OPEN ACCESS
Potravinarstvo Slovak Journal of Food Sciences
Crossref Similarity Check Powered by iThenticate

potravinárstvo®

Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 11, 2017, no. 1, p. 403-409
doi: https://dx.doi.org/10.5219/763
Received: 20 February 2017. Accepted: 15 May 2017.
Available online: 29 June 2017 at www.potravinarstvo.com

ISSN 1337-0960 (online)

SPECIES OF GENERA *BOTRYTIS*, *FUSARIUM* AND *RHIZOPUS* ON GRAPES OF THE SLOVAK ORIGIN

Dana Tančinová, Zuzana Mašková, Ľubormír Rybárik, Viera Michalová

ABSTRACT

Our research was focused to identify the *Botrytis*, *Fusarium* and *Rhizopus* species from grapes of the Slovak origin. A further goal of the project was to characterized toxinogenic potential of chosen strains of species *Fusarium*. 50 samples of grapes, harvested in years 2011, 2012 and 2013 from various wine-growing regions were analyzed in this study. For the isolation of species the of direct plating method was used: a) surface-sterilized berries (using 1% freshly pre-pared chlorine) b) berries and c) damaged berries on DRBC (Dichloran Rose Bengal Chloramphenicol agar). For each analysis were used 50 berries (or all damaged berries from sample). The cultivation was carried at 25 ±1°C, for 5 to 7 days in dark. After incubation, the colonies of *Botrytis*, *Fusarium* and *Rhizopus* were transferred to identification media and after incubation strains were identified to species level. Thirteen species of fusaria (*F. acuminatum, F. avenaceum, F. culmorum, F. equiseti, F. graminearum, F. oxysporum, F. proliferatum, F. semitectum, F. solani, F. sporotrichioides, F. subglutinans F. tricinctum* and *F. verticilioides*) were identified. Frequency of fusaria isolation was 92 %. *Botrytis cinerea* was determined from 86% samples and *Rhizopus* from 94%. Chosen strains of species of genus *Fusarium* were able to produce following mycotoxins: deoxynivalenol, T-2 toxin, HT-2 toxin and diacetoxyscirpenol in *in vitro* conditions as determinated by thin-layer chromatography. Thirty-two (68%) of tested isolates of *Fusarium* species were able to produce at least one mycotoxin.

Keywords: *Botrytris; Fusarium; Rhizopus*; grapes; trichothecenes

INTRODUCTION

Grapes have a complex microbial ecology including filamentous fungi, yeasts and bacteria with different physiological characteristics and effects upon wine production **(Barata et al., 2012)**. Moulds commonly isolated from grapes are *Alternaria*, *Cladosporium* and *Botrytis cinerea*, the latter causing bunch rot. Pathogenic and opportunistic species of *Fusarium*, *Penicillium* and *Aspergillus* can also colonize inducing grape disease **(Oliveri and Catara, 2011)**.

The concern about filamentous fungi in the vineyasrd has traditionally been linked to spoilage of grapes due to fungal growth. The main fungus responsible for grape rot is *Botrytis cinerea*, a pathogen that damages the berries and had a detrimental effect on the organoleptic properties **(Serra et al., 2006)**. *Botrytis* is a common genus in the temperate zones, where it occurs mainly as a pathogen on a variety of plant crops. Vegetable and small berry fruits are particularly susceptible. Invasion may occur before maturity or postharvest, both in transport and in storage. Onions and other allium species and grapes are the most susceptible crops. In the latter, it is notable that the disease is sometimes encouraged. Grapes affected by *Botrytis*, in this ciccumstance called “the noble rot”, are used int the production of certain high quality sweet wines in France, Germatny, Australia and other countries **(Pitt and Hocking, 2009)**.

Rhizopus stolonifer is one of the most common and fastest-growing species in the *Zygomycota* phylum. Disease caused by this fungus is known as soft rot, black mould and *Rhizopus* rot **(Bautista-Baños et al., 2014)**. *Rhizopus* rot is common on soft fruits, more abundant in warm, humid climates than in cool climate viticulture. In several fruits and crops such as strawberry, tomato, cucumber and table grapes Rhizopus rot causes soft rot during transport and storage **(Kassemeyer and Berkelmann-Löhnertz, 2009)**.

Fusarium species are renowned for their role as plant pathogens, causing a wide renage of diseases such as vascular wilts, root and stem rots, pre- and post-emergence blight and many others **(Pitt and Hocking, 2009)**.

Our research was focused to identify the *Botrytis*, *Fusarium* and *Rhizopus* species from grapes of the Slovak origin. A further goal of the project was to identify toxinogenic potential of chosen strains of species *Fusarium.*

MATERIAL AND METHODOLOGY

Samples

Fifty samples of wine grapes, harvested in years 2011, 2012 and 2013 from various wine-growing regions of Slovakia, from small and medium-sized vineyards were analysed.

Slovak wine region is characterized as a territory of the Slovak Republic, where grapes are grown. It is divided into 6 viticulturally regions, which are divided into 40 wine area and 690 wine-growing villages. The samples which were analysed were taken from all viticulturally regions (Table 1).

White and red grape varieties were analysed. White grape: Chardonnay (4), Grüner Veltliner (5), Müller Thurgau (1), Riesling (3), Velsch Riesling (7), Pálava (1), Pinot blanc (2), Pinot gris (2), Sauvignon (2), Tramin (2), Zala gyöngye (1) and grape using for special Tokay vine

Table 1 List of analysed samples of grapes and their origin.

Number of sample	Town or willage	Wine-growing area	Vineyard region	Variety
1.	Báb	Šintavský	Nitra	Chardonnay
2.	Nitra	Nitriansky	Nitra	Velsch Riesling
3.	Oponice	Radošinský	Nitra	Chardonnay
4.	Beladice	Zlatomoravecký	Nitra	Velsch Riesling
5.	Vinodol	Vrábeľský	Nitra	Chardonnay
6.	Komjatice	Žitavský	Nitra	Riesling
7.	Čaka	Želiezovský	Nitra	Velsch Riesling
8.	Nová Dedina	Tekovský	Nitra	Grüner Veltliner
9.	Brhlovce	Pukanecký	Nitra	Pinot Blanc
10.	Hontianske Moravce	Hontiansky	Central Slovak	Konkordia
11.	Gbelce	Strekovský	Southern Slovak	Velsch Riesling
12.	Mužla	Štúrovský	Southern Slovak	Velsch Riesling
13.	Pribeta	Hurbanovský	Southern Slovak	Riesling
14.	Veľký Krtíš	Modrokamenský	Central Slovak	Pinot Gris
15.	Veľký Krtíš	Modrokamenský	Central Slovak	Pinot Noir
16.	Veľký Krtíš	Modrokamenský	Central Slovak	Sauvignon
17.	Modra	Modranský	Small Carpathian	Pinor Blanc
18.	Zeleneč	Trnavský	Small Carpathian	Cabernet Sauvignon
19.	Báb	Šintavský	Nitra	Tramin
20.	Báb	Šintavský	Nitra	Blaufrankise
21.	Svätý Martin	Senecký	Small Carpathian	André
22.	Doľany	Dolanský	Small Carpathian	Pinot Noir
23.	Doľné Orešany	Orešanský	Small Carpathian	Blaufrankise
24.	Dvorníky	Hlohovecký	Small Carpathian	Sauvignon
25.	Pezinok	Pezinský	Small Carpathian	Blaufrankise
26.	Moravany nad Váhom	Vrbovský	Small Carpathian	Grüner Veltliner
27.	Vinica	Vinický	Central Slovak	Blaufrankise
28.	Šahy	Ipeľský	Central Slovak	Zala gyöngye
29.	Sebechleby	Hontiansky	Central Slovak	Saint Laurent
30.	Gajary	Záhorský	Small Carpathian	André
31.	Skalica	Skalický	Small Carpathian	Blaufrankise
32.	Zeleneč	Trnavský	Small Carpathian	Cabernet Sauvignon
33.	Nové Zámky	Palárikovský	Southern Slovak	Grüner Veltliner
34.	Abrahám	Galanstký	Southern Slovak	Velsch Riesling
35.	Čamovce	Fiľakovský	Central Slovak	Pálava
36.	Rimavská Sobota	Gemerský	Central Slovak	Blaufrankise
37.	Kráľ	Tornaľský	Central Slovak	Müller Thurgau
38.	Orechová	Sobranecký	Eastern Slovak	Pinot Gris
39.	Vinné	Michalovský	Eastern Slovak	Grüner Veltliner
40.	Streda na Bodrogom	Kráľovsko-chlmecký	Eastern Slovak	Tramin
41.	Hrušov	Moldavský	Eastern Slovak	Alibernet
42.	Viničky	Tokajský	Tokaj	Furmint
43.	Viničky	Tokajský	Tokaj	Lipovina
44.	Viničky	Tokajský	Tokaj	White Frontignan
45.	Bratislava - Rača	Bratislavský	Small Carpathian	Riesling
46.	Bratislava - Rača	Bratislavský	Small Carpathian	Blaufrankise
47.	Stupava	Stupavský	Small Carpathian	Grüner Veltliner
48.	Nesvady	Komárňanský	Southern Slovak	Velsch Riesling
49.	Veľký Meder	Dunajsko-Stredský	Southern Slovak	Chardonnay
50.	Šamorín	Šamorínsky	Southern Slovak	Blaufrankise

Table 2 Preparation of the chromatographic plates before visualisation of the diacetoxyscirpenol (DAS), deoxynivalenol (DON), HT-2 toxin (HT-2) and T-2 toxin (T-2) and the manifestation of visualization.

Mycotoxin	Chromatographic plate preparation	Visualisation under UV light with a wavelenght of 366 nm
DAS, DON	• application of 20% $AlCl_3$ in 60% ethanol • heating-up	light blue fluorescet spot
HT-2, T-2	• application of 20% H_2SO_4 in water • heating-up for 8 min.at 130 ^oC	green-blue fluorescent spot

Furmit (1), White Frontignan (1) and Lipovina (1). Red grape: Alibernet (1 sample), André (2 samples), Blaufrankise (8), Cabernet Sauvignon (2), Konkordia (1), Pinot noir (2), Saint Laurent (1). Informations about analysed samples are shown in Table 1. Samples (3 kg) were collected at the time of technological ripeness. Picked grapes were stored at 4 ±1 °C and analysed within 24 h after harvest.

Mycological analysis

For the isolation of *Botrytis* sp., *Fusarium* sp. and *Rhizopus* sp. was used the method of direct plating berries: surface-sterilized berries, non-sterilized and damaged berries on DRBC (Dichloran Rose Bengal Chloramphenicol agar) (Biolife, Italia) according **Samson et al. (2002)**.

The endogenous mycobiota was determined by the method of direct placing of superficially sterilized berries on agar plates **(Samson et al., 2002)**. More than 50 pieces of undamaged berries from each sample were superficially sterilized (using 1% freshly pre-pared chlorine). Sterilization was carried out for 2 minutes. Berries were rinsed 3 times with sterile distilled water and dried on sterile filter paper. Exactly 50 berries from each sample were placed on DRBC plates **(Samson et al., 2002)**. The total mycobiota (non-sterilized berries) was determined by the method of direct placing of grape berries on DBRC plates, also. Only the undamaged berries were used for analysis. For determination fungal colonization of damaged berries all berries (from 7 to 15 berries from sample) with some evident defect were used. Berries from each sample were placed on DRBC plates.

Cultivation lasted from 5 to 7 days in darkness at 25 ±1 °C (in the all cases).

Grown micromycetes were classified into the genera and then isolated by reinoculation on the identification nutrient media and identified through macroscopic and microscopic observation in accordance with accepted mycological keys and publications.

Identification of Fusarium species

Potato Dextrose agar (PDA) **(Samson et al., 2002)** was used for observation of colony characteristics. "Synthetischer nährstoffarmer agar" (SNA) **(Samson et al., 2002)** was used for micromorphological features. Cultures were incubated at the room temperature and natural light. Species identification was done after 10 days according to **Leslie and Summerell (2006), Nelson et al.**

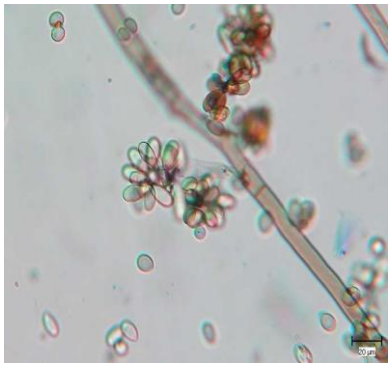

Figure 1 *Botrytis cinerea*

Figure 2 *Rhizopus stolonifer*

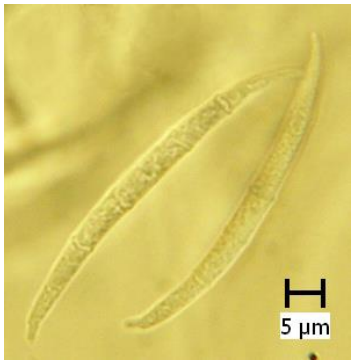

Figure 3 *Fusarium proliferatum* (macroconidia)

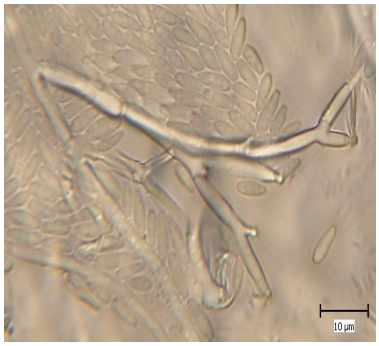

Figure 4 *Fusarium proliferatum* (conidiophres and microconidia)

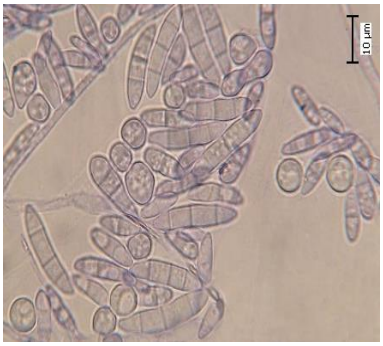

Figure 5 *Fusarium sporotrichioides* (microconidia and mesoconidia)

Figure 6 *Fusarium solani*

(1983), Pitt and Hocking (2009) and **Samson et al. (2002, 2010)**.

CYA (Czapek yeast extract agar) **(Samson et al., 2002)** and MEA (Malt extract agar) (Biomark, India) were used for species identification of *Botrytis*. Species identification was done after 10 days cultivation at 20 ±1 °C according to **Samson et al. (2002, 2010)** and **Pitt and Hocking (2009)**.

Obtained results were evaluated and expressed in isolation frequency (Fr) at the species level. The isolation frequency (%) was defined as the percentage of samples within which the species occurred at least once. These values were calculated according to **González et al. (1996)** as follows:

$$Fr\ (\%) = (ns / N) \times 100$$

Where: ns = number of samples with a species; N = total number of samples.

Mycotoxins screening by a modified agar plug method

For the determination of toxigenity a modified agar plag method using thin-layer chromatography according to the **Samson et al. (2002)**, modified by **Labuda and Tančinová (2006)** was used. A total of 47 randomly selected strains of the *F. acuminatum, F. culmorum, F. equiseti, F. graminearum, F. oxysporum, F. proliferatum, F. semitectum, F. sporotrichioides* (the most important species according to their occurrence), *F. tricinctum* and *F. verticillioides* were re-inoculated on YES (yeasts extract agar) **(Samson et al., 2002)**, cultured in the dark at a temperature of 25 ±1 °C from 7 to 14 days and then tested for the ability to produce mycotoxins deoxynivalenol (DON), diacetoxyscirpenol (DAS), HT-2 toxin (HT-2) and T-2 toxin (T-2). From the grown colonies squares of the approximate size 2 x 2 cm were cut and put them in small chunks to Eppendorf tube with 1 ml of extraction reagent chloroform : methanol, 2 : 1 (for DON) and acetonitrile (Fischer, Slovakia) : water, 50 : 50 (for DAS, HT-2, T-2). After a 5 minute mixing the extract was applied to the chromatographic plate (Alugram®SIL G, Macherey – Nagel, Germany). Subsequently, developing solution toluene : acetone : methanol (5 : 3 : 2) (Centralchem, Slovakia) were used. Before visualisation, chromatographic plates were processed as is given in Table 2. Mycotoxins were confirmed by comparison with standards (Merck, Germany) under UV light with a wavelength of 366 nm.

RESULTS AND DISCUSSION

Botrytis cinerea and *Erysiphe necator* are among the most relevant fungi in viticulture **(Lopez Pinar et al., 2016)**. *Botrytis cinerea* (Figure 1) was identified in 86% of samples in our research (Table 3, 4). Significant difference was observed in the number of isolates from Tokaj viticulturally region. From 3 samples from this region were isolated 257 isolates and only 187 isolates from 47 samples from other regions. The grapes from Tokaj viticulturally region are used for production of typical

Table 3 List of the isolated moulds of genera *Botrytis, Fusarium* and *Rhizopus* from wine grapes berries of the Slovak origin, isolated from berries, berries superficially sterilized and damaged berries.

Isolated species	Number of positive samples	Number of isolates	Isolation frequency (%)
Botrytis cinerea	1, 2, 3, 5, 6, 7, 8, 9, 11, 12, 13, 14, 15, 16, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 31, 32, 33, 35, 36, 37, 38, 39, 40, 41, **42*, 43*, 44***, 45, 46, 47, 48, 49, 50	183 +257*	86
F. acuminatum	18, 24, 25, 32, 35, 40	18	12
F. avenaceum	6, 9, 12, 19, 24, 31, 37, 41	26	16
F. culmorum	9	13	2
F. equiseti	1, 3, 4, 5, 6, 13, 20, 28, 33, 40, 46, 50	42	24
F. graminearum	1, 3, 5, 19,20, 28, 30, 48	21	16
F. oxysporum	7, 11, 12, 16, 17, 19, 20, 28, 30, 39, 48	43	22
F. proliferatum	11, 22, 23, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 37, 39, 40, 41, **44*,** 49	97 +1*	38
F. semitectum	8, 10, 18, 20, 24, 26, 34, 46	12	16
F. solani	18, 24, 31, 47	46	8
F. sporotrichioides	3, 5, 7, 8, 10, 11, 14, 15, 19, 25, 27, 28, 30, 31, 32, 33, 34, 40, 41, 45, 46, 47, 48, 49, 50	63	50
F. subglutinans	1, 7, 20, 29	27	8
F. tricinctum	4, 8, 12, 22, 29, 50	38	12
F. verticillioides	4, 12, 22, 29, 50	9	10
F. sp.	1, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 19, 20, 21, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 39, 40, 41, **44***, 46, 47, 48, 49, 50	48	82
Fusarium together	1, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, **44***, 45, 46, 47, 48, 49, 50	504	92
Rhizopus	1, 2, 3, 5, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, **43*, 44***, 45, 46, 47, 48, 49, 50	356 +17*	94

Note: ***** – samples and isolates from Tokaj viticulturally region.

Table 4 Species of *Botrytis, Fusarium* and *Rhizopus* isolated from berries of the Slovak origin determinated by using plate direct method on DRBC agar from 50 samples.

Species	Superficially sterilized berries	Berries without sterilization	Damaged berries
Botrytis cinerea	183 +257*****	354 +334*****	210 +79*****
F. acuminatum	4	10	4
F. avenaceum	16	9	1
F. culmorum	7	4	2
F. equiseti	41	1	
F. graminearum	5	13	3
F. oxysporum	18 +1*****	22	2
F. proliferatum	14	63	21
F. semitectum	3	8	1
F. solani	39	7	
F. sporotrichioides	3	49	11
F. subglutinans	21	2	4
F. tricinctum	35	3	
F. verticillioides	4	4	1
Fusarium sp.	16	25	7
Rhizopus stolonifer	89	217	67

Note: *F.* – *Fusarium,* ***** - isolates from Tokaj viticulturally region.

Table 5 Potential ability of *Fusarium* species isolates to produce mycotoxins in *in vitro* conditions, tested by TLC method.

Species	Number of tested isolates	Number of isolates without the production of mycotoxins	Mycotoxins			
			DAS	DON	HT-2 toxin	T-2 toxin
F. acuminatum	1	1	0			
F. culmorum	1	1				
F. equiseti	2	1		1		
F. graminearum	2	0	2		2	2
F. oxysporum	1	0			1	1
F. proliferatum	15	10	2		1	3
F. semitectum	2	1			1	1
F. sporotrichioides	20	0	8		11	15
F. tricinctum	1	0	1			
F. verticillioides	2	1	1			

Note: *F.* – *Fusarium,* TLC – thin layer chromatography, DON – deoxynivalenol, DAS – diacetoxyscirpenol.

sweet wine – Tokaj. It was confirmed by high incidence of isolates of *Botrytis cinerea* in the grapes from this region. "Noble rot" is a historic term indicating the *Botrytis cinerea* stage of development in grapes that has a positive impact on the overall quality of particular wines that include the famous sweet white wines **(Tosi et al., 2012)**. On the other hand, an uncontrolled growth of the pathogen in the vineyard causes losses in wine production. Undesirable effects of *Botrytis* growth then decrease the quality and quantity of grapes available for winemaking. As a result of the damaging infection, the wine making process is complicated by the formation of a haze of white wines and oxidative browning of red wines **(Perutka et al., 2016)**. Acording to **Tournas and Katsoudas (2005)** *Botrytis cinerea* is one of the most common fungi spoiling grapes and **Felšöciová et al. (2015)** reported occurrence of genus *Botrytis* in 71% of samples of grapes from Small Carpatian area.

R. stolonifer (Table 3, Table 4 and Figure 2) was identified in 94% of samples in our research. Isolates of *R. stolonifer* were detected in undamaged grape berries, and during storage or transport can be source of soft rot. *R. stolonifer* develops on mature berries in the field, during storage at temperatures above 8 °C and during shelf-life. It was isolated from naturally contaminated soils throughout the year, and from fruits. The airborne spore population increased in vineyards at the time of fruit maturation and was related to the proximity of stone-fruit orchards. The size of this population was highly correlated with disease incidence and thus may be a satisfactory tool for disease prediction. Intact young berries were more resistant than mature ones to *Rhizopus* inoculation, in both the vineyard and in the laboratory **(Lisker et al., 1996)**.

Therteen species of fusaria (*F. acuminatum, F. avenaceum, F. culmorum, F. equiseti, F. graminearum, F. oxysporum, F. proliferatum* (Figure 2 and Figure 3), *F. semitectum, F. solani* (Figure 5), *F. sporotrichioides* (Figure 4), *F. subglutinans F. tricinctum* and *F. verticilioides*) were identified (Table 3 and Table 4) (48 isolates were not determinate to the species level). **Serra et al. (2005)** shown that *Fusarium* strains were primarily detected at the early maturation stages of grapes, with and without surface disinfection. Isolation frequency of *Fusarium* was very high – 92%. Isolates were detected

from superficially sterilized berries (227 isolates), berries without sterilization (220) and damaged berries (57). Occurrence of species of genus *Fusarium* reported: **Bellí et al. (2006)** from Spanish regions, **Serra et al. (2006)** from Portuguese vineyards, **Magnoli et al. (2003)** from Mendoza (region of Argentina), **Tournas and Katsoudas (2005)** from local supermarkets in the Washington DC area, **Chunmei et al. (2013)** from Shaanxi province (China), **Lorenzini and Zapparoli (2015)** and **Lorenzini et al. (2016)** from Northern Italy, too. According to these authors, species of *Fusarium* are not classified like dominant mycobiota of grapes.

Mycotoxins are abiotic hazards produced by certain fungi that can grow on a variety of crops **(Marin et al., 20013)**. Mycotoxin risk in the grape product chain is primarily due to ochratoxin A occurrence in wine and dried vine fruits **(Somma et al., 2012)**. The regulation levels in food products are established at 10 $\mu g.kg^{-1}$ in dry grapes **(EC No. 472/2002)**, 2 $\mu g.kg^{-1}$ in must and wine **(EC No. 123/2005)**. Ochratoxin A is a secondary metabolite produced by filamentous fungi of the two genera *Aspergillus* and *Penicillium* present in a wide variety of foodstuffs **(Amézqueta et al., 2012; Vega et al., 2012)**. These two genera are main genera responsible for mycotoxin production in grapes **(Serra et al., 2006)**.

Species of genus *Fusarium* are important producers of mycotoxins, too. **Serra et al. (2005)** reported that species described as producers of mycotoxins represented 8.0% of the grape mycobiota, distributed as follows: potential produceres of aflatoxins (0.3%), ochratoxin A (6.0%), patulin (0.5%) and trichothecenes (1.2%). Selected isolates (47) of ten species were tested for their ability to produce relevant mycotoxins - trichothecenes in *in vitro* condition, by means of thin-layer chromatography. The results are presented in Table 5. Thirty-two (68%) of tested isolates were able produce at least one mycotoxin. All isolates of *F. sporotrichioides* were able to produce some mycotoxin in *in vitro* conditions. Isolates of potential producers of mycotoxins can produce more than one mycotoxin. 11 isolates of *F. sporotrichioides* produced T-2 toxin and HT-2 toxin, 4 isolates T-2 toxin, HT-2 toxin and diacetoxyscirpenol. *F. graminearum* (2 tested isolates) produced T-2 toxin, HT-2 toxin and diacetoxyscirpenol.

CONCLUSION

From 2500 surface-sterilized berries, 2500 berries without sterilization and 550 damaged berries (50 samples) wine grape berries were isolated 440 strains of *Botrytis cinerea*, 504 strains of *Fusarium* spp. and 373 strains of *Rhizopus stolonifer*. Significant difference was observed in the number of strains of *Botrytis cinerea* from Tokaj viticulturally region (3 samples – 257 strains) to another samples (47 samples – 183 strains). Chosen strains of species of genus *Fusarium* were able to produce following mycotoxins: deoxynivalenol, T-2 toxin, HT-2 toxin and diacetoxyscirpenol in *in vitro* conditions by means of thin-layer chromatography. In another research would be advisable to follow occurrence of these mycotoxins in grapes, must, wine and another products from grapes.

REFERENCES

Amézqueta, S., Schorr-Galindo, S., Murillo-Arbizu, M., González-Peñas, E. 2012. OTA-producing fungi in foodstuffs: A review. *Food Control*, vol. 26, no. 2, p. 259-268. https://doi.org/10.1016/j.foodcont.2012.01.042

Barata, A., Malfeito-Ferreira, M., Loureiro, V. 2012. The microbial ecology of wine grape berries. *International Journal of Food Microbiology*, vol. 153, no. 3, p. 243-259. https://doi.org/10.1016/j.ijfoodmicro.2011.11.025

Bautista-Baños, S., Bosquez-Molina, E., Barrera-Necha, L. L. 2014. *Rhizopus stolonifer* (soft rot). In Bautista-Baños, S. *Postharvest Decay*. Amsterdam, Netherland : Elsevier, p. 1-44. https://doi.org/10.1016/B978-0-12-411552-1.00001-6

Bellí, N., Bau, M., Marín, S., Abarca, M. L. Ramos, A. J., Bragulat, M. R. 2006. Mycobiota and ochratoxin A producing fungi from Spanish wine grapes. *International Journal of Food Microbiology*, vol. 111, suppl. no. 1, p. S40-S45.

Chunmei, J., Junling, S., Qi´an, H., Yanlin, L. 2013. Occurrence of toxin-producing fungi in intact and rotten table and wine grapes and related influencing factors. *Food Control*, vol. 31, no. 1, p. 5-13. https://doi.org/10.1016/j.foodcont.2012.09.015

Commission Regulation (EC) No. 472/2002 of 12 March 2002 amending Regulation (EC) No. 466/2001 setting maximum levels for certain contaminants infoodstuffs.

Commission Regulation (EC) No. 123/2005 of 26 January 2005 amending Regulation (EC) No. 466/2001 as regards ochratoxin A.

Felšöciová, S., Tančinová, D., Rybárik, Ľ., Mašková, Z., Kačániová, M. 2015. Mycobiota of Slovak wine grapes with emphasis on *Aspergillus* and *Penicillium* species in the Small Carpatian area. *Potravinarstvo*, vol. 9, no. 1, p. 501-508. https://dx.doi.org/10.5219/529

González, H. H. L., Pacin, A., Resnik, S. L., Martinez, E. J. 1996. Deoxynivalenol and contaminant mycoflora in freshly harvested Argentinean wheat in 1993. *Mycopathologia*, vol. 135, no. 2, p. 129-134. https://doi.org/10.1007/BF00436463

Kassemeyer, H. H., Berkelmann-Löhnertz, B. 2009. Fungi of grapes. In König, H. et al. *Biology of Microorganisms on Grapes, in Must and in Wine*. Berlin, Germany : Heidelberg : Springer – Verlag, p. 61-87. https://doi.org/10.1007/978-3-540-85463-0_4

Labuda, R., Tančinová, D. 2006. Fungi recovered from Slovakian poultry feed mixtures and their toxinogenity. *Annals of Agricultural and Environmental Medicine*, vol. 13, no. 2, 193-200. PMid:17195991

Leslie, J. F., Summerell, B. A. 2006. *The Fusarium Laboratory Manual*. Australia : Blackwell Publishing. 388 p. ISBN 978-0-8138-1919-8. https://doi.org/10.1046/j.1365-3059.1996.d01-10.x

Lisker, N., Keren-Shacham, Z., Sarig, P., Zutkhi, Y., Ben-Aire, R. 1996. The biology and pathology of the fungus *Rhizopus stolonifer*, cause of black mould disease of table grapes in Israel. *Plant Pathology*, vol. 45, no. 6, p. 1099-1109. http://dx.doi.org/10.1046/j.1365-3059.1996.d01-10.x

Lopez Pinar, A., Rauhut, D., Ruehl, E., Buettner, A. 2016. Effects of Botrytis cinerea and Erysiphe necator fungi on the aroma character of grape must: A comparative approach. *Food Chemistry*, vol. 207, p. 251-260. https://doi.org/10.1016/j.foodchem.2016.03.110 PMid:27080903

Lorenzini, M., Zapparoli, G. 2015. Occurrence and Infection of *Cladosporium*, *Fusarium*, *Epicoccum* and

Aureobasidium in withered rotten grapes during post-harvest dehydration. *Antonie van Leeuwenhoek*, vol. 108, no. 5, p 1171-1180. https://doi.org/10.1007/s10482-015-0570-8 PMid:26459338

Lorenzini, M., Cappello, M. S., Logrieco, A., Zapparoli, G. 2016. Polymorphism and phylogenetic species delimitation in filamentous fungi from predominant mycobiota in withered grapes. *International Journal of Food Microbiology*, vol. 238, p. 56-62. https://doi.org/10.1016/j.ijfoodmicro.2016.08.039 PMid:27591387

Magnoli, C., Violante, M., Combina, M., Palacio, G., Dalcero, A. 2003. Mycoflora and ochratoxin-producing strains of *Aspergillus* section *Nigri* in wine grapes in Argentina. *Letters in Applied Microbiology*, vol. 37, no. 2, p. 179-184. https://doi.org/10.1046/j.1472-765X.2003.01376.x

Marin, S., Ramos, A. J., Cano-Sancho, G., Sanchis, V. 2013. Mycotoxins: Occurrence, toxicology, and exposure assessment. *Food and Chemical Toxicology*, vol. 60, p. 218-237. https://doi.org/10.1016/j.fct.2013.07.047

Nelson, P. E., Toussoun, T. A., Marasas, W. F. O. 1983. *Fusarium species. An illustrated manual for identification*. USA : The Pennsylvania State University. 193 p. ISBN 0-271-00349-9.

Oliveri, C., Catara, V. 2011. Mycoflora and biodiversity of black Aspergilli in vineyard eco-systems. In Grillo, O. et al. *The Dynamical Processes of Biodiversity – Case Studies of Evolution and Spatial Distribution*. Rijeka, Croatia : InTech, p. 259-276. ISBN 978-953-307-772-7. https://doi.org/10.5772/24611

Perutka, Z., Šufeisl, M., Strnad, M., Šebela, M. 2016. Protein profiling of a white wine produced from grapes damaged by *Botrytis cinerea*. *New Biotechnology*, vol. 33, p. 180. https://doi.org/10.1016/j.nbt.2016.06.1346

Pitt, J. I., Hocking, A. D. 2009. *Fungi and food spoilage*. 3rd ed. London, New York : Springer Science & Business Media, LLC, 519 p. ISBN 978 0-387-92206-5. https://doi.org/10.1016/j.ijfoodmicro.2010.08.005

Samson, R. A., Houbraken, U., Thrane, U., Frisvad, J. C., Andersen, B. 2010. *Food and Indoor Fungi*. Utrecht, Netherlands : CBS-KNAW Fungal Biodiversity Centre, 390 p. ISBN 978-90-70351-82-3.

Samson, R. A., Hoekstra, E. S., Frisvad, J. C., Filtenborg, O. 2002. Introduction to food- and airborne fungi. Utrecht, Netherlands : Centraalbureau voor Schimmelcultures. 389 p. ISBN 90-70351-42-0. https://doi.org/10.5580/104b

Serra, R., Braga, A., Venâncio, A. 2005. Mycotoxin-producing and other fungi isolated from grapes for wine production, with particular emphasis on ochratoxin A. *Research in Microbiology*, vol. 156, no. 4, p. 515-521. http://dx.doi.org/10.1016/j.resmic.2004.12.005

Serra, R., Lourenço, A., Alípio, P., Venâncio, A. 2006. Influence of the region of origin on the mycobiota of grapes with emphasis on *Aspergillus* and *Penicillium* species. *Mycological research*, vol. 110, no. 8, p. 971-978. https://doi.org/10.1016/j.mycres.2006.05.010

Somma, S., Perrone, G., Logrieco, A. 2012. Diversity of black Aspergilli and mycotoxin risk in grape, wine and dried vine fruits. *Phytopathologia*, vol. 51, no. 1, p. 131-147.

Tosi, E., Fedrizzi, B., Azzolini, M., Finato, F., Simonato, B., Zapparoli, G. 2012. Effects of noble rot on must composition and aroma profile of Amarone wine produced by the traditional grape withering protocol. *Food Chemistry*, vol 130, no, 2, p. 370-375. https://doi.org/10.1016/j.foodchem.2011.07.053

Tournas, V. H., Katsoudas, E. 2005. Mould and yeast flora in fresh berries, grapes and citrus fruits. *International Journal of Food Microbiology*, vol. 105, no. 1, p. 11-17. https://doi.org/10.1016/j.ijfoodmicro.2005.05.002

Vega, M., Ríos, G., von Baer, D., Mardones, C., Tessini, C., Herlitz, E., Saelzer, R., Ruiz, M. A. 2012. Ochratoxin A occurrence in wines produced by Chile. *Food Control*, vol. 28, no. 1, p. 147-150. https://doi.org/10.1016/j.foodcont.2012.04.032

Acknowledgments:

This work was supported by Research Center AgroBioTech built in accordance with the project Building Research Centre „AgroBioTech" ITMS 26220220180 and KEGA 015SPU-4/2015.

Contact address:

Dana Tančinová, Slovak University of Agriculture, Faculty of Biotechnology and Food Sciences, Department of Microbiology, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia, E-mail: dana.tancinova@uniag.sk

Zuzana Mašková, Slovak University of Agriculture, Faculty of Biotechnology and Food Sciences, Department of Microbiology, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia, E-mail: zuzana.maskova@uniag.sk

Ľubomír Rybárik, Báb 49, 951 34 Báb, Slovakia, E-mail: alcedo1245@gmail.com

Viera Michalová, Slovak University of Agriculture, Faculty of Biotechnology and Food Sciences, Department of Microbiology, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia, E-mail: x.michalova@is.uniag.sk