy vs. fruit vegetables Z = 3.588 P = 0.00033 Difference highly significant	Leafy vegetables vs. berries Z = - 2.079 P = 0.0376 Difference significant	Fruit vegetables vs. berries Z = - 5.669 P = 0.000000 Difference highly significant
Concentration of aflatoxin B1 ( $\mu\text{g kg}^{-1}$ ) * Median (range)	0.280 (<LOD – 0.778) Prevalence = 58.9%	<LOD (<LOD – 0.329) Prevalence = 18.0%	<LOD Prevalence = 0.00
Statistical differences between concentrations of aflatoxin B1 in the individual classes of vegetables or fruits (Mann-Whitney test)	Leafy vs. fruit vegetables Z = 5.481 P = 0.000000 Difference highly significant	Leafy vegetables vs. berries Z = 8.960 P = 0.000000 Difference highly significant	Fruit vegetables vs. berries Z = 3.867 P = 0.00011 Difference highly significant
Concentration of deoxynivalenol ( $\mu\text{g kg}^{-1}$ ) Median (range)	<LOD (<LOD – 627.0) Prevalence = 2.2%	<LOD Prevalence = 0.00	<LOD (<LOD – 1060.0) Prevalence = 30.8%
Statistical differences between concentrations of deoxynivalenol in the individual classes of vegetables or fruits (Mann-Whitney test)	Leafy vs. fruit vegetables Z = 1.418 P = 0.156 Difference not significant	Leafy vegetables vs. berries Z = - 5.078 P = 0.000000 Difference highly significant	Fruit vegetables vs. berries Z = - 5.645 P = 0.000000 Difference highly significant

LOD - limit of detection, \*50 samples each of leafy and fruit vegetables were tested

of positive samples) of selected mycotoxins in the individual groups of vegetables and fruits: total aflatoxins (AFT), aflatoxin B1 (AFB1) and deoxynivalenol (DON). AFT occurred in the tested samples with a relatively high prevalence, exceeding 50% in all groups, and moderate concentrations ranging from <LOD-7.848  $\mu\text{g kg}^{-1}$ . Their concentrations in leafy vegetables were significantly higher compared to fruit vegetables, but significantly lower compared to berry fruits. As regards AFB1, both the prevalence and concentration of this mycotoxin was significantly higher in leafy vegetables compared to fruit, vegetables and to berry fruits in which it was not detected (Tab. 3). The prevalence of DON in leafy vegetables was very low, whereas in fruit vegetables this mycotoxin was not detected at all. In contrast, both the average prevalence and concentration of this mycotoxin in berry fruits was relatively high, and significantly greater compared to leafy and fruit vegetables. However, these results were due to a high prevalence and concentration of DON in raspberry fruits (58.5% and <LOD-1060.0  $\mu\text{g kg}^{-1}$ , respectively), while in strawberry fruits this mycotoxin was not detected [7].

## DISCUSSION

As no widely accepted values exist for permissible concentrations of filamentous fungi in vegetables and/or fruits, the levels of fungi found and assessed in this study, averaging between 2.5–4.5  $\log_{10}$  CFU  $\text{g}^{-1}$ , were compared with the results obtained in other studies. In this respect, the results obtained in the current study were similar to those obtained in the USA [8], Iran [9], Turkey [10] and Poland [11], but lower compared to results from Venezuela [12] and Italy [13], and generally were close to the concentrations reported by other authors. As shown above, the significantly highest concentrations of filamentous fungi were found in root vegetables. The ratios of the median fungal concentration in root vegetables to the concentrations in other examined

classes of vegetables and fruits, e.g. berry fruits, leafy, and fruit vegetables (all expressed in linear scale) were 8:1, 9:1 and 22:1, respectively. These discrepancies could be explained by the differences in the environment surrounding the edible parts of examined plants. In the case of root vegetables, the edible roots were surrounded by soil, which contains the organic and inorganic compounds enabling reproduction of filamentous fungi, as well as water enabling the diffusion of fungal conidia and/or mycelium fragments through the vegetable epidermis [14]. As evidenced in the current and several other studies, the main fungi penetrating into root vegetables are the *Penicillium* and *Fusarium* species commonly present in soil and posing a risk for consumers as mycotoxin producers [5]. The limitation of this study is the lack of determination of the mycotoxin concentrations in root vegetables to support this thesis. The authors intend to fill this gap in the future.

In turn, the edible parts of other vegetables and fruits grew above the soil, without being surrounded by anything else; hence, the concentrations of fungi were distinctly lower compared to root vegetables. The atmospheric air serves only as a medium for transporting fungal conidia and hyphae, of which the most numerous were 'field fungi' of plant origin belonging to the genera *Cladosporium* and *Alternaria* and exhibiting allergenic properties [15, 16], which are easily deposited on the surfaces of many edible fruits and vegetables and after the penetration develop in their tissues. It is noteworthy that the *Alternaria* species produce about 70 mycotoxins revealing strong toxic properties [17].

Although the concentrations of filamentous fungi in the examined vegetables and fruits do not by themselves pose a health hazard for consumers, a much greater risk is associated with the marked biodiversity of mycobiota in this particular ecological niche. Nearly 90% of the species identified in this study could be classified as potentially pathogenic, mostly as producers of mycotoxins. Mycotoxins may exhibit, at favourable external conditions, nephrotoxic, genotoxic,

teratogenic, carcinogenic, and cytotoxic properties, and, as a consequence, may cause liver carcinomas, renal dysfunctions, and immunosuppressed states [18, 19]. Immunocompromised persons and those with concomitant chronic diseases are most at risk.

The potential risk from mycotoxins based on mycological characteristics was confirmed by the detection of mycotoxins in the examined vegetables and fruits with an immunoenzymatic method. A special risk is associated with the constant presence of the total aflatoxin (the sum of aflatoxins B1, B2, G1, G2, known as remarkably hazardous) in all classes of the examined vegetables and fruits. Although the levels of the total aflatoxins recorded in this study can be generally classified as moderate, in 8 samples of lettuce and spinach the maximal allowable concentration of  $4.0 \mu\text{g kg}^{-1}$ , proposed by the European Union (EU) [20] for dried fruits, was exceeded. The prevalence of very hazardous aflatoxin B1 was high in leafy vegetables (58.9%), but in this case a maximal concentration proposed by the EU ( $2.0 \mu\text{g kg}^{-1}$ ) was not exceeded [6]. The prevalence of the trichothecene toxin DON in leafy and fruit vegetables, as well as in strawberries, was low or none. Unexpectedly, a high prevalence (58.5%) and a high median concentration ( $242.0 \mu\text{g kg}^{-1}$ ) of DON was noted in raspberry. In 7 out of 41 samples of raspberry fruits (17.0%), the DON concentration exceeded the maximal allowable value ( $750.0 \mu\text{g kg}^{-1}$ ) proposed by the EU [20]. To the best of the authors' knowledge, this is the first report indicating raspberry as a potential source of mycotoxins.

Besides mycotoxin production, fungi isolated in the course of this study may also reveal, either alone or in conjunction with other pathogenic properties, infectious properties (altogether in 30 out of 59 species; 50.8%) and allergenic (altogether in 20 out of 59 species; 33.9%). Among allergenic fungi, the most important are *Cladosporium* and *Alternaria* species, prevailing in vegetables and berries growing above the soil surface. The majority of fungal species revealing infectious properties were described as opportunistic pathogens (altogether 25 out of 30 species; 83.3%). Fungi isolated from vegetables and fruits were described most often as a cause of cutaneous and/or ocular mycoses [6,7].

The risk of food allergy symptoms after consumption of vegetables and/or fruits contaminated with allergenic fungi, mainly from the *Alternaria* and *Cladosporium* genera, could be considerably increased by the preceding sensitization to this fungi by the respiratory route. Because of similar allergenic determinants and IgE-mediated mechanisms causing both types of allergy, consumption of even low doses of allergenic fungi may trigger gastrointestinal or respiratory symptoms in persons previously sensitized (subclinically or clinically) to these fungi by inhalation [21, 22].

## CONCLUSIONS

1. The concentrations of filamentous fungi in 4 classes of vegetables and fruits were moderate, ranging between  $2.813\text{--}4.146 \log_{10} \text{CFU g}^{-1}$ . The higher values compared to the remaining 3 classes were noted in the root vegetables, whereas the values smaller compared to the remaining 3 classes were noted in fruit vegetables. In all cases, the differences proved to be highly significant ( $P=0.000000$ ).
2. The mycobiota of examined vegetables and fruits revealed

a marked biodiversity shown by the presence of 67 species from 33 genera. *Penicillium* and *Fusarium* prevailed in the mycobiota of root vegetables, whereas *Cladosporium* and *Alternaria* in the remaining classes. The vast majority of identified species (88.1%) have been described in earlier studies as potentially pathogenic, mostly as mycotoxin producers.

3. The importance of mycotoxins as a potential health risk factor for vegetable consumers has been confirmed by the detection of the high prevalence (>55%) of total aflatoxins in all classes of the examined vegetables and fruits. The level of total aflatoxins in berry fruits was significantly higher than in other classes ( $P<0.05$ ), whereas in 8.9% of the samples from leafy vegetables the exceeding of the maximal value of  $4.0 \mu\text{g kg}^{-1}$  proposed by the European Union (EU) was noted. In raspberries, a high prevalence (58.5%) of deoxynivalenol (DON) was noted, exceeding the maximal value proposed by the EU ( $750 \mu\text{g kg}^{-1}$ ) in 17% of the studied samples.
4. In spite of moderate levels, filamentous fungi present in the examined vegetables and fruits may pose a health risk for consumers because of the high prevalence of potentially pathogenic species, mostly those producing mycotoxins. The high-risk groups include persons with decreased immunity and/or chronic diseases.

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