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FUNGI ASSOCIATED WITH TOMATO SPOILAGE FROM RETAIL MARKETS AND MYCOTOXIN PRODUCTION POTENTIAL

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ABSTRACT

Tomatoes represent a suitable substrate for the growth of microscopic filamentous fungi and yeasts, including potentially toxigenic species. The aim of this study was to compare the mycocenosis of tomatoes from retail chains in Slovakia and Hungary, with an emphasis on species diversity and the ability of selected isolates to produce mycotoxins. A total of 30 tomato samples (Slovakia, n = 21; Hungary, n = 9) were analysed. Fungal genera were identified based on macro- and micromorphological characteristics, while identification at the species, section, or group level was performed using standard taxonomic keys. Selected isolates of the genera *Penicillium*, *Aspergillus*, *Alternaria*, and *Fusarium* were tested for their ability to produce selected mycotoxins *in vitro* using thin-layer chromatography (TLC). Yeasts were identified using MALDI-TOF MS. In total, nine genera of filamentous fungi were identified. The dominant genus was *Penicillium* (73.3%), followed by *Alternaria* (30.0%). The most frequently isolated taxa were *Penicillium olsonii* (56.7%) and the *Alternaria arborescens* species group (36.6%), both detected in Slovak and Hungarian samples. Several isolates of *Penicillium*, *Aspergillus*, *Alternaria*, and *Fusarium* demonstrated the ability to produce at least one mycotoxin, including aflatoxins B_1 and G_1, patulin, citrinin, roquefortine C, penitrem A, cyclopiazonic acid, griseofulvin, alternariol, alternariol monomethylether, T-2 toxin, HT-2 toxin, and zearalenone. The results revealed differences in fungal species diversity and toxigenic profiles between Slovak and Hungarian tomatoes and highlight the potential food safety risks associated with the presence of mycotoxigenic fungi in fresh tomatoes.

Keywords: microorganisms, mycobiota, postharvest spoilage, secondary metabolites

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the most widely consumed vegetables worldwide and represents an important component of the human diet due to its high nutritional value. Tomatoes are a rich source of bioactive compounds, including lycopene, β-carotene, flavonoids, vitamin C, and phenolic acids, which contribute to their antioxidant and health-promoting properties **(Gerszberg *et al.*, 2015)**. Due to their high-water activity, soft tissue structure, and nutrient-rich composition, tomatoes represent a favorable substrate for the growth of microorganisms, particularly microscopic filamentous fungi and yeasts **(Oladiran & Iwu, 1993; Tijjani *et al.*, 2014)**. During cultivation, harvesting, transportation, storage, and retail distribution, tomatoes may become contaminated with fungi originating from soil, air, water, packaging materials, and handling practices **(Verhoeff, 1970; Wani, 2011)**. Post-harvest fungal contamination is considered one of the major causes of quality deterioration and economic losses in tomatoes worldwide **(Tijjani *et al.*, 2014)**.

Numerous studies have identified genera such as *Penicillium*, *Alternaria*, *Aspergillus*, *Fusarium*, *Cladosporium*, *Botrytis*, and *Rhizopus* as the most frequent agents of tomato spoilage **(Oladiran & Iwu, 1993; Wani, 2011; Tijjani *et al.*, 2014)**. These fungi can colonize tomatoes through mechanical injuries or natural openings and may proliferate under a wide range of storage conditions, including refrigeration.

Beyond visible spoilage, several fungal species associated with tomatoes are of particular concern due to their ability to produce mycotoxins. Mycotoxins are secondary metabolites with well-documented toxic, carcinogenic, mutagenic, and immunosuppressive effects on humans and animals **(Samson *et al.*, 2002; Frisvad *et al.*, 2019)**. Species of the genera *Penicillium*, *Aspergillus*, *Alternaria*, and *Fusarium* are known producers of a broad spectrum of mycotoxins, including aflatoxins, ochratoxin A, patulin, citrinin, cyclopiazonic acid, alternariol, alternariol monomethylether, T-2 toxin, HT-2 toxin, and zearalenone **(Samson *et al.*, 2002; Frisvad *et al.*, 2007)**. Although fresh tomatoes are not traditionally regarded as a high-risk commodity for mycotoxin contamination, the presence of mycotoxigenic fungi on tomatoes represents a potential food safety hazard, particularly when they are damaged or stored under unfavorable conditions **(Tijjani *et al.*, 2014)**. The *Alternaria arborescens* species group, frequently associated with tomato diseases, has been reported to produce several mycotoxins, including alternariol and alternariol monomethylether **(Vaquera *et al.*, 2014)**. Similarly, various *Penicillium* species isolated from tomatoes have demonstrated toxigenic potential under suitable environmental conditions **(Samson *et al.*, 2002, 2019; Živković *et al.*, 2021)**.

Despite increasing interest in fungi associated with fresh produce, relatively limited data are available on the diversity and toxigenic potential of fungi responsible for tomato spoilage, particularly in retail environments in Central Europe. Moreover, many studies focus primarily on fungal occurrence, while fewer address the actual mycotoxin-producing capacity of isolates, which is essential for a realistic assessment of food safety risks. Therefore, the aim of this study was to characterize fungi associated with mould spoilage of retail tomatoes obtained from markets in Slovakia and Hungary, with particular emphasis on the diversity of microscopic filamentous fungi and yeasts and on the ability of selected isolates to produce mycotoxins under *in vitro* conditions. We hypothesized that tomatoes from retail chains in Slovakia and Hungary differ in fungal species diversity and in the toxigenic potential of associated isolates.

MATERIAL AND METHODS

Samples and mycological analysis

Tomato samples from retail chains in Slovakia (n = 21) and Hungary (n = 9) were analysed (Tab 1). The samples were collected between 2023 and 2024 and consisted of commercially packaged consumer units obtained from retail outlets. The packages remained unopened until laboratory analysis. Only tomatoes showing visible signs of fungal spoilage (e.g. mycelial growth or lesions) were included in the study, in order to assess the diversity and toxigenic potential of fungi actively involved in spoilage.

Tomato samples (*Solanum lycopersicum* L.) were classified according to the product labelling provided by the manufacturers (e.g. cherry, cocktail tomatoes). Information on specific tomato varieties was not available from the product labelling. A piece of mycelium was collected from visibly mouldy parts of each tomato sample and inoculated onto MEA (malt extract agar; HiMedia, India). Subsequently, cultivation was carried out at 25 ± 1 °C for 7 days in a thermostatically controlled incubator.

Table 1 Overview of tomato samples analysed from retail chains in Slovakia and Hungary, including sample type and country of origin

No.	Sample	Country of origin	No.	Sample	Country of origin
1S	Cherry tomatoes	Slovakia	16S	Cherry tomatoes	Slovakia
2S	Summer cherry tomatoes	Slovakia	17S	Cherry tomatoes	Slovakia
3S	Cherry tomatoes	Slovakia	18S	Cherry tomatoes	Slovakia
4S	Cherry tomatoes	Slovakia	19S	Cherry tomatoes	Netherlands
5S	Tomatoes	Slovakia	20S	Cherry tomatoes	Tunisia
6S	Tomatoes	Slovakia	21S	Cherry tomatoes	Tunisia
7S	Tomatoes	Slovakia	1H	Tomatoes	Hungary
8S	Tomatoes	Slovakia	2H	Cherry tomatoes	Hungary
9S	Cherry tomatoes	Slovakia	3H	Cherry tomatoes	Hungary
10S	Cherry tomatoes	Netherlands	4H	Tomatoes	Hungary
11S	Cherry tomatoes	Slovakia	5H	Cherry tomatoes	Hungary
12S	Cherry tomatoes	Slovakia	6H	Cocktail tomatoes	Morocco
13S	Tomatoes	Slovakia	7H	Tomatoes	Turkey
14S	Cherry tomatoes	Slovakia	8H	Tomatoes	Turkey
15S	Cherry tomatoes	Tunisia	9H	Tomatoes	Spain

Legend: S – samples from retail chains in Slovakia, H – samples from retail chains in Hungary

Figure 1 Mouldy tomatoes samples

Genera identification of micromycetes

After 5–7 days of cultivation, genus-level identification of fungi was performed based on microscopic observation of slides prepared in a drop of lactic acid stained with cotton blue using an Olympus BX51 microscope. Following genus identification, isolates of the genus *Alternaria* were inoculated onto the identification medium PCA (potato carrot agar; HiMedia, India), isolates of the genus *Aspergillus* onto MEA and CYA (Czapek yeast extract agar; **Pitt *et al.*, 1979**), *Fusarium* isolates onto SNA (synthetic nutrient agar; **Nirenberg, 1976**) and PDA (potato dextrose agar; **Pitt & Hocking, 2009**), and *Penicillium* isolates onto MEA, CYA, YES (yeast extract sucrose agar**; Samson & Frisvad, 2004**), and CREA (creatine sucrose agar; **Frisvad, 1985**). Cultures of *Aspergillus* and *Penicillium* genera were incubated at 25 ± 1 °C for 7 days in a thermostatically controlled incubator, whereas cultures of *Alternaria* and *Fusarium* were incubated at room temperature under natural light conditions.

Species/sections/species groups identification of micromycetes

After cultivation of micromycetes (5–7 days) on identification media, isolates were identified at the species level (genera *Fusarium* and *Penicillium*), at the species group level (*Alternaria*), and at the section level (*Aspergillus*). Identification was based on macro- and micromorphological, physiological, and chemotaxonomic characteristics of the isolates, using standard taxonomic keys and monographs.
For individual genera, identification was performed according to commonly accepted taxonomic references, including those for *Aspergillus* (e.g. **Klich, 2002**; **Samson *et al*., 2014**; **Visagie *et al*., 2014b**), *Alternaria* (e.g. **Andersen *et al.*, 2001, 2004; Simmons & Roberts, 1993; Lawrence *et al.*, 2016**), *Fusarium* (e.g. **Leslie & Summerell, 2008**), and *Penicillium* (e.g. **Pitt & Hocking, 2009**; **Visagie *et al*., 2014a**).

Frequency of occurrence

The occurrence of fungal genera/species/species groups/sections (frequency of occurrence, Fr) was calculated according to the formula described by **Hyde & Jones (1988)**. This approach allows for a standardized comparison of fungal prevalence across the analysed samples.

$$\textit{Frequency of occurrence}\ (\%) = \frac{\textit{Number of samples in which the fungus was detected}}{\textit{Total number of samples examined}} \times 100$$

Statistical analysis

Data were evaluated using descriptive statistical methods. The occurrence of individual fungal genera and species was expressed as relative frequencies (percentages) based on the total number of analysed samples. No inferential statistical analyses were performed due to the limited and unequal sample size, which was not considered sufficient for a robust statistical comparison.

Assessment of Toxigenicity in Selected Micromycete Isolates

Selected isolates of potentially toxigenic species were tested by TLC (thin-layer chromatography) for their ability to produce selected mycotoxins *in vitro.* Cultures for screening of intracellular mycotoxins were cultivated on CYA, and for screening of extracellular mycotoxins on YES, for 14 days at 25 ± 1 °C. TLC was performed according to **Samson *et al.* (2002)**, as modified by **Labuda & Tančinová (2006)**.
For fungi of the genera *Aspergillus* and *Penicillium*, three agar plugs (0.5 × 0.5 cm) were cut from each colony; each plug contained both mycelium and the underlying culture medium. The plugs were transferred into 1.5 mL microtubes (Eppendorf, Germany), and 0.5 mL of a chloroform–methanol extraction solvent (2:1, v/v) was added. To facilitate the release of metabolites into the extraction solvent, the samples were homogenized by vortexing (Genie 2, Scientific Industries, USA) for 5 min. Subsequently, aliquots of each extract (30–50 µL) were applied onto chromatographic plates (Merck, Germany). The developing solvent system consisted of toluene–ethyl acetate–formic acid (5:4:1, v/v/v).
For *Alternaria* isolates, agar plugs (approximately 2 × 2 cm) were cut from the colonies together with the culture medium, divided into smaller pieces, placed into microtubes, and extracted with 0.5 mL of chloroform–methanol (2:1, v/v).
For *Fusarium* isolates, squares of approximately 2 × 2 cm were cut from the grown colonies, fragmented, and transferred into microtubes containing 1 mL of extraction solvent. Methanol–water (80%, v/v) was used for the extraction of zearalenone, while acetonitrile–water (84%, v/v) was used for the extraction of deoxynivalenol, HT-2 toxin, and T-2 toxin. After 5 min of mixing, the extracts were applied onto chromatographic plates. The following developing systems were used: toluene–acetone–ethyl acetate (5:4:1, v/v/v) for T-2 and HT-2 toxins, chloroform–acetonitrile–methanol (6:3:1, v/v/v) for deoxynivalenol, and toluene–acetone–acetic acid (90:9:1, v/v/v) for zearalenone.
Subsequently, visualisation of selected mycotoxins was performed. Citrinin appeared under UV light (365 nm) as a yellow–green fluorescent stain with a characteristic tail. Roquefortine C was visualised under daylight as an orange spot after spraying the chromatographic plate with $Ce(SO_4)_2 \cdot 4H_2O$. Ochratoxin A was observed under UV light as a blue–green fluorescent stain. Patulin was visualised under daylight as a yellow–orange stain after spraying the plate with a 0.5% solution of MBTH (3–methyl–2–benzothiazolinone hydrazone) in methanol and heating at 130 °C for 8 min. Penitrem A was visualised as a dark green to black stain after spraying the plate with a 20% solution of $AlCl_3$ in 60% ethanol followed by heating at 130 °C for 8 min. Cyclopiazonic acid was detected under daylight as a purple tail stain after application of Ehrlich's reagent and subsequent heating at 130 °C for 8 min. Aflatoxin B_1 was observed under UV light as a blue fluorescent stain, while aflatoxin G_1 appeared as a green fluorescent stain. Altenuene was observed under UV light as a turquoise stain, whereas alternariol and alternariol

monomethylether appeared as light blue fluorescent stains. T-2 toxin, HT-2 toxin, and deoxynivalenol were visualised under UV light; T-2 and HT-2 toxins appeared as green–blue fluorescent stains, while deoxynivalenol was detected as a light blue stain. Zearalenone was observed under UV light as a blue–purple fluorescent stain.

Identification of yeasts by MALDI-TOF

The identification of yeast isolates was performed using the MALDI-TOF MS system (Bruker Daltonik GmbH, Germany), a widely recognized tool for microbial analysis. The procedure was supported by the proprietary Biotyper software (Bruker Daltonik GmbH, Germany), which facilitates accurate classification based on protein fingerprinting. Detailed information about the specific steps involved in protein extraction, sample preparation, and the identification workflow is available in a previously published study **(Hleba *et al*., 2020)**.

RESULTS AND DISCUSSION

Genera identification

Table 2 and Supplementary Table S1 document the diversity of microscopic filamentous fungi and yeasts isolated from tomato samples obtained from retail chains in Slovakia and Hungary. In total, representatives of nine genera of filamentous fungi as well as yeasts were identified, with their occurrence varying among individual samples. The fungal genera detected in the present study, namely *Aspergillus, Alternaria, Botrytis, Cladosporium, Fusarium, Mucor, Penicillium, Rhizopus*, and *Trichoderma*, correspond to those most frequently reported in the literature as principal agents of post-harvest tomato spoilage. These fungi are well known for their ability to colonize tomatoes, facilitated by the production of degradative enzymes such as pectinases, their tolerance to a wide range of storage conditions, and their capacity to exploit structural weaknesses of the tomato, including mechanical damage and high moisture levels. The consistent detection of these genera across multiple studies highlights their pervasive role in tomato spoilage and suggests the influence of similar ecological strategies and environmental factors promoting their proliferation **(Oladiran & Iwu, 1993; Wani, 2011; Tijjani *et al*., 2014)**.

The occurrence frequency of the dominant fungal genera in tomato samples from Slovak and Hungarian retail chains is summarized in Figure 2. As shown in Figure 2, *Penicillium* was the most frequently detected genus in samples from retail chains in both countries, confirming its dominant position in the mycocenosis of tomatoes. In total, *Penicillium* was isolated from 22 out of 30 samples (73.3%), with a higher frequency in Slovak samples (81.0%) compared to Hungarian samples (55.6%). The second most frequently detected genus was *Alternaria*, identified in 9 samples (30.0%), showing a higher frequency of occurrence in tomatoes obtained from Hungarian retail chains. Representatives of the genus *Aspergillus* were detected in 6 samples (20.0%), while *Cladosporium* and *Botrytis* were each present in 5 samples (16.7%). In contrast, *Fusarium* species were isolated exclusively from tomato samples obtained from Hungarian retail chains.

Table 2 Occurrence frequency (Fr, %) of fungal genera in tomato samples

Genus	Slovakia (n = 21)	Hungary (n = 9)	Total (n = 30)	Fr (%)
Penicillium	17 (81.0%)	5 (55.6%)	22	73.3
Alternaria	5 (23.8%)	4 (44.4%)	9	30.0
Aspergillus	4 (19.0%)	2 (22.2%)	6	20.0
Cladosporium	4 (19.0%)	1 (11.1%)	5	16.7
Botrytis	4 (19.0%)	1 (11.1%)	5	16.7
Fusarium	0 (0.0%)	3 (33.3%)	3	10.0
Rhizopus	2 (9.5%)	1 (11.1%)	3	10.0
Mucor	2 (9.5%)	0 (0.0%)	2	6.7
Trichoderma	2 (9.5%)	0 (0.0%)	2	6.7
Yeasts	6 (28.6%)	2 (22.2%)	8	26.7

Yeasts were detected in 8 samples (26.7%). Among them, *Geotrichum* was the most frequently identified genus, although in some cases yeast isolates could not be reliably classified at the genus level based on the available identification methods. The co-occurrence of multiple fungal genera within individual samples indicates mixed microbial contamination, which may reflect cultivation practices, post-harvest handling, storage conditions, or distribution processes involved in tomato production and marketing.

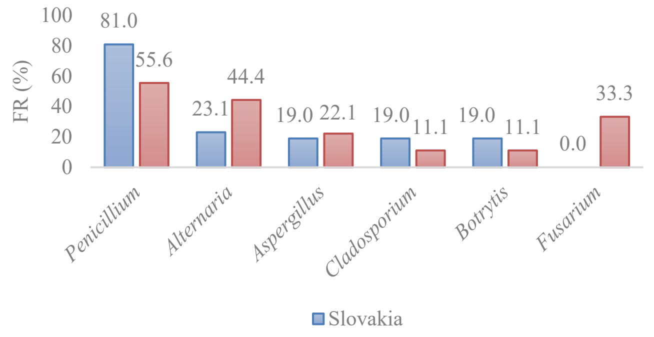

Figure 2 Occurrence frequency (Fr, %) of dominant fungal genera isolated from tomatoes obtained from retail chains in Slovakia (n = 21) and Hungary (n = 9). Only visibly mouldy tomatoes were analysed.

Species identification

Overall, eight *Penicillium* species were recorded (Tab 3, Tab S2). *P. olsonii* was the dominant species, occurring in 17 samples (56.7%), including 12 of 21 Slovak samples (57.1%), and 5 of the Hungarian samples (55.6%). The second most frequently detected species was *P. griseofulvum*, which occurred in 3 samples (10.0%) of Slovak origin. Other species were detected only sporadically and exclusively in samples from Slovak retail chains. In contrast, samples from Hungarian retail chains showed lower species diversity, with a dominance of *P. olsonii* and the absence of other *Penicillium* species. In several samples from Slovak markets (e.g., sample 11S), the co-occurrence of multiple *Penicillium* species was observed, indicating mixed contamination, whereas samples from Hungarian retail chains were typically colonized by a single *P. olsonii* species. In the studies by **Barboráková *et al.* (2023)** and **Mašková *et al.* (2023)**, similar to our findings, *P. olsonii* was the most frequently identified fungal species at the species level among *Penicillium* isolates from tomatoes. Similarly, *P. olsonii*, frequently detected in our samples, was recently reported by **Živković *et al.* (2021)** as a post-harvest tomato contaminant in Serbia. Their study demonstrated that *P. olsonii* caused grey lesions on tomatoes under experimental conditions, with morphological and molecular characterization matching those of our isolates.

Table 3 Occurrence frequency (Fr, %) of *Penicillium* species in tomato samples

Species	Slovakia (n = 21)	Hungary (n = 9)	Total isolates	Fr (%)
P. olsonii	12	5	17	56.7
P. griseofulvum	3	0	3	10.0
P. expansum	1	0	1	3.3
P. crustosum	1	0	1	3.3
P. camemberti	1	0	1	3.3
P. sublectaticum	1	0	1	3.3
P. thomii	1	0	1	3.3
P. waksmanii	1	0	1	3.3

The genus *Alternaria* was detected in 9 analysed samples (Fr 30.0%). The *A. arborescens* species group was present in 5 samples from Slovak retail chains (Fr 23.8%) and 4 samples from Hungarian retail chains (Fr 44.4%) (Tab 4, Tab S3). The fungal species identified in this study align with findings from authoritative mycological research. *A. arborescens* species group, isolated from tomato samples in this work, has been explicitly documented as a tomato pathogen by **Vaquera *et al.* (2014)**, who stated that *A. arborescens* is the causal agent of tomato stem canker and can produce several mycotoxins, such as alternariol, alternariol monomethylether, and tenuazonic acid.

Representatives of the genus *Aspergillus* were isolated only from three samples (Fr 10.0%) and belonged to three sections: *Circumdati, Flavi*, and *Nigri*. Similarly, species of the genus *Fusarium* were isolated from three samples (Fr 10.0%), exclusively from tomatoes obtained from Hungarian retail markets. The identified species included *F. acuminatum*, *F. incarnatum*, and *F. subglutinans*. No *Fusarium* species were detected in tomatoes from Slovak retail chains. The yeast *Geotrichum candidum*, identified using MALDI-TOF MS, was detected in 5 samples (Fr 16.7%).

These findings are consistent with previously published data. As emphasized by **Vaquera *et al.* (2014)**, environmental factors such as water activity and temperature critically influence fungal proliferation, which may explain the high occurrence rates observed in our samples. These findings further support the ecological and agricultural relevance of the fungal species identified in this study. The observed differences in fungal species diversity and toxigenic profiles between Slovak and Hungarian samples may be influenced by several factors. In the Slovak samples, a greater diversity of countries of origin (e.g. the Netherlands, Tunisia) was recorded, which may have contributed to a more diverse mycobiota. In contrast, Hungarian samples included a greater proportion of tomatoes originating from countries such as Turkey, Morocco, and Spain, which may reflect differences in supply chains and sources of contamination.

In addition, variations in transportation distance, storage conditions, and handling practices within retail chains may have influenced the composition of fungal communities and their toxigenic potential. Differences in shelf life and turnover rates between retail markets in Slovakia and Hungary may also contribute to the extent of fungal colonization and spoilage. Environmental factors, particularly temperature and humidity during post-harvest handling, are likely to further affect fungal growth and the potential for mycotoxin production.

Table 4 Occurrence frequency (Fr, %) of selected fungal species, groups and sections

Taxon / Group	Slovakia	Hungary	Total	Fr (%)
Alternaria arborescens species group	5	4	9	30.0
Aspergillus section *Flavi*	1	0	1	3.3
Aspergillus section *Circumdati*	1	0	1	3.3
Aspergillus section *Nigri*	0	1	1	3.3
Fusarium acuminatum	0	1	1	3.3
Fusarium incarnatum	0	1	1	3.3
Fusarium subglutinans	0	1	1	3.3
Geotrichum candidum	4	1	5	16.7

Toxigenicity of isolates

A total of eight selected isolates of the genus *Penicillium* and three isolates of the genus *Aspergillus* were tested for their ability to produce selected mycotoxins by TLC *in vitro* (Tab 5). Among the five tested isolates of *P. griseofulvum*, all produced griseofulvin, four isolates produced patulin, and two isolates each produced roquefortine C and cyclopiazonic acid. The *P. expansum* isolate produced all tested mycotoxins (roquefortine C, patulin, and citrinin), the *P. crustosum* isolate produced penitrem A and roquefortine C, while the isolate of *P. camemberti* produced cyclopiazonic acid (Tab S4). The toxigenic potential of fungal isolates associated with tomatoes has been addressed in several previous studies; however, comparative data focusing on isolates from retail chains remain limited. In the present study, all tested *Penicillium* isolates demonstrated the ability to produce at least one mycotoxin *in vitro*, which is consistent with findings reported by **Samson *et al.* (2002)** and **Frisvad *et al.* (2007)**, who described *Penicillium* species as prolific producers of a wide range of secondary metabolites. Similar results were reported by **Živković *et al.* (2021)**, who identified P. *olsonii* as a post-harvest pathogen of tomatoes with confirmed spoilage capability. Although toxigenicity was not the primary focus of their study, the species detected correspond closely to those identified in our work. The production of patulin, griseofulvin, roquefortine C, cyclopiazonic acid, and citrinin by *Penicillium* isolates in the present study aligns with observations reported for *Penicillium* species isolated from fruits and vegetables by **Barboráková *et al.* (2023)** and **Mašková *et al.* (2023)**. These previous studies also highlighted that the toxigenic profiles of *Penicillium* isolates can vary considerably depending on environmental conditions and substrate, which may explain differences in the spectrum and frequency of detected mycotoxins.

In the case of *Aspergillus,* toxigenic potential was detected only in the isolate belonging to section *Flavi*, which produced aflatoxins B_1 and G_1. This finding aligns well with numerous studies identifying *Aspergillus* section *Flavi* as the primary source of aflatoxin production in food commodities **(Samson *et al.*, 2002; Frisvad *et al.,* 2019)**. In contrast, isolates belonging to sections *Circumdati* and *Nigri* did not produce the tested mycotoxins under the applied conditions, which corresponds with reports by **Visagie *et al.* (2014b)**, indicating that mycotoxin production is species-specific and dependent on suitable environmental conditions.

Table 5 Toxigenic potential of selected fungal genera

Genus	No. of tested isolates	≥1 mycotoxin (%)	Main detected mycotoxins
Penicillium	8	100.0	PAT, G, RC, CPA, C
Aspergillus	3	33.3	AFB_1, AFG_1
Alternaria	11	81.8	AOH, AME, ALT
Fusarium	3	33.3	T-2, HT-2, ZON

Legend: AFB_1 – aflatoxin B_1, AFG_1 – aflatoxin G_1, C – citrinin, CPA – cyclopiazonic acid, G – griseofulvin, PAT – patulin, RC – roquefortine C, ALT – altenuene, AME – alternariol monomethylether, AOH – alternariol, ZON – zearalenone

Alternaria isolates exhibited a high toxigenic potential in the present study, with 81.8% of the tested isolates producing at least one *Alternaria* toxin. This result is comparable to findings by **Vaquera *et al.* (2014)**, who reported that members of the *A. arborescens* species group can produce alternariol, alternariol monomethylether, and other toxins on tomato-based media. Similarly, **Andersen *et al.* (2002)** and **Lawrence *et al.* (2016)** highlighted the frequent occurrence of toxigenic *Alternaria* species on plant substrates and their adaptability to diverse environmental conditions. The high proportion of toxigenic *Alternaria* isolates observed in our study supports the importance of this genus as both a spoilage organism and a potential source of mycotoxins in tomatoes.

Compared to *Penicillium* and *Alternaria, Fusarium* isolates showed a lower toxigenic potential in the present study, with mycotoxin production detected only in one isolate of *F. incarnatum*. This observation partially corresponds with previous reports by **Leslie & Summerell (2008)**, who emphasized pronounced interspecific variability in toxigenic capacity within the genus *Fusarium.* Similar variability has been reported in studies focusing on *Fusarium* isolates from vegetables, in which only a subset of isolates could produce trichothecenes or zearalenone under experimental conditions.

It should be noted that the thin-layer chromatography (TLC) method used in this study provides only screening evidence of the ability of fungal isolates to produce mycotoxins under *in vitro* conditions. This does not confirm the actual presence or concentration of mycotoxins in tomato tissues. Therefore, the results should be interpreted strictly as evidence of *in vitro* toxigenic potential and not as proof of actual mycotoxin contamination in tomato tissues.

A limitation of the present study is the unequal number of analysed samples obtained from Slovak (n = 21) and Hungarian (n = 9) retail chains. This imbalance significantly limits the robustness of direct cross-country comparisons and reduces the generalizability of the findings. It may also have influenced the observed differences in fungal species diversity and toxigenic profiles. Therefore, the comparative results should be interpreted with caution.

Another limitation is that fungal identification was based on morphological, physiological, and chemotaxonomic characteristics without molecular confirmation (e.g. ITS sequencing). Although classical taxonomic approaches are widely used and remain reliable for routine identification, the absence of molecular methods may have affected the accuracy of species-level identification, particularly in taxonomically complex groups, which may have resulted in possible misidentification.

Overall, the comparison of our results with published data indicates that the toxigenic potential of fungi associated with tomatoes is strongly species- and strain-dependent and influenced by environmental conditions.

CONCLUSION

This study demonstrated that tomatoes from retail chains in Slovakia and Hungary are colonized by a wide spectrum of filamentous fungi and yeasts, including species with known toxigenic potential. The *Penicillium* genus dominated the mycocenosis of the analysed samples, with *P. olsonii* being the most frequently isolated species. Tomatoes from Slovak retail chains exhibited higher species diversity, whereas samples from Hungarian retail chains were characterized by a more uniform fungal composition. A substantial proportion of selected isolates of the *Penicillium*, *Aspergillus*, *Alternaria*, and *Fusarium* genera could produce at least one mycotoxin *in vitro*, indicating a potential risk under favorable conditions rather than confirmed contamination. The detected toxigenic potential does not necessarily indicate the presence of mycotoxins in tomato tissues. Differences in toxigenic profiles among individual genera and species emphasize the importance of species-level identification in food safety assessments. The obtained results highlight the need for regular monitoring of fungal contamination and toxigenic potential in fresh vegetables, particularly tomatoes, throughout the entire production and distribution chains. Further research should focus on the quantitative determination of mycotoxins in tomato products and on identifying critical factors influencing fungal growth and toxin production during post-harvest handling and storage, as well as on the application of molecular methods (e.g. ITS sequencing) to improve the accuracy of fungal identification. These conclusions should be interpreted in the context of the study limitations, particularly the unequal sample size and the absence of molecular confirmation.

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