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TOXIN PRODUCING MICROMYCETES OF THE GENUS *PENICILLIUM* AND *ASPERGILLUS* ON BERRIES, GRAPES, AND TOMATO FRUITS IN SLOVAK STORES

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ABSTRACT

In this study, contamination by microscopic fungi (focused on *Penicillium* and *Aspergillus* species) of 75 fruit samples (strawberries, blackberries, blueberries, raspberries, grapes) and 29 tomato samples, which were obtained from Slovak stores was investigated. Totally 93.3% of fruit samples were contaminated with micromycetes (70.6% *Botrytis* spp., 52.0% *Cladosporium* spp., 42.7% *Penicillium* spp., 13.3% *Rhizopus* spp., 8.0% *Alternaria* spp., 6.7% *Mucor* spp., 5.3% *Aspergillus* spp., 4.0% *Fusarium* spp., and 1.3% *Epicoccum* spp.) and 6.7% of samples were contaminated with yeasts. The presence of 15 species of the genus *Penicillium* in fruits, namely *P. atramentosum*, *P. aurantiogriseum*, *P. brevicompactum*, *P. bialowiezense*, *P. citrinum*, *P. crustosum*, *P. digitatum*, *P. expansum*, *P. fellutanum*, *P. glabrum*, *P. hordei*, *P. italicum*, *P. kiamense*, *P. olsonii*, and *P. purpurescens* was found. With the highest frequency, *P. brevicompactum* (12.0%) and *P. expansum* (10.7%) were isolated from fruits. Four isolates of the genus *Aspergillus* were isolated from fruit samples (*A.* section *Clavati*, *A.* section *Flavi*, and two isolates of *A.* section *Nigri*). Thin layer chromatography (TLC) was used for testing of mycotoxin production by selected fungal isolates. Overall, 93.3% of tested *Penicillium* isolates and one tested *Aspergillus* of section *Clavati* proved the ability to produce at least one of the tested mycotoxins. As for tomato samples, 93.1% were contaminated with microscopic filamentous fungi (79.3% *Penicillium* spp., 24.1% *Botrytis* spp. and *Alternaria* spp., 10.3% *Cladosporium* spp., *Mucor* spp. and *Rhizopus* spp., 6.9% *Aspergillus* spp. and 3.4% *Fusarium* spp.) and 6.9% samples were contaminated with yeasts. In 65.5% of tomato samples, the occurrence of 8 species of the genus *Penicillium* (*P. brevicompactum*, *P. crustosum*, *P. expansum*, *P. griseofulvum*, *P. italicum*, *P. olsonii*, *P. sublectaticum*, *P. thomii*), and two species of the genus *Aspergillus* (from section *Circumdati* and *Flavi*) was recorded. *P. olsonii* (58.6%) and *P. griseofulvum* (10.3%) were isolated with the highest frequency. All 7 tested *Penicillium* isolates (100.0%) and 1 isolate of the genus *Aspergillus* (section *Flavi*), which were obtained from tomatoes, had the ability to produce at least one of the tested mycotoxins. *Aspergillus* section *Flavi* isolate (from tomatoes of Slovak origin) produced 5.5 $\mu g.mL^{-1}$ of aflatoxin B_1, 4.2 $\mu g.mL^{-1}$ of aflatoxin B_2, 154.4 $\mu g.mL^{-1}$ of aflatoxin G_1, and 5.6 $\mu g.mL^{-1}$ of aflatoxin G_2 on 14th day of cultivation on liquid YES medium at 25±1 °C in darkness, and detected by high pressure liquid chromatography (HPLC).

Keywords: fruit, tomato, *Penicillium*, *Aspergillus*, mycotoxins, microscopic fungi, TLC, HPLC

INTRODUCTION

Fruits and vegetables have a high nutritional value, and they help to prevent diseases (e.g., cancer, cardiovascular, eye diseases...) **(Mritunjay *et* Kumar, 2015)**. For its nutrition, humans can use several plant origin commodities grown locally, as well as those that are imported from different countries. These products diversify the daily diet, making it healthier and more balanced. These commodities of plant origin mainly include fruits and vegetables. Fruits and vegetables are grown on different types of soils, using various agrotechnical and agrochemical means. However, some conditions promote the development of different groups of microorganisms that contaminate these commodities already during growth, maturation and harvesting **(Carlile *et* Watkinson, 1994)**. Fruits and vegetables are an easily accessible substrate for microorganisms and are often degraded by their activities to such an extent that they are unsuitable for consumption and associated with considerable economic losses **(Lugauskas *et* Stakėnienė, 2002)**. It is estimated that about 20% of all fruits and vegetables produced is lost each year due to spoilage **(Barth *et al.*, 2009)**. Some authors state, that 30 to 50% of stored fruits and vegetables are spoiled by the activity of microorganisms **(Bautista-Baños, 2014)**. Many fruits and vegetables present nearly ideal conditions for the survival and growth of many types of microorganisms **(Miedes *et* Lorences, 2004)**. The fruit contains a lot of sugars and other nutrients and has an ideal water activity for microbial growth. The low pH makes it especially sensitive to spoilage by fungi, since much of the bacterial competition that prefers an almost neutral pH, is eliminated **(Tournas *et* Katsoudas, 2005)**. Microscopic filamentous fungi produce large amounts of extracellular pectinases and hemicellulases, which are important factors influencing the deterioration of fruits and vegetables **(Tančinová *et al.*, 2022)**. Due to its composition and pH, vegetables are an ideal breeding ground for microorganisms. Tomatoes with a pH from 4.2 to 4.5 are prone to rotting caused mainly by micromycetes of the genera *Alternaria*, *Cladosporium*, *Rhizopus*, *Mucor* **(Pitt *et* Hocking, 2009)** and *Penicillium* **(Samson *et al.*, 2019)**. Active contaminants of fruits and vegetables are micromycetes that contaminate these commodities during harvest and during storage **(Lugauskas *et al.*, 2001; Tournas *et* Katsoudas, 2005)**. Contamination of fruits by fungi not only causes high post-harvest losses, but fruits contaminated by microscopic fungi can also be a source of toxic substances harmful to humans **(De Vries *et al.*, 2002)**. Vegetables and fruits damaged by micromycetes turn into a watery mass. Brownish or grey spots appear on the skin of the fruits, and the mycelium of the fungus gradually penetrates deeper of tissues. Some micromycetes cause dry rot, in which part of the vegetables or fruits damaged by fungi gradually dry out **(Lugauskas *et al.*, 2005)**. Micromycetes belonging to the genera *Aspergillus*, *Penicillium* **(Stakeniene *et al.*, 2001)**, and *Alternaria* are major contributors to fruit decomposition and are also producers of mycotoxins during the different stages of fruits pathogenesis **(Barkai-Golan *et* Paster, 2008)**. Mycotoxins are toxic secondary metabolites produced by fungi **(Coulombe, 1993; Stakeniene, *et al.*, 2001).** The mycotoxins commonly associated with fruits and vegetables and with products made from them include patulin, aflatoxins, toxins of the genus *Alternaria* and ochratoxin A **(Jackson *et* Al-Taher, 2008; Barkai-Golan *et* Paster, 2008)**. Most mycotoxins are highly stable during the processing of fruits into fruit products; therefore, they can reach the consumer. Although consumers reject visibly rotten fruits, processed fruit products can still constitute a significant source of these mycotoxins and pose a serious threat to human and animal health worldwide **(Barkai-Golan *et* Paster, 2008)**. In contaminated fruits, the simultaneous appearance of various mycotoxins is often present **(Barkai-Golan, 2008)**.

The objective of our study was to detect micromycetes (with an emphasis on *Aspergillus* and *Penicillium* spp.), which are responsible for the decay of fruits

and tomatoes obtained from Slovak stores, and to test representative fungal isolates for their ability to produce mycotoxins.

MATERIAL AND METHODS

Samples information

In the study, the occurrence of microscopic fungi (focused on the *Penicillium* and *Aspergillus* species) in 75 fruit (raspberries 18, blackberries 6, strawberries 26, blueberries 16, grapes 9) and in 29 tomato samples, which were visibly contaminated with microscopic fungi (mouldy), was monitored. Fruit and tomato samples came from various countries (Morocco, Spain, Italy, Tunisia, Egypt, Peru, Chile, Poland, Belgium), and from Slovakia, as well. Samples were obtained from different Slovak stores during the period 2021 and 2022 and mycologically investigated.

Mycological analysis

The mycelium of micromycetes, which was grown on the berries, grapes, and tomatoes, was inoculated on MEA (malt extract agar; **Samson *et al.*, 2002**). Cultivation was performed at a temperature 25±1 °C for 7 days in the darkness. Identification was carried out based on an assessment of the micro- and macromorphological features of micromycetes. For species identification of *Penicillium* isolates MEA, CYA (Czapek yeast extract agar; **Pitt 1979**), YES (yeast extract sucrose agar; **Samson *et* Frisvad, 2004**) and CREA (creatine sucrose agar; **Frisvad, 1985**) was used. MEA and CYA were used as an identification media for the *Aspergillus* isolates, as well. Isolates were cultivated for 7 days in the darkness at a temperature 25±1 °C. Publications used for identification of *Penicillium* species were Pitt *et* Hocking (2009), Samson *et* Frisvad (2004), Samson *et al.* (2002, 2010, 2019), Visagie *et al.* (2014) and for identification of *Aspergillus* species were **Klich (2002)**, **Samson *et* Varga (2009)**, **Houbraken *et al.* (2007)**, **Samson *et al.* (2009, 2014)**, **Varga *et al.* (2011)**, **Visagie *et al.* (2014)**. Microscopes Olympus CX21FS1 and Olympus BX51TF with Olympus Nomarski DIC for higher contrast were used for microscopic observation. Microscopic preparations were prepared in lactic acid with cotton blue (**Tančinová *et al.*, 2012)**. For evaluating the results of the fungal occurrence, the indicator "frequency" (Fr, the proportion of samples in which a given species/section occurred at least once) was used.

TLC analysis

Isolates of potentially toxigenic species of the *Penicillium* and *Aspergillus* genera were tested by TLC (thin-layer chromatography) **(Samson *et al.*, 2002** modified by **Labuda *et* Tančinová, 2006**) for the ability to produce selected mycotoxins. Cultivation for screening of patulin, citrinin, ochratoxin A, and aflatoxins (B_1, B_2, G_1, G_2) was realized on YES and for screening of penitrem A, roquefortine C and cyclopiazonic acid on CYA for 14 days in the darkness at 25±1 °C. Three plugs with mycelium and agar (0.5 x 0.5 cm) were added in Eppendorf tubes (1.5 mL). For extraction 500 µL of chloroform/methanol (2/1, vol/vol) was used and samples were 5 min mixed by vortex (Vortex Genie® 2, MO BIO Laboratories, Inc. – Carlsbad, CA). Totally 30 – 50 µL of extracts and of reference standards were applied on TLC plates (20 x 20 cm, Alugram® SIL G, Macherey – Nagel, Germany). As an eluent system TEF solution (toluene/ethyl acetate/formic acid, 5/4/1, vol/vol/vol) was used.
The visualization of patulin was performed by spraying the TLC plate with 0.5 % MBTH solution (3-methyl-2-benzothiazolinone hydrazone hydrochloride in methanol) and then heating at 130 °C for 8 min (yellow-orange spot) and the visualization of penitrem A was done by applying 20% $AlCl_3$ (20% in 60% ethanol) and then heating at 130 °C for 8 min (blackish blue spot). The visualization of roquefortine C was realized applying $Ce(SO_4)_2$ (1% in 50% sulphuric acid) on the TLC plate (orange spot), while the visualization of cyclopiazonic acid was performed applying of Ehrlich reagent on the plate and then heating at 130 °C for 8 min (violet spot with a tail). Aflatoxins, ochratoxin A and citrinin were examined in UV light (366 nm) (aflatoxins B_1 and B_2 as blue spots, aflatoxins G_1 and G_2 as green spots, ochratoxin A as a greenish blue spot, and citrinin as a yellow-green spot with a tail).

HPLC analysis

The amount of aflatoxins produced by the isolate of *Aspergillus* section *Flavi* (*A. parasiticus* KMi1040, NR121219.1) was quantified by HPLC-FLD (high-performance liquid chromatography with FLD detector) according to **Jakabová *et al.* (2022)**. Measurements were performed on the device Agilent 1260 Infinity (Agilent Technologies, Palo Alto, USA). For the separation, the column Eclipse XDB-C18 (3.5 µm, 3.0×150 mm, Agilent, USA) was used. The column temperature was set at 40 °C. The isocratic mode was applied with a composition of the mobile phase water/methanol (60/40, vol/vol). The injected volume was 10 µL and the flow rate was 1 $mL.min^{-1}$. Excitation wavelength was 362 nm and emission wavelength was 455 nm. The LOD (limit of detection) was 0.03 $\mu g.mL^{-1}$.

The *Aspergillus* section *Flavi* isolate was isolated from cherry tomatoes of Slovak origin and production of aflatoxins was firstly confirmed by TLC method. The isolate was cultivated in 40 mL of liquid YES medium (in 50 mL centrifuge tubes, VWR International) in 3 repetitions. Cultivation was carried out on Orbital Shaker PSU-10i (Biosan) at 220 RPM. For inoculation of media 1 mL of spore suspension in Tween 80 (0.5%) was used. Concentration of spores was $2.0x10^8.mL^{-1}$. The inoculated media were incubated at 25±1 °C during 14 days in darkness.
On the 14th day of cultivation, 20 mL of the liquid medium were taken into 50 mL centrifuge tubes and centrifuged (Rotina 420 centrifuge, Hettich Zentrifugen, Tuttlingen, Germany) for 3 minutes at a speed of 5,000 × g. Consequently, 2 mL of the centrifuged liquid medium were filtered through a 25 mm Nylon syringe filter (0.2 µm, Agilent Captiva) into microtubes (1.5 ml, Eppendorf). The filtrate (500 µL) was extracted by adding 500 µL ethyl acetate (Fisher Scientific, Leics, UK) and mixed for 5 minutes using an IKA MS 3 digital vortex (IKA-Werke GmbH & Co., Staufen, Germany). The prepared extract (100 µL) was added into HPLC vials (2 ml, Agilent) and then dried. Before HPLC analysis, the extract was reconstructed by adding 500 µL of methanol/water solution (40/60, vol/vol).

RESULTS AND DISCUSSION

Berries and grapes

Out of a total of 75 analysed berries and grape samples, 70 samples (93.3%) were contaminated with microscopic filamentous fungi and 5 samples (6.7%) with yeasts. Overall, 53 samples (70.6%) were contaminated with microscopic fungi of the genus *Botrytis*, 39 samples (52.0%) with micromycetes of the genus *Cladosporium*, 30 samples (42.7%) with microscopis fungi of the genus *Penicillium*, 10 samples (13.3%) with species of the genus *Rhizopus*, 6 samples (8.0%) with species of the genus *Alternaria*, 5 samples (6.7%) with species of the genus *Mucor*, 4 samples (5.3%) with microscopic fungi of the genus *Aspergillus*, three samples (4.0%) with the species of the genus *Fusarium* and one sample (1.3%) with the fungi of the genus *Epicoccum*. The overall fungal contamination of tested berries (Fig. 1) and grapes is summarized in Table 1. Totally 164 isolates of microscopic filamentous fungi were isolated from berries and grapes. **Tournas *et* Katsoudas (2005)** reported that 100.0% of blackberry and raspberry, 97.0% of strawberry and 95.0% of blueberry samples showed some sort of fungal contamination. Samples were purchased from local supermarkets in the Washington, DC area. In our study 100.0% of strawberry and blueberry, 94.0% of raspberry and 66,7% of blackberry samples were contaminated with filamentous micromycetes.

Table 1 Isolation frequency of micromycetes on berries and grapes

Fruit	Total no. of samples	Genus	Contaminated samples (%)
Strawberries	26	*Botrytis*	92.8
		Penicillium	42.3
		Cladosporium	30.8
		Rhizopus	26.9
		Mucor	11.5
		Fusarium	3.8
Raspberries	18	*Cladosporium*	83.3
		Botrytis	61.1
		Penicillium	50.0
		Mucor	11.1
		Rhizopus	5.6
		Fusarium	5.6
Blackberries	6	*Botrytis*	33.3
		Cladosporium	33.3
		Penicillium	16.6
Blueberries	16	*Botrytis*	93.8
		Penicillium	37.5
		Cladosporium	37.5
		Alternaria	31.3
		Aspergillus	12.5
		Fusarium	6.3
		Epicoccum	6.3
Grapes	9	*Penicillium*	55.6
		Botrytis	44.4
		Rhizopus	33.3
		Cladosporium	22.2
		Aspergillus	22.2
		Alternaria	11.1

Tournas *et* Katsoudas (2005) confirmed that the most common fungi found in berries in their study were *Botrytis cinerea*, *Alternaria*, *Cladosporium*, *Penicillium*, *Fusarium*, and *Rhizopus*, what matches with our results. *B. cinerea*

was by far the most common spoiler of berries in study of **Tournas *et* Katsoudas (2005)** and **Pitt *et* Hocking (2009)** stated, that *Rhizopus stolonifer* was the cause of major rot in berries. **Barth *et al.* (2009)** mentioned as an important pathogens of berries micromycetes of the *Penicillium*, *Botrytis*, *Colletotrichum*, *Monillia*, *Phytophthora*, and *Mucor* genera, in the United States. **Lugauskas *et* Stakėnienė (2002)** stated, that the micromycetes of the *Penicillium*, *Aspergillus*, *Mucor*, and *Rhizopus* genera were dominated on fruit, berries, vegetables, and food articles of plant origin grown in Lithuania, as well. Rotting and spoilage of grape berries before harvest can be caused by a variety of fungal species. Molds that contaminate wine grapes include species of *Botrytis*, *Penicillium*, *Aspergillus*, *Mucor*, *Rhizopus*, *Cladosporium*, *Alternaria*, *Uncinula*, and *Plasmopara* genera. *Botrytis cinerea* is regarded as the most serious cause of spoilage in grapes **(Doneche, 1993)**.

Figure 1 Contamination of berries by microscopic filamentous fungi

Barkai-Golan *et* Paster (2008) reported that several *Penicillium* species are highly prevalent agents of postharvest diseases, and they attack a wide range of fruits and vegetables. We isolated 38 isolates of *Penicillium* genus and 4 isolates of *Aspergillus* genus from 75 samples of fruits. We recorded the presence of 15 species of the genus *Penicillium* in berries and grapes (Tab. 2): *P. atramentosum*, *P. aurantiogriseum*, *P. brevicompactum*, *P. bialowiezense*, *P. citrinum*, *P. crustosum*, *P. digitatum*, *P. expansum*, *P. fellutanum*, *P. glabrum*, *P. hordei*, *P. italicum*, *P. kiamense*, *P. olsonii*, and *P. purpurescens*. *P. brevicompactum* (12.0%) and *P. expansum* (10.7%) were isolated with the highest Fr. **Barkai-Golan (2008)** stated that *P. brevicompactum* was found on ginger rootand was responsible for production of the mycotoxin mycophenolic acid. *P. expansum* is the main reason for development of so-called, "blue rot" of apples, pears of plums, peaches, apricots, cherries, blackberries, melons, and strawberries **(Snowdon, 1990)** and is commonly found on several vegetables such as onions, garlic, and cabbage **(Lugauskas *et al.*, 2005)**. *P. expansum* species produces various mycotoxins, mainly patulin. Human exposure to patulin is primarily via apple-based products, coming from fruit infection by *Penicillium expansum*. Attention has been drawn especially to patulin contamination in infant apple products, and in organic fruits versus conventional ones **(Barkai-Golan *et* Paster, 2008)**. **Tournas *et* Katsoudas (2005)** stated that species of the genus *Penicillium* were present in all types of berries tested, which is in an agreement with our results.

We found four isolates belonging to the genus *Aspergillus* from 75 berries and grape samples: one isolate of *A.* section *Clavati* (blueberry sample), two isolates of aspergilli section *Nigri* (two grape samples), and one of section *Flavi* (blueberry sample). *Aspergillus* species are widespread in nature. These saprophic species can be found on a wide range of substrates, including food and feeds, and several species are typical pathogens of harvested fruits and vegetables **(Barkai-Golan, 2001)** and they are major producers of mycotoxins. The black aspergilli (*Aspergillus* section *Nigri*) are an important group in food and medical mycology and biotechnology **(Varga *et al.*, 2000)**. *A. niger* (species of the *A.* section *Nigri*) is by far the most common *Aspergillus* species responsible for postharvest decay of fresh fruit, including apples, pears, peaches, citrus, grapes, figs, strawberries, mangoes, and melons **(Snowdon, 1990)**. *A. niger* has also been reported to cause serious losses in tomatoes in Sokoto, in northwestern Nigeria **(Muhammad *et al.*, 2004)**. Black aspergilli are potential producers of ochratoxin A and cyclopiazonic acid. Aspergilli from section *Flavi* are important producers of aflatoxins, cyclopiazonic acid, mycophenolic acid, and other mycotoxins **(Frisvad *et al.*, 2019)**.

Table 2 Spectrum of micromycetes of the *Penicillium* and *Aspergillus* genera isolated from berries and grapes

Species	Sample	Fr (%)	Species/Section	Sample	Fr (%)
P. atramentosum	strawberries (1*)	1.3	*P. glabrum*	raspberries (1	1.3
P. aurantiogriseum	strawberries (1*)	1.3	*P. hordei*	strawberries (1*)	1.3
P. brevicompactum	blackberries (1*), raspberries (4*), grapes (2*), strawberries (2*)	12.0	*P. italicum*	grapes (2*)	2.7
P. bialowiezense	raspberries (1*)	1.3	*P. kiamense*	raspberries (1	1.3
P. citrinum	raspberries (2*), grapes (1*)	4.0	*P. olsonii*	strawberries (2*)	2.7
P. crustosum	strawberries (1*), blueberries (1*)	2.7	*P. purpurescens*	raspberries (1*	1.3
P. digitatum	strawberries (2*), raspberries (1*)	4.0	*A. sec. Clavati*	blueberries (1*	1.3
P. expansum	blueberries (5*), raspberries (1*), strawberries (2*)	10.7	*A.* sec. *Flavi*	blueberries (1*)	1.3
P. fellutanum	strawberries (1*)	1.3	*A.* sec. *Nigri*	grapes (2*)	2.7

Legend: sec. – section, *A.* – *Aspergillus*, *P.* – *Penicillium*, Fr –frequency, * number of contaminated samples

Toxigenity of *Penicillium* and *Aspergillus* isolates from berries and grapes

Most of the studies on mycotoxins in fruits are focused on patulin (produced mainly by *P. expansum*) in apple products, and ochratoxin A (produced mainly by *A. carbonarius*) in grapes and in wines **(Paster *et* Barkai-Golan, 2008)**. **Lugauskas *et* Stakeniene (2002)** mentioned, that the most active in production and excretion of secondary metabolites, under the research conditions, were micromycetes ascribed to various *Penicillium* species, their occurrence in the investigated berries, fruit, and vegetables was rather wide but irregular. The ability to produce selected mycotoxins was tested within 15 isolates of the genus *Penicillium* and 4 isolates of the genus *Aspergillus*, which were isolated from berries and grape samples (Tab. 3). We used a simple TLC method for identification of selected mycotoxins produced by fungal isolates. Production of at least one mycotoxin was confirmed in 14 *Penicillium* isolates (93.3%) and in one isolate of the *Aspergillus* genus. All three of tested *P. citrinum* isolates produced citrinin and both *P. crustosum* isolates produced roquefortine C and penitrem A.

Table 3 Potential toxigenicity of *Aspergillus* and *Penicillium* isolates obtained from mouldy berries and grapes

Tested isolate	C	RC	P	PA	OTA	CPA	AFB_1	AFB_2	AFG_1	AFG_2
P. citrinum	3*/3**									
P. crustosum		2*/2**		2*/2**						
P. expansum	9*/9**	9*/9**	8*/9**							
P. hordei		0*/1**								
A. sec. *Clavati*			1*/1**							
A. sec. *Flavi*						0*/1**	0*/1**	0*/1**	0*/1**	0*/1**
A. sec. *Nigri*					0*/2**	0*/2**				

Legend: sec. - section, * number of positive isolates, ** number of tested isolates, C - citrinin, RC - roquefortine C, P - patulin, PA - penitrem A, OTA - ochratoxin A, CPA - cyclopiazonic acid, AFB_1 - aflatoxin B_1, AFB_2 - aflatoxin B_2, AFG_1 - aflatoxin G_1, AFG_2 - aflatoxin G_2

All 9 tested *P. expansum* isolates produced both citrinin and roquefortine C, 8 isolates produced patulin. In our study, the tested isolate of *A.* section *Clavati* produced patulin, as well. Patulin is a relatively simple lactone produced mainly by species of the genera *Penicillium*, *Aspergillus*, and some species of *Paecilomyces* sp. (White et al., 2006). *Paecilomyces lilacinus* is not producer of patulin, it is keratinophilic fungi **(Javoreková *et al.*, 2012)**. *P. expansum* is considered as its main producer. The production of patulin was found to be mediated at the level of gene transcription in *P. expansum* **(White *et al.*, 2006)**. Human exposure to patulin through the consumption of contaminated fruits and products derived from them can lead to severe toxicoses, which include mutagenic, teratogenic, hepatotoxic, nephrotoxic, and genotoxic effects. Although recent safety assessments have concluded that patulin is unlikely to be carcinogenic

(Speijers, 2004), however, the ability of patulin to cause gene mutations in mammalian cells may affect its carcinogenicity **(Schumacher *et al.*, 2005)**. Patulin can also be formed during food storage and remains stable during food processing. Therefore, constant monitoring of various fruit products should be carried out to obtain a correct estimation of human exposure to this toxin **(Sadok *et al.*, 2019)**. **Barkai-Golan (2008)** reported that the major mycotoxins associated with *Aspergillus* species in fruits and vegetables are aflatoxins, produced mainly by strains of *A. flavus* and *A. parasiticus* (*A.* section *Flavi*), and ochratoxin A, produced by *A. carbonarius* (*A.* section *Nigri*) and other ochratoxigenic aspergilli. In our study, none of the *Aspergillus* from the section *Nigri* produced ochratoxin A or cyclopiazonic acid, nor did the tested isolate from the section *Flavi* produced aflatoxins or cyclopiazonic acid.

Tomato fruits

Tomatoes are highly susceptible to contamination by fungi during growth in the field, and during transportation, processing, and storage also **(Mariuttti *et* Soares, 2009)**. Contamination with microscopic filamentous fungi was confirmed in 93.1% cases from a total of 29 tomato samples analysed (Tab. 4, Fig. 2). Totally 155 isolates of microscopic filamentous fungi were isolated from tomatoes. Contamination with yeast was confirmed in 6.9% of samples. Totally 23 samples (79.3%) were contaminated with micromycetes of the genus *Penicillium*, 7 samples (24.1%) with the micromycetes of the genus *Botrytis* and *Alternaria*, three samples (10.3%) with species of the genus *Cladosporium*, *Mucor*, and *Rhizopus*, two samples (6.9%) with micromycetes of the genus *Aspergillus*, and one sample (3.4%) with the genus *Fusarium*. **Barth *et al.* (2009)** mentioned as an important tomato pathogen in the United States the micromycetes of *Rhizopus*, *Phytophthora*, *Colletotrichum*, *Botrytis*, *Fusarium*, *Alternaria*, *Sclerotinia*, and *Mucor* genera. **Pitt *et* Hocking (2009)** reported that the postharvest pathogens of tomato fruit are *Alternaria* (mainly *Al. solani*), *Cladosporium*, *Botrytis cinerea*, *Rhizopus*, *Mucor*, *Fusarium*, *Trichothecium*, *Sclerotinia*, *Phytophthora*, *Pythium*, and *Diaporthe* species.

In 19 tomato samples (65.5%), we found the presence of 8 species of the genus *Penicillium* (Tab. 5): *P. brevicompactum*, *P. crustosum*, *P. expansum*, *P. griseofulvum*, *P. italicum*, *P. olsonii*, *P. sublectaticum*, and *P. thomii*. *P. olsonii* (58.6%), and *P. griseofulvum* (10.3%) species were isolated with the highest Fr. **Samson *et al.* (2019)** linked the occurrence of *P. olsonii* with tomatoes. We obtained two isolates of the genus *Aspergillus* from 29 analysed tomato samples. One isolate belonged to the section *Circumdati* and one isolate to the section *Flavi*.

Muhammad *et al.* (2004) stated that the associated fungi on tomato fruits from markets in Sokoto (northwestern Nigeria) were *Aspergillus niger*, *A. ochraceus*, *A. flavus*, *A. fumigatus*, *P. citrinum*, *Helminthosporium fulvum*, *Curvularia lunata*, and *Sclerotium rolfsii*. *A. flavus* and *A. niger* had the highest rate of occurrence among the isolated fungi. *A. flavus* and *A. parasiticus* have been isolated from tomatoes and tomato products in the study of **Mariutti *et* Soares (2009)** in Brazil.

Table 4 Frequency of micromycetes on tomato fruits

Sample	Total no. of samples	Genera	Contaminated samples (%)
Tomatoes	29	*Penicillium*	79.3
		Botrytis	24.1
		Alternaria	24.1
		Rhizopus	10.3
		Mucor	10.3
		Cladosporium	10.3
		Aspergillus	6.9
		Fusarium	3.4

Figure 2 Contamination of tomato fruits by microscopic filamentous fungi (*Penicillium olsonii* in the left picture, *Rhizopus* spp. in the right picture)

Table 5 Spectrum of micromycetes of the genus *Aspergillus* and *Penicillium* isolated from tomato fruits

Species	Number of contaminated samples	Fr (%)	Species/Section	Number of contaminated samples	Fr (%)
P. brevicompactum	1	3.4	*P. olsonii*	17	58.6
Penicillium sp.	1	3.4	*P. sublectaticum*	2	6.9
P. crustosum	1	3.4	*P. thomii*	1	3.4
P. expansum	1	3.4	*A.* sec. *Circumdati*	1	3.4
P. griseofulvum	3	10.3	*A.* sec. *Flavi*	1	3.4
P. italicum	1	3.4			

Legend: sec. - section, *A.* - *Aspergillus*, *P.* - *Penicillium*, Fr - frequency

Toxigenity of *Penicillium* and *Aspergillus* isolates from tomatoes

We tested 7 isolates of the genus *Penicillium* and two isolates of the genus *Aspergillus* (Tab. 6) for their ability to produce selected mycotoxins. All 7 *Penicillium* isolates (100.0%) and one isolate of the genus *Aspergillus* was confirmed to be able to produce at least one mycotoxin. The tested *P. crustosum* isolate produced both roquefortine C and penitrem A, and the *P. expansum* isolate produced all three tested mycotoxins (citrinin, roquefortine C and patulin). All tested isolates (5) of *P. griseofulvum* produced griseofulvin, roquefortine C and patulin was produced in 4 of them, as well. Production of cyclopiazonic acid was not confirmed in any of them. *Aspergillus* from the section *Circumdati* in our study did not produce ochratoxin A. Many filamentous species belonging to the genera *Aspergillus* and *Penicillium* produce ochratoxin A as a secondary metabolite **(Miller *et* Trenholm, 1994)**. The main producers of ochratoxin A belong to the sections *Circumdati* and *Nigri*. *Aspergillus carbonarius*, belonging to *A.* section *Nigri* (black aspergilli), is a major ochratoxin A producer. This species is commonly present in grapes, and it is responsible for production and presence of ochratoxin A in grape products, including grape juice, wines, and dried vine fruits **(Magnoli *et al.*, 2003)**. Ochratoxin A can be produced by the genus *Penicillium*, especially *P. verrucosum* and *P. nordicum* species **(Geisen *et al.*, 2004).** The isolate from the section *Flavi*, tested in this study, produced all 4 monitored aflatoxins (B_1, B_2, G_1 and G_2), but did not produce cyclopiazonic acid. **Muhammad *et al.* (2004)** reported occurrence of toxigenic *A. flavus* species and aflatoxins in tomato fruits in Sokoto (Nigeria) markets.

Table 6 Potential toxigenicity of *Aspergillus* and *Penicillium* isolates obtained from mouldy tomato fruits

Tested isolate	C	RC	P	PA	OTA	CPA	AFB_1	AFB_2	AFG_1	AFG_2	G
P. crustosum		1*/1**		1*/1**							
P. griseofulvum		4*/5**	4*/5**			0*/5**					5*/5**
P. expansum	1*/1**	1*/1**	1*/1**								
A. sec. *Circumdati*					0*/1**						
A. sec. *Flavi*						0*/1**	1*/1**	1*/1**	1*/1**	1*/1**	

Legend: sec. - section, * number of positive isolates, ** number of tested isolates, C - citrinin, RC - roquefortine C, P - patulin, PA - penitrem A, OTA - ochratoxin A, CPA - cyclopiazonic acid, AFB_1 - aflatoxin B_1, AFB_2 - aflatoxin B_2, AFG_1 - aflatoxin G_1, AFG_2 - aflatoxin G_2, G - griseofulvin

The aflatoxins are undoubtedly the most documented of all mycotoxins and have a wide product presence. However, the aflatoxins have been detected in most agricultural commodities, their presence with particular significance is important in corn, cottonseed, groundnuts, and treenuts. Aflatoxins can be acutely toxic, carcinogenic, mutagenic, teratogenic, and immunosuppressive to most mammalian species **(Smith, 1997).** Quantification of levels of aflatoxins, produced by *Aspergillus* isolate from section *Flavi* (*A. parasiticus* KMi1040, NR121219.1, isolated from tomatoes of Slovak origin), was carried out by HPLC-FLD method. This isolate produced 5.5 $\mu g.mL^{-1}$ of aflatoxin B_1, 4.2 $\mu g.mL^{-1}$ of aflatoxin B_2, 154.4 $\mu g.mL^{-1}$ of aflatoxin G_1,and 5.6 $\mu g.mL^{-1}$ of aflatoxin G_2 on 14th day of cultivation on liquid YES medium at 25±1 °C in a darkness.

CONCLUSION

Microscopic filamentous fungi frequently cause the spoilage of fruits and vegetables in the stores, as these commodities are an ideal substrate for the development of microscopic fungi due to their composition and properties. The identification of microscopic filamentous fungi in these commodities is very important. Many of them can produce diverse toxic secondary metabolites (mycotoxins), which have serious negative effects on the health of consumers (some of them are carcinogenic, or potentially carcinogenic), many of them can cause allergies or infections of consumers. Micromycetes of the genera *Penicillium* and *Aspergillus* are very often involved in spoilage of berries, grapes, and tomato fruits, and many of them are significant producers of mycotoxins, as was confirmed by our results.

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