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THE NATURAL OCCURRENCE OF *PENICILLIUM* SPP. METABOLITES IN MAIZE KERNELS ORIGINATING FROM SERBIA

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Abstract: Maize can be contaminated with a wide range of fungal secondary metabolites that decrease the quality and safety of maize and maize-derived products. The increase of natural fungal metabolites occurrence in maize, influenced by climate changes, is recognized as a significant issue in recent years. Therefore, the main aim of this study was to investigate the influence of weather conditions on the natural occurrence of *Penicillium* spp. metabolites in maize kernel samples. The survey was conducted for two maize vegetation seasons 2016 and 2017. In total 458 maize samples were analyzed by liquid chromatography-tandem mass spectrometry method. The samples originated from the Autonomous Province of Vojvodina, and regions of Bačka, Srem, and Banat. Among 45 *Penicillium* metabolites investigated 16 and 18 were detected in samples from 2016 and 2017, respectively. The most commonly occurring *Penicillium* metabolite in both years was oxaline, which was detected in more than 90% of analyzed samples. Questiomycin A, 7-hydroxypestalotin, pestalotin, and mycophenolic acid were also very frequently detected *Penicillium* metabolites. This is one of the unique studies in the Republic of Serbia, as well as in this part of Europe, investigating the occurrence of a great number of *Penicillium* metabolites in maize samples.

Key words: *safety, quality, weather conditions, Vojvodina Province, LC-MS/MS, oxaline*

INTRODUCTION

The *Penicillium* genus is one of the most common fungi occurring worldwide in a diverse range of habitats, from soil to vegetation, air, indoor environments, and various food products. *Penicillium* species may have diverse physiological properties and their metabolites are of great medical and industrial significance. The most significant medical application of *Penicillium* metabolites is for the treatment of bacterial diseases. A diverse physiological property of *Penicillium* metabolites is a key requirement for their widespread use in several following application areas: production of fermented foods, pharmaceuticals,

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organic acids, enzymes, and other multiple agricultural and biotechnological applications. Due to the wide industrial applications of *Penicillium* metabolites, *Penicillium* fungi are considered one of the most economically important genera (Houbraken, de Vries & Samson, 2014; Perrone & Susca, 2017; Toghueo & Boyom, 2020).

On the other hand, besides the numerous positive applications of *Penicillium* metabolites mentioned above, many negative aspects are linked to the *Penicillium* species. The main risk is related to their ability to produce a great number of different harmful metabolites, some of which belong to contaminants of worldwide concern called mycotoxins. Mycotoxins, as well as other *Penicillium* secondary metabolites, are recognized as a significant threat to human and animal health worldwide. These fungal metabolites contaminate a variety of food and feed. When ingested *Penicillium* metabolites may cause numerous negative effects on human and animal health, manifesting as acute or chronic diseases. Further, the presence of *Penicillium* species and further synthesis of secondary metabolites could influence the damage of several agronomical important foods and feed raw materials, with significant economic losses as consequences of decreased production, yield, quality, and safety (Barkai-Golan, 2008; Tola & Kebede, 2016).

Several *Penicillium* species are capable to produce many potent mycotoxins and other secondary metabolites. *Penicillium expansum, P. cyclopium, P. viridacatum,* and *P. chrysogenum* are *Penicillium* species with the greatest ability for similar production of several different metabolites (Barkai-Golan, 2008). Some of these *Penicillium* species are frequently been isolated from maize around the world (Degani, Regev & Dor, 2021). Contamination of maize with fungal species and different fungal metabolites could represent a significant risk since maize represents one of the most common worldwide used staple food and feed ingredients. The results in the available literature indicate that in Serbia *Penicillium*, besides *Aspergillus* and *Fusarium*, are the most common mycotoxigenic fungi genera isolated both from the field and stored maize (Medić-Pap, Maširević & Šofhauzer, 2011; Maširević, Medić-Pap & Birvalski, 2012; Krnjaja et al., 2015; Kos et al., 2021). Furthermore, in terms of Serbia, maize has also high socioeconomic value, since it represents one of the most significant agricultural products and export items of the country. In the last decade, Serbia is classified among the leading countries in terms of maize production and export, both in Europe and other parts of the globe. Unfortunately, due to the recent climate changes and low levels of irrigation (7-9%), maize production in Serbia is highly dependent on weather conditions (Maslac, 2019). Since weather conditions represent factors with the strongest influence on the growth and development of fungi and consequent synthesis of fungal metabolites (Medina, Rodríguez & Magan, 2015) natural occurrence of fungal metabolites in maize in Serbia also is mainly affected and highly dependent on weather conditions recorded during the maize growing season (Kos et al., 2020; Janić Hajnal et al., 2020).

Among *Penicillium* metabolites, ochratoxin A (OTA) is one of the well-known, prevalent, and toxic compounds from the ochratoxins group which includes at least nine different compounds. It was originally described as a metabolite of *Aspergillus ochraceus* (Van der Merwe, Steyn, Fourie, Scott & Theron, 1965), while later studies confirmed that *Penicillium viridicatum*, as well as other *Penicillium* species, are very common fungi that synthesize OTA. The International Agency for Research on Cancer classified OTA in group 2B as a possible carcinogenic compound to humans (IARC, 2012). OTA is detected worldwide in various food and feed sources. Studies show that this molecule can have several toxicological effects such as nephrotoxic, heaptotoxic, neurotoxic, teratogenic, and immunetoxic (Pfohl-Leszkowicz, Petkova-Bocharova, Chernozemsky & Castegnaro, 2002). Furthermore, several decades of research investigate the role of OTA in the aetiology of Balkan endemic nephropathy (BEN) and its association with urinary tract tumours (Stiborová, Arlt & Schmeiser, 2016). Scientific results from the Balkan region, published before the year 2000, reported that there were relative risk calculations indicated an association between OTA and BEN in animals and humans (Pepeljnjak & Cvetnić, 1985; Plestina et al., 1990). Although over the last 50 years, several hypotheses on the cause of BEN have been formulated, including mycotoxins (in particular OTA), heavy elements, viruses, and trace-element insufficiencies, the recent molecular epidemiological studies provide a strong case that chronic dietary exposure to aristolochic acid is the main cause of BEN (Stiborová et al., 2016).

Ochratoxin B (OTB) is the non-chlorinated ester of OTA, which could be transformed into OTA even though at a low level. OTA and OTB often co-exist in food raw materials, easily transforming into each other under special environmental conditions (El Khoury & Atoui, 2010).

Citrinin is a nephrotoxin, mainly produced by *P. verrucosum*, *P. expansum*, *P. odoratum*, and *P. westlingii*. The toxicity studies showed that it was implicated, together with OTA, as a potential causative agent in human endemic Balkan nephropathy (IARC, 1986). The European Food Safety Authority (EFSA) indicates that based on the available data concern for genotoxicity and carcinogenicity could not be excluded at the level of no concern for nephrotoxicity (EFSA, 2012). Citrinin is mainly detected as a natural contaminant of cereals, different food and feedstuffs, as well as biological fluids (Perrone & Susca, 2017).

There is no evidence of the significant important toxicity of penicillic acid. It has been claimed that penicillic acid may have cytotoxic (Gräbsch, Wichmann, Loffhagen, Herbarth & Müller, 2006), hepatotoxic, carcinogenic, and cardiotoxic effects, while their major toxicity effect could be related to their possible synergistic toxic effect with OTA (Frisvad, 2018).

Mycophenolic acid is mainly produced by *P. roqueforti*. It is a weak organic acid with antifungal, antibacterial, and antiviral activities (Cline, Nelson, Gerzon, Williams & Delong, 1969). Mycophenolic acid has well-known immunosuppressive properties. Consumption of immunosuppressive compounds increases the risk of infectious diseases in livestock, but this risk cannot be accurately estimated without knowledge of naturally occurring immunosuppressants such as mycophenolic acid in feed materials (Schneweis, Meyer, Hörmansdorfer & Bauer, 2000). Other toxigenic *Penicillium* secondary metabolites including pestalotin, 7-hydroxypestalotin, and ques-tiomycin A have been reported to be produced mainly by *P. nordicum* (Wierzbinska, 2017) while recent research indicates that *P. krskae, P. silybi*, and *P. chalabudae* also produce these metabolites (Labuda et al., 2021). Although many *Penicillium* metabolites can have toxic effects, some of them can possess positive effects. Thus, questiomycin A is desirable since it has anticarcinogenic, cardio-preventive, and antimicrobial properties (Machihara, Tanaka, Hayashi, Murakami, & Namba, 2017).

Besides *Penicillium* metabolites, listed above, there is a long list of other *Penicillium* metabolites which require more investigation in terms of producing fungi, occurrence, toxicity, positive and/or negative effects on human and animal health, etc. In general, there is a lack of data on the natural occurrence of *Penicillium* metabolites in agricultural products, as well as in food and feed, worldwide. Within the last several years, available information related to the natural occurrence of *Penicillium* metabolites in agricultural products around the world has slowly increased, particularly due to the development of analytical methods for their determination (Sulyok, Krska & Schuhmacher, 2007; Sulyok, Stadler, Steiner & Krska, 2020). Contrary to this, there are still limited studies that investigate and confirm the toxicity of different *Penicillium* metabolites. In terms of Serbia, there is only one study about the occurrence of a great number of differrent *Penicillium* metabolites in maize (Janić Hajnal et al., 2020).

Since the natural occurrence of *Penicillium* metabolites in food is recognized as significant health and economic issue worldwide, this study aimed to investigate the natural occurrence of the following 45 *Penicillium* metabolites in maize from Serbia: 7-hydroxypestalotin, anacin, andrastin A, andrastin B, aurantine, aurantiamin A, chanoclavin, citreoviridin, citreohybridinol, citrinin, cyclopenin, cycloaspeptide A, cyclopenol, dimethylsulochrin, dihydrocitrinone, desoxyverrucosidin, F01 1358-A, flavoglaucin, griseophenone B, griseophenone C, griseofulvin, meleagrin, mycophenolic acid, mycophenolic acid IV, norverrucosidin, O-methylviridicatin, OTA, OTB, oxaline, quinolactacin A, penicillic acid, penicillide, pentoxyfylline, pestalotin, pinselin, purpactin A, purpuride, pyrenocin A, puestiomycin A, puinolactacin A, roquefortine C, rugulovasine A, secalonic acid D, verrucofortine, and viridicatol.

MATERIALS AND METHODS

Samples

Samples of harvested maize were collected in 2016 and 2017 from the agricultural advisory services of Bačka, Banat, and Srem regions located in the Autonomous Province of Vojvodina, Northern Serbia. A total of four hundred and fifty-eight (n = 458) maize kernel samples, 277 from the year 2016, and 181 from 2017 were included in this study. The sampling procedure was performed according to the requirements of the European Union for sampling for the official control of the levels of mycotoxins in foodstuffs (European Commission, 2006). After sampling, approximately 10-15 kg of aggregate maize samples, were transported to the laboratory of the Institute of Food Technology in Novi Sad. The aggregate samples were homogenized (Nauta mixer, model 19387, Nauta patenten, Netherlands) and quartered to get 0.5-1.0 kg of a laboratory sample. The laboratory samples were further milled (KnifetecTM 1095 mill, Foss, Hoganas, Sweden) and stored before analysis at -20°C in the freezer.

Sample preparation

Maize samples were analyzed at the Department for Agrobiotechnology (IFA-Tulln, Austria). Before transportation of samples, the laboratory samples were taken out from the freezer, and further homogenized and quartered to give 30 g subsamples, which are placed in sealable plastic storage bags and transported into a cooler to Austria. Sample preparations were conducted following the method published by Sulyok et al. (2020) without any modifications. In brief, five grams of maize samples were extracted with 20 mL of extraction solvent (acetonitrile/water/acetic acid 79:20:1, v/v/v) for 90 min on a rotary shaker and subsequently centrifuged for 2 min at 3000 rpm. The 350 μL of the extract was diluted with the same volume of dilution solvent (acetonitrile/water/acetic acid 20:79:1, v/v/v), and further analyzed by the liquid chromatography-tandem mass spectrometry method (LC–MS/MS).

Analysis of samples by LC-MS/MS

The analysis of 45 *Penicillium* metabolites was conducted following the method published by Sulyok et al. (2020) without any modifications, as well as with the same equipment and chemicals. Detection and quantification were performed with a QTrap 5500 MS/MS system (Applied Biosystems, Foster City, CA), equipped with a TurboV electrospray ionization (ESI) source and a 1290 series UHPLC system (Agilent Technologies, Waldbronn, Germany). Both mobile phases contained 5 mM ammonium acetate and were composed of methanol/water/acetic acid 10:89:1 (v/v/v; eluent A) and 97:2:1 (v/v/v; eluent B), respectively. Quantification was based on linear, 1/x weighed calibration using serial dilutions of an external multicomponent stock solution.

Weather conditions analysis

Weather conditions parameters were taken from the official Republic Hydrometeorological Service of Serbia (http://www.hidmet.gov.rs/) for the period of maize growing seasons from 1st April to 30th September in 2016 and 2017 years. Deviations in weather condition parameters were analyzed in comparison to the data from the long-term period (1981-2010).

RESULTS AND DISCUSSION

Among 45 examined *Penicillium* metabolites a total of 16 and 18 metabolites were detected in maize samples collected in Serbia in 2016 and 2017 years, respectively. Figure 1 shows the presence of the most commonly occurring *Penicillium* metabolites. As can be seen, questomycin A (97%), oxaline (93%), 7-hydroxypestalotin (82%), and pestalotin (53%) were dominant *Penicillium* metabolites in maize samples collected in 2016. With the contamination frequency of 72, 97, 50, and 60% these four metabolites were also most frequently present in maize samples from 2017. Besides the four most frequently detected *Penicillium* metabolites the following ones were detected in maize samples from 2016: mycophenolic acid (34%), mycophenolic acid IV (27%), F01 1358-A (16%), secalonic acid D (13%), and quinolactacin A (10%). Aurantine, andrastin A, meleagrin, anacin, penicillic acid, citreohybridinol, and griseofulvin, were detected in less than 10% of maize samples from 2016 year. Further, in maize samples from 2017 secalonic acid D and meleagrin were detected with contamination frequencies of 46 and 12%, respectively; while OTA, citrinin, mycophenolic acid, pentoxyfylline, quinolactacin A, andrastin A, griseofulvin, flavoglaucin, quinolactacin A, andrastin B, and penicillide detected in less than 10% of samples. The other examined, but not previously listed, metabolites were not detected in maize samples from both years. Weather conditions are a significant factor influencing the absence or presence of some fungal metabolites. Therefore, for better interpretation of the data related to the prevalence of *Penicillium* metabolites, the analysis of the most important weather condition parameters was conducted. Figures 2 and 3 show the average air temperature and the sum of precipitation in Serbia during the period of maize planting, growing, and harvesting (from April to September 2016-2017) regarding long-term averages (1981-2010). Weather conditions analysis indicates that in the period from April to September (2016-2017), in almost every month, recorded air temperatures were higher than long-term averages air temperatures (1981-2010), which is in line with the trend of increasing air temperatures observed in the previous decade in Serbia (Kos et al., 2020). In 2016, deviations in air temperatures were between 0.5 ºC (August) to 2.3 ºC (September). Furthermore, the sum of precipitation was in line with long-term average values, except in June, during which 143% of the average sum of precipitation was recorded.

Hot and dry weather conditions dominated during the most of the maize growing season in 2017, especially during the generative phase of maize from June to August. The recorded air temperatures in April and May were around long-term average values, while in June, July, August, and September, air temperatures were 2.7, 2.0, 3.6, and 1.6 ºC above long-term average values. High air temperatures were followed by a lack of precipitation. During June, July, and August, 52, 58, and 75% of long-term precipitation averages were recorded. In 2017, high air temperatures and lack of precipitation increased the sensitivity of maize to water stress, especially during the generative phase in which maize needs the greatest amount of water. Even though during maize growing seasons in 2016 and 2017 there were deviations in weather condition parameters compared to long-term averages, extreme conditions were not recorded as in some previous years (Kos et al., 2020). The concentrations of the most frequently detected *Penicillium* metabolites in maize harvested in Serbia in 2016 and 2017 are shown in Table 1. As can be seen, only in maize from 2016, mycophenolic acid occurred at higher concentrations, while the majority of the other determined *Penicillium* metabolites were present at the same level of average concentrations, lower than 100 µg/kg.

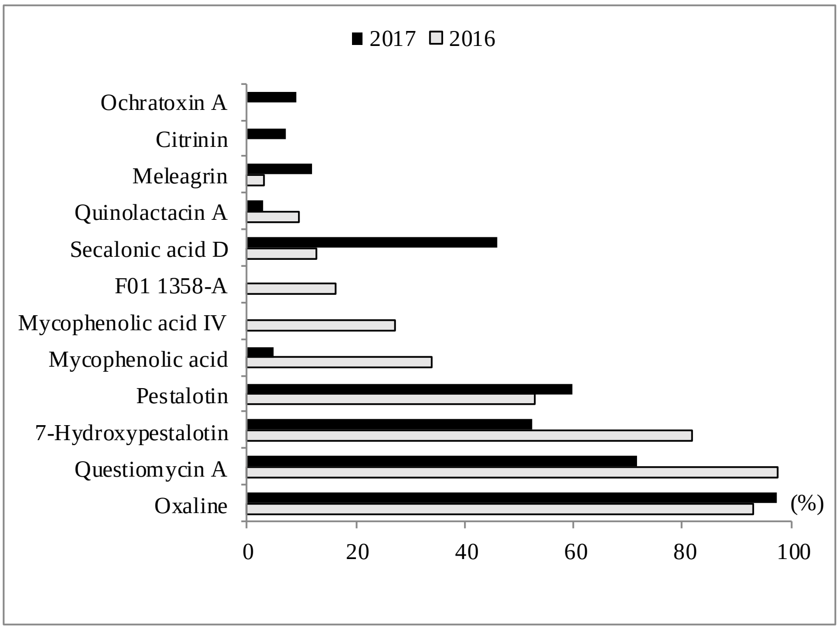

Figure 1. The frequency (%) of the most commonly occurring *Penicillium* metabolites in maize collected in the Republic of Serbia in 2016 and 2017 years

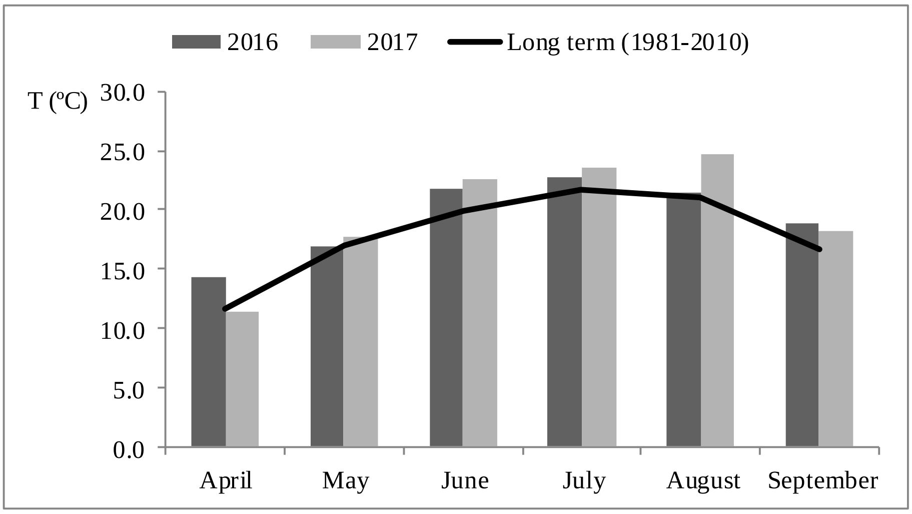

Figure 2. The average air temperature in the Republic of Serbia in the period from April to September in 2016 and 2017 years regarding long-term averages (1981-2010)

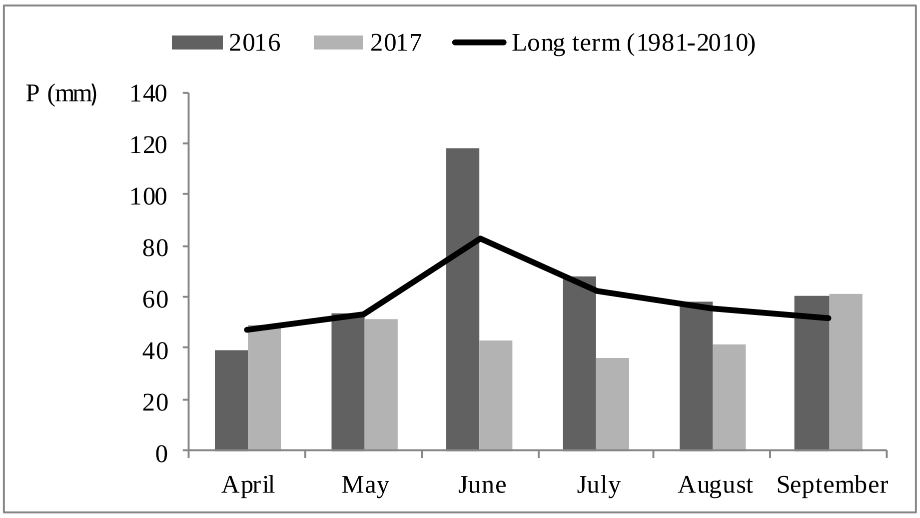

Figure 3. The average sum of precipitation in the Republic of Serbia in the period from April to September in 2016 and 2017 years regarding long-term averages (1981-2010)

Among detected concentrations, quinolactacin A occurred at the lowest level of concentrations in maize samples from both years, while average concentrations of pestalotin and 7-hydroxypestalotin in 2017 were also less than 10 μg/kg. From the obtained results it can be seen that mycophenolic acid and secalonic acid were detected in higher concentrations in maize samples in 2016 than in 2017. F01 1358-A was detected only in maize samples collected in 2016. OTA and citrinin were detected only in maize samples from 2017, while in 2016 no one maize sample was contaminated with OTA. Oxaline was detected in maize from 2017 in higher concentrations in comparison to the 2016 year. It can be assumed that differences in weather conditions, recorded in 2016 and 2017, influenced differences in the occurrence as well as the level of contamination of certain *Penicillium* metabolites. In the case of other detected *Penicillium* metabolites, there were no significant differences detected between the 2016 and 2017 growing seasons in terms of determining concentrations.

There is only one previously published study investigating the natural occurrence of *Penicillium* metabolites, other than OTA, in maize from Serbia (Janić Hajnal et al., 2020). This study included maize samples from seasons with extreme drought (2012), hot and dry conditions (2013 and 2015), and extreme precipitation (2014).

Table 1.
The concentrations of the most frequently detected *Penicillium* metabolites in maize harvested in the Republic of Serbia in 2016 and 2017.

***Penicillium* metabolite**	**Year**			
	2016		**2017**	
	Range (µg/kg)	**Average ± SD** (µg/kg)	**Range** (µg/kg)	**Average ± SD** (µg/kg)
Ochratoxin A	nd	nd	1.2–149.6	20.9 ± 38.7
Citrinin	nd	nd	12.6–102.8	38.5 ± 31.3
Oxaline	0.1–197.4	6.3 ± 19.3	0.3–710.5	32.3 ± 72.3
F01 1358-A	0.9–105.3	16.3 ±22.7	nd	nd
Quinolactacin A	0.04–6.0	0.3 ± 1.2	0.04–1.0	0.5 ± 0.4
Secalonic acid D	13.3–356.4	69.7 ± 78.6	1.7–644.0	35.7 ± 88.2
Mycophenolic acid	4.0–11540.5	975.1 ± 1924.1	17.–187.6	48.8 ± 55.0
Pestalotin	5.2–49.0	12.6 ± 9.0	0.3–146.6	7.9 ± 14.0
7-Hydroxypestalotin	4.8–91.2	22.4 ± 16.4	1.6–55.7	6.5 ± 8.0
Questomycin A	1.4–236.3	47.3 ± 37.1	5.0–163.2	34.9 ± 29.4
Meleagrin	18.5–100.2	41.1 ± 28.2	4.9–68.9	17.8 ±15.1

Average ± SD - average concentrations (µg/kg) ± standard deviation (µg/kg);
nd - not detected

Janić Hajnal et al. (2020) reported that even though different weather conditions were recorded in investigated four years the most frequently detected *Penicillium* metabolites were the same as the *Penicillium* metabolites detected in samples from 2016 and 2017 years. Figure 4 shows a comparison of the frequency of the most prevalent *Penicillium* metabolites in maize from Serbia over a period of six years (2012-2017). As can be seen from Figure 2, in maize samples collected in Serbia in the period 2012-2015 the most frequently occurring *Penicillium* metabolites were questomycin A (prevalence in 94–100% of samples), oxaline (84-100%), 7-hydroxypestalotin (84-100%), and pestalotin (74-96%).

Obtained results in this study related to secalonic acid D are comparable with the results published by Janić Hajnal et al. (2020). In 2012 year, characterized by extreme drought, the greatest prevalence (96%) of secalonic acid D was detected, followed by years also dominated by hot and dry conditions: 2013 (47%), 2017 (46%), and 2015 (14%).

The lowest prevalence of secalonic acid D was observed in the extreme wet 2014 year. Extreme drought in 2012 was also favourable for meleagrin production since 96% of maize samples were contaminated. The absence of extremely dry conditions in 2013 (39%), 2017 (12%), and 2015 (10%) was less favourable for meleagrin production, while contamination frequency of 4 and 3% indicate that weather conditions in 2014 and 2016 did not favour meleagrin production, respectively. Results obtained in this study, as well as results from our previous study (Kos et al., 2020), indicate that OTA was detected in years characterized by hot and dry conditions, while the absence of dry conditions (2016) and extreme wet conditions (2014) are not favourable for OTA synthesis. The highest concentrations, as well as the highest contamination frequency of OTA, were noticed in maize samples originating from the extreme drought maize growing season in 2012. As can be seen from Fig. 4, even though OTA was detected in maize samples from four of the six investigated years, its contamination frequency was not very high in comparison to other detected fungal metabolites. OTA was detected in 25, 2, 18, and 9% of maize samples collected in 2012, 2013, 2015, and 2017 years, respecttively. In the review study by Udovicki, Audenaert, De Saeger and Rajkovic (2018), the overview of the incidence of OTA in food from Serbia was investigated. Based on the presented results, the natural occurrence of OTA in maize in the period from 2008 to 2015 was not frequently investigated. Matić et al. (2008) reported that 8% of maize and maize product samples were contaminated with OTA, while Ljubojević et al. (2014) detected OTA in 11 out of 28 analyzed maize samples from the year 2012.

The obtained results in this study are comparable with the only available study from a neighbouring country, Croatia, investigating the occurrence of *Penicillium* metabolites in differrent grains (Kifer, Sulyok, Jakšić, Krska & Šegvić Klarić, 2021).

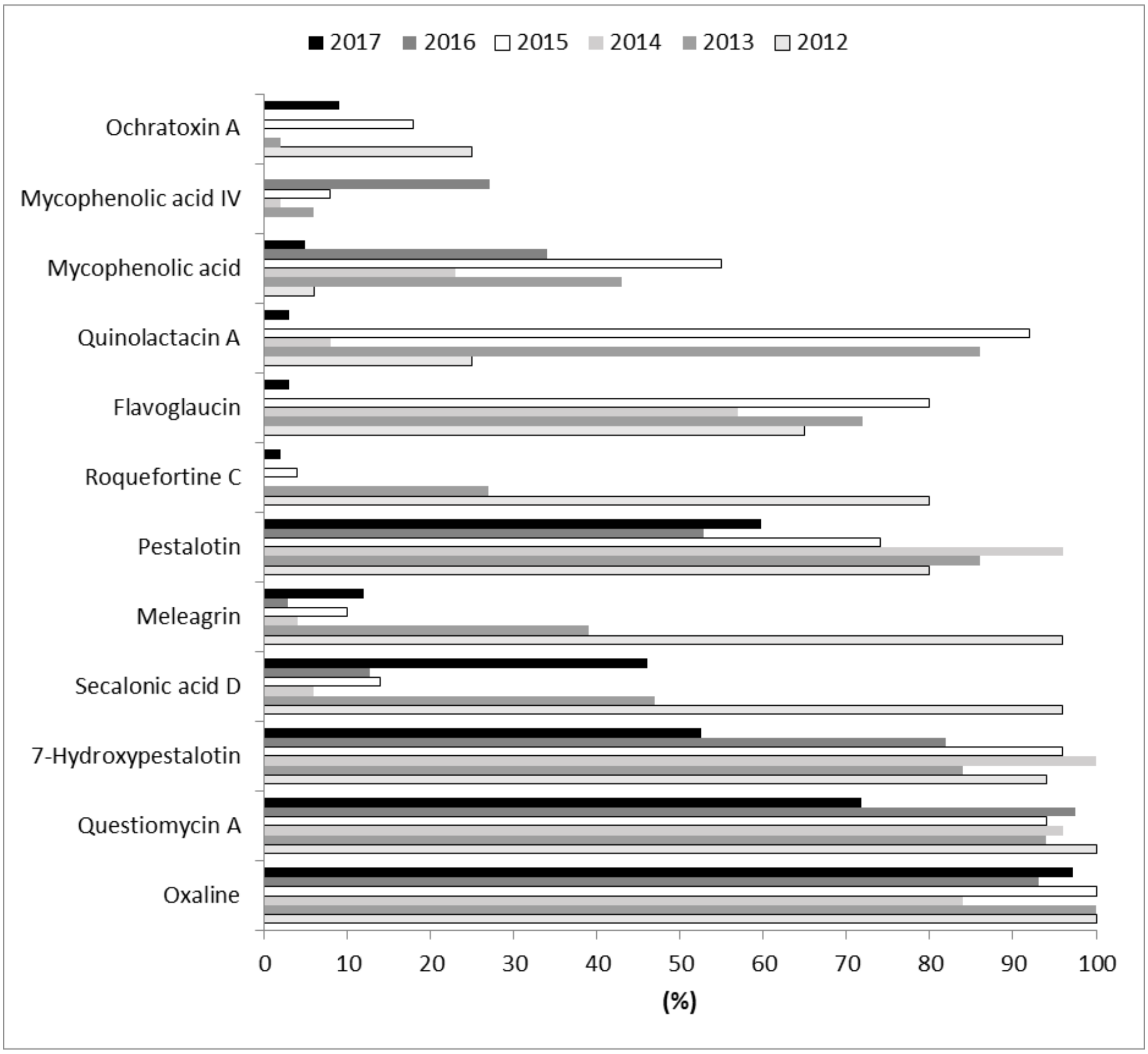

Figure 4. Comparison of the frequency (%) of the most prevalent *Penicillium* metabolites in maize from the Republic of Serbia, in the period 2016-2017 with the period 2012-2015 (Kos et al., 2020; Janić Hajnal et al., 2020)

Table 2.
The number of different *Penicillium* metabolites detected in maize harvested in Serbia in 2016 and 2017, in comparison to the years 2012, 2013, 2014, and 2015 (Janić Hajnal et al., 2020)

Number of detected *Penicillium* metabolites	**Year**					
	2012	**2013**	**2014**	**2015**	**2016**	**2017**
	20	29	17	25	16	18

The most frequent *Penicillium* metabolites detected in grains from Croatia were questionmycin A, chanoclavin, mycophenolic acid, quinolactacin A, and 7-hydroxypestalotin. The majority of the *Penicillium* metabolites occurred at relatively low median concentrations, except roquefortine C, secalonic acid D, cyclopiazonic acid, meleagrin, and mycophenolic acid in certain locations.

To the best of the author's knowledge, there are only a few other available studies investigating the occurrence of Penicillium metabolites in maize. However, these studies are mainly from African countries, and due to the differences in agricultural practices, as well as in weather conditions they cannot be completely compared with the results obtained in this study (Matumba, Sulyok, Monjerezi, Biswick & Krska, 2015; Abdallah, Girgin, Baydar, Krska & Sulyok, 2017; Getachew et al., 2018).

Table 2 shows the number of differrent *Penicillium* metabolites detected in maize examined in this study, collected in the period

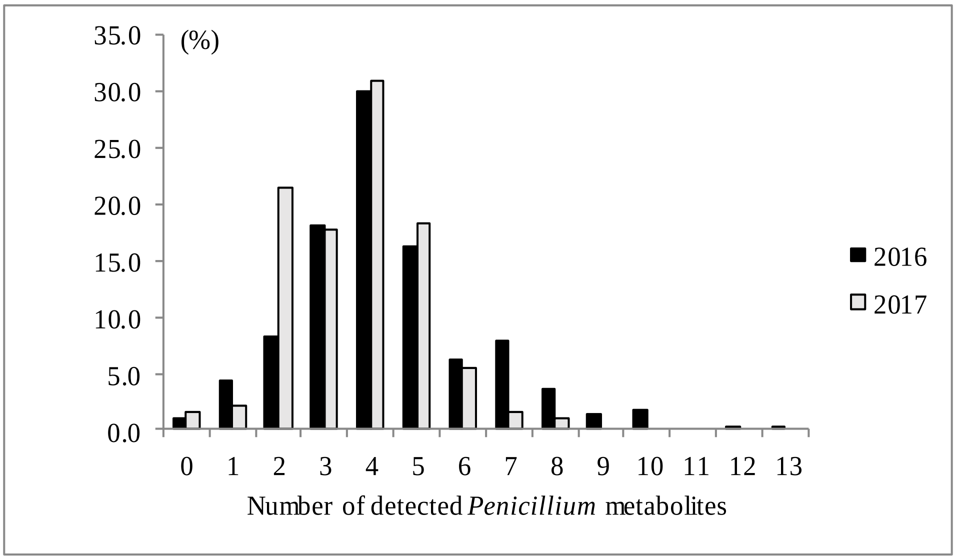

Figure 5. Percentage of contaminated samples per year with different numbers of *Penicillium* metabolites

2016-2017, in comparison to the years 2012-2015 (Janić Hajnal et al., 2020). It can be seen that maize samples, in each of the six examined years, were a mixture of between 16 to 29 different *Penicillium* metabolites.

Fig. 5 shows percentages of contaminated samples per year with a different number of detected *Penicillum* metabolites. It can be seen that the greatest percentage of contaminated samples from both years were contaminated with four different *Penicillium* metabolites, followed by three and five different metabolites. Further, in 2017 a great percentage of contaminated samples contained two *Penicillium* metabolites, while a combination of more than seven different *Penicillium* metabolites occurred very rarely. Maize samples from both years were rarely (<5%) free of *Penicillium* metabolites or contaminated with one metabolite or more than nine different metabolites. Within the maize samples collected in 2016, the maximum number of detected *Penicillium* metabolites was 13.

Although a mixture of different *Penicillium* metabolites was found in maize samples collected in Serbia for a period of six years (2012-2016), differences in weather conditions recorded in that period had a significantly higher influence on the natural occurrence of *Aspergillus* and *Fusarium* metabolites in maize in comparison to *Penicillium* metabolites (Janić Hajnal et al., 2020; Kos et al., 2021; Radić et al., 2021). Extreme drought conditions in maize growing seasons in 2012 had the greatest influence on high contamination frequency, as well as high concentrations of certain *Aspergillus* metabolites (aflatoxins, kojic acid, 3-nitropropionic acid, meleagrin, etc.). Further, hot and dry conditions in 2013, 2015, and 2017 influenced the frequent occurrence of mentioned *Aspergillus* metabolites in comparison to conditions recorded in 2014 and 2016. In terms of *Fusarium* metabolites, certain metabolites (fumonisins, moniliformin, bikaverin, and beauvericin) were detected with very high frequency (>95%) in maize samples from each year regardless of different weather conditions. On the other hand, deoxynivalenol, zearalenone, and its derivatives had the greatest frequency, as well as detected concentrations in samples from the extremely rainy 2014 year.

CONCLUSIONS

Analysis of 45 *Penicillium* metabolites in maize kernel from Serbia indicates that 16 and 18 different *Penicillium* metabolites occurred in maize samples harvested in the 2016 and 2017 years, respectively. The highest percentage of maize samples from both years was contaminated with 3 to 5 different *Penicillium* metabolites. Although extreme weather conditions were absent in the investigated period (2016-2017), in comparison to the previous years, obtained results from this study indicate that recorded weather conditions during maize growing seasons influenced the natural occurrence, as well as a level of contamination of some *Penicillium* metabolites.

The limited previous studies from Serbia indicate that several different *Penicillium* metabolites contaminated maize samples collected during different years. These studies are of great importance because, besides investigating the natural occurrence of fungal metabolites concerning weather conditions changes, they also gain insight into which *Penicillium* metabolites represent a risk due to their frequent occurrence and, consequently, which need additional examination in terms of their toxicity and possible adverse effects on human and animal health. Further, due to the frequent occurrence of different fungal metabolites, there is a need for more investigations of maize and other field crops, as well as for the development of more methods for their determination which will significantly contribute to the increase of available information.

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RASPROSTRANJENOST *PENICILLIUM* METABOLITA U KUKURUZU GAJENIM U SRBIJI

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Sažetak: Kukuruz može biti kontaminiran sa velikim brojem različitih metabolita plesni čije prisustvo znatno utiče na smanjenje kako zdravstvene ispravnosti tako i kvaliteta kukuruza i proizvoda od kukuruza. Poslednjih godina uočen je znatan uticaj klimatskih promena na sve učestaliju pojavu različitih metabolita plesni u kukuruzu. Usled navedenog, osnovni cilj ove studije bio je da se ispita uticaj vremenskih prilika na prirodnu pojavu metabolita *Penicillium* vrste u uzorcima kukuruza sakupljenih tokom 2016. i 2017. godine. Istraživanje je sprovedeno na ukupno 458 uzoraka kukuruza sakupljenih na teritoriji Autonomne Pokrajine Vojvodine, u regionima Bačka, Srem i Banat. Uzorci su analizirani primenom tečne hromatografije sa tripl kvadrupol masenim detektorom. Od ukupno 45 ispitanih *Penicillium* metabolita, u uzorcima iz 2016. godine detektovano je 16, dok je u uzorcima iz 2017 godine detektovano 18 različitih metabolita. Sa učestalosti pojave u više od 90% analiziranih uzoraka, oksalin je bio najčešće detektovan *Penicillium* metabolit u uzocima kukuruza iz obe godine. Pored oksalina, detektovana je i česta pojava kvestomicina A, 7-hidroksipestalotina, pestalotina i mikofenolne kiselina. Ovo je jedna od veoma retkih studija, kako u Republici Srbiji, tako i u ovom delu Evrope, koja se bavi istraživanjima pojave *Penicillium* metabolita u uzorcima kukuruza.

Ključne reči: *bezbednost, kvalitet, vremenske prilike, Vojvodina, LC-MS/MS, oksalin*

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