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# OCCURRENCE OF POTENTIALLY TOXIGENIC MOULD SPECIES IN FRESH SALADS OF DIFFERENT KINDS OF READY-FOR-USE VEGETABLES

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*In the mycological survey of fresh salads of different vegetables, the collected samples were tested for total counts of moulds with special attention paid to the presence of potentially toxigenic species. The survey also included the isolation and the identification of species, as well as the evaluation of mycotoxin biosynthesis ability of potential producers of ochratoxin A (OA) and sterigmatocystin (STC). Mould counts ranged from 10.0 to* $4.7\times10^2$ *cfu* $g^{-1}$. *The most common moulds found in fresh salads were Cladosporium (42.89%), Penicillium (25.78%), Aspergillus (14.67%) and Alternaria (6.89%). C. cladosporioides (40.44%), followed by A. niger (10.22%), P. aurantiogriseum (7.55%), A. alternata (6.89%) and Fusarium spp. (3.11%) were the most dominating species. Other species were represented with 2.22% (Eurotium spp.), 1.56% (Botrytis spp.), 0.67% (Phoma spp.), 0.44% (Geotrichum spp., Mucor spp., Phialophora spp.) and 0.22% (Emericella spp., Paecilomyces spp., Trichoderma spp., Xeromyces spp.). Twenty-two of 41 identified mould species were potentially toxigenic, which accounted for 46.18% of the total isolated population. The most frequent were the potential producers of ochratoxin A (17.77%). Potential producers of moniliformin were isolated in 3.11% of samples, while producers of fumonisin and STC were found in 2.67% and 2.44% of samples, respectively. The tested isolates of OA producers did not demonstrate the ability to biosynthetise this mycotoxins, but two out of five isolates of A. versicolor were found to biosynthesise STC in doses of 109.2 ng* $mL^{-1}$ *and 56.3 ng* $mL^{-1}$. *The obtained results indicate that such products may threaten human health, considering that isolated species were potentially toxigenic, while isolates of A. versicolor also biosynthesised STS.*

KEYWORDS**:** Toxigenic moulds, mycotoxins, fresh salads, vegetables.

## INTRODUCTION

Moulds comprise a large group of microorganisms, which are frequent contaminants, and causes of spoilage in many food commodities. They are not only responsible for the

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formation of offensive odours and off-flavours in food, but for the production of very toxic secondary metabolites - mycotoxins and allergenic compounds. Moulds can excrete mycotoxins into the substrate and then disappear due to unfavourable growth conditions, but the excreted toxins remain in the substrate. By consuming such food, toxic metabolites are deposited in human and animal organisms where they can cause diseases called mycotoxicoses. Mycotoxicoses are manifested with carcinogenic, hepatotoxic, mutagenic, teratogenic, cytotoxic, immunosuppressive, estrogenic and anabolic effects (1, 2). Large body of data reported worldwide and here confirms the occurrence of mycotoxins in almost all types of food commodities of either animal or plant origin, as well as in animal feed (2 -14).

By broadening our knowledge about mould species profiles, their properties, stimulatory or inhibitory factors that affect their growth, more effective methods for the control and elimination of negative effects could be established. Therefore, the aim of this study was to determine the profile of fungal population in different types of fresh salads.

## EXPERIMENTAL

### Materials

A mycological survey included samples of ready-to-use (RTU) salads made of different types of vegetables, which were diced and packed. Seventeen randomly chosen samples were obtained from supermarkets in Novi Sad (Serbia).

### Isolation and determination of total mould count

The isolation and mould counting was performed on Dichloran Rose Bengal Chloramphenicol agar (DRBC, Himedia, India). The presence of dichloran in the medium limited the growth of fast-growing moulds such as *Mucor* and *Rhizopus*, whereas chloramphenicol inhibited the bacterial growth. The dilution method by Koch was used to determine total mould counts. Serial dilutions were performed with 0.1% peptone. The inoculated medium was stored at 25°C for 5-7 days. The total mould count represented a mean of triplicate measurements.

### Mould identification

In order to obtain pure cultures and perform the identification, after the determination of total mould counts, colonies that were suspected to belong to genera *Penicillium, Aspergillus, Eurotium* and *Emericella* were re-inoculated on Chapek agar, whereas the others were inoculated on Malt Extract Agar (MAE). The inoculated media were incubated at 25°C for 7 days. Isolates belonging to the genus *Fusarium* were cultivated on Potato Dextrose Agar (PDA, Himedia, India) and Carnation Leaf Agar (CLA) to obtain monospore cultures (13-16). These cultures were incubated at 25°C under an alternating 12h UV light/12 h dark regime for 10-14 days, to stimulate the formation of conidiogenic structures.

The identification of *Fusarium* species was performed according to the methods and keys described by Nelson et al. (15), Leslie and Summerell (16) and Lević (13). Criteria proposed by Pitt and Hocking (17), Samson et al. (1), Samson and Frisvad (18) were used to identify *Penicillium* species. *Aspergillus, Eurotium* and *Emericella* species were identified by the methods of Pitt and Hocking (17) and Klich (19).

## Determination of OA and STC biosynthesis

Pure cultures of potentially toxigenic *Penicillium, Aspergillus*, *Eurotium* and *Emericella* species, isolated from the samples, were cultivated stationary in a liquid medium with the yeast extract and saccharose at 25°C for 21 days. Tests were performed in 300 mL Erlenmeyer flasks and the medium volume was 100 mL. The inocula were prepared in the following manner: conidia were skimmed from the surface of cultures grown on PDA, using approximately 5 mL of Yeast extract saccharose (YES) broth and the suspension was transferred to the Erlenmeyer flasks with YES broth. After 21-day inoculation, cultures were filtered through Whatman filter No. 1. Then, the pH (potentiometric, pH meter MA 5730 Iskra) of each filtrate was measured, as well as the mycelia weight (using data on the content of dry solids). Total content of dry solids was calculated as the difference in the mycelia weight before and after drying. Drying was conducted at 60°C 6h and at 40°C, overnight (20). The content of mycotoxin was determined in the obtained filtrate.

From the potentially ochratoxigenic species, eight isolates of *A. niger*, 10 of *P. aurantiogriseum,* and three of *P. chrysogenum* were chosen to determine ochratoxin A. The ability to synthetise STC was evaluated in two isolates of *A. versicolor*, two of *E. herbariorum* and one of *E. nidulans*.

## Qualitative OA and STC determination

The qualitative determination of ochratoxin A (OA) was conducted according to the multimycotoxin method described by Balzner et al. (21) and the STC determination was performed by thin-layer chromatography (TLC) according to van Egmond (22). The samples which were found positive to the presence of mycotoxins by the TLC method were further analysed by HPLC, to quantitatively determine the content of toxins.

## Quantitative STC determination

The STC content was determined by liquid chromatography coupled with tandem mass spectrometry, using Agilent Technologies 1200 Series Rapid Resolution liquid chromatograph with G6410A QqQ MS-MS detector with electrospray ionisation (ESI). One μL of filtered samples was injected into the system, and components were separated on Rapid Resolution HT Zorbax Eclipse XDB-C18 50 mm × 4.6 mm × 1.8 μm column (Agilent Technologies) held at 30°C. The mobile phase A consisted of 0.04 % V/V aqueous formic acid with 2 mmol $L^{-1}$ $CH_3COONH_4$, while phase B was acetonitrile. The mobile phase was delivered in the gradient mode: 0 min 75% B, 5 min 100% B, 7 min 100% B, post time 3 min (run time 10 min) with a constant flow of 0.5 mL $min^{-1}$. During the

first 3 min, the eluate was forwarded to MS without any flow splitting, and afterwards it was diverted to waste. The ESI parameters were as follows: drying gas flow ($N_2$) 9 L $min^{-1}$ , temperature 350°C, nebuliser gas pressure 40 psi, capillary voltage 4 kV (optimised). The signal was acquired in the MRM (multiple reactions monitoring) mode, using a positive polarity (resulted in fewer unwanted adducts formation, as well as, better sensitivity). The protonated ion *m/z* = 325 was used as a precursor, with products $[M+H-CO_2]^+$ *m/z* = 281 and $[M+H-CH_3^{\bullet}]^+$ *m/z* = 310 chosen as the target and qualifier ions, respectively. For both transitions, optimised conditions were: fragmentor voltage 120 V, collision cell voltage 35 V. Using reference standards, the calibration curve in 7.8-1000 ng $mL^{-1}$ range was prepared and the STC concentration was determined by the external standard method. The peak identity was confirmed by using a qualifier-to-target peak area ratio, which amounted to 0.52 ± 0.01 for standards and to 0.52 ± 0.03 for samples.

## RESULTS AND DISCUSSION

### Mycopopulation in tested samples

The presence of moulds was detected in all tested salad samples. Total mould counts ranged from 10.0 to $4.7\times10^2$ cfu $g^{-1}$. Forty one species were detected and they belonged to the genera of *Alternaria, Aspergillus, Botrytis, Cladosporium, Geotrichum, Emericella, Eurotium, Fusarium, Mucor, Paecilomyces, Phialophora, Phoma, Penicillium, Trichoderma* and *Xeromyces*. The most frequently isolated mycopopulations were *Cladosporium, Penicillium, Aspergillus* and *Alternaria* (Table 1).

**Table 1**. Frequency of mould species in fresh salads of different vegetables

| Genus | Frequency of genus (%) | Species | Frequency of species (%) |
|---|---|---|---|
| *Alternaria* | 6.89 | A. alternata (Fr.) Keissler | 6.89 |
| *Aspergillus* | 14.67 | *A. glaucus* Link | 0.67 |
| | | *A. niger* van Tieghem | 10.22 |
| | | *A. restrictus* G. Smith | 1.11 |
| | | *A. versicolor* (Vuill.) Tiraboschi | 1.78 |
| | | *A. wentii* Wehmer | 0.89 |
| *Botrytis* | 1.56 | *B. cinerea* Pers. | 1.56 |
| *Cladosporium* | 42.89 | *C. cladosporioides* (Fres.) de Vries | 40.44 |
| | | *C. macrocarpum* Preuss | 1.56 |
| | | *C. sphaerospermum* Penzig | 0.89 |
| *Geotrichum* | 0.44 | *G. candidum* Link | 0.44 |
| *Emericella* | 0.22 | *E. nidulans* (Edam) Vuill. | 0.22 |
| *Eurotium* | 2.22 | *E. amstelodami* L. Mangin | 1.33 |
| | | *E. chevalieri* L. Mangin | 0.22 |
| | | *E. herbariorum* Link | 0.44 |
| | | *E. rubrum* Jos. König et. al. | 0.22 |

**Table 1**. Continuation

| Genus | Frequency of genus (%) | Species | Frequency of species (%) |
|---|---|---|---|
| *Fusarium* | 3.11 | *F. oxysporum* Schlecht.:Fr. | 0.44 |
| | | *F. proliferatum* (Matsushima) Nirenberg | 1.78 |
| | | *F. subglutinans* (Wollenw. & Reinking) Nelson, Toussoun & Marasas | 0.89 |
| *Mucor* | 0.44 | *M. circinelloides* v. Tieghem | 0.22 |
| | | *M. hiemalis* Wehmer | 0.22 |
| *Paecilomyces* | 0.22 | *P. variotii* Bain | 0.22 |
| **Phialophora** | 0.44 | *P. fastigiata* (Legerb. & Melin) Conant | 0.44 |
| *Phoma* | 0.67 | *Ph. glomerata* (Corda) Wollenweber & Hochapfel | 0.67 |
| *Penicillium* | 25.78 | *P. aurantiogriseum* Dierckx | 7.55 |
| | | *P. bilaii* Kucey | 0.67 |
| | | *P. brevicompactum* Dierckx | 3.55 |
| | | *P. corylophilum* Dierckx | 0.22 |
| | | *P. chrysogenum* Thom | 0.44 |
| | | *P. citrinum* Thom | 0.89 |
| | | *P. commune* Thom | 0.44 |
| | | *P. expansum* Link | 3.55 |
| | | *P. funiculosum* Thom | 1.33 |
| | | *P. glabrum* (Wehmer) Westling | 2.67 |
| | | *P. implicatum* Biourge | 2.67 |
| | | *P. janthinellum* Biourge | 0.44 |
| | | *P. rugulosum* Thom | 0.22 |
| | | *P. solitum*Westling | 1.11 |
| *Trichoderma* | 0.22 | *T. harzianum* Rifai | 0.22 |
| *Xeromyces* | 0.22 | *X. bisporus* L.R. Fraser | 0.22 |

The genus *Cladosporium* dominated with three isolated species or 42.89% of total samples (Table 1). The most frequent was *C. cladosporioides* (40.44%). This species has been frequently isolated from different foods including fresh salads, wheat, flour, barley, rice, dry fish, etc. (17). Due to the psychrophilic nature of this species (able to grow at minus 5ºC) it can spoil refrigerated food such as cheese and meat (23, 24, 25). It decomposes cellulose, pectin and lipids. It is capable to grow at water activity ($a_w$) values below 0.86 at 25ºC (26), and shows resistance against microwave heating (27). *Cladosporium* has not been known to produce mycotoxins (17). Besides this species, which dominated in the tested samples, *C. macrocarpum* and *C. sphaerospermum* were also found, but with much lower frequency (from 1.56% to 0.89%). They have not been recognised as toxin producers.

The *Penicillium* population was present with the highest number of isolated species (14) (Table 1). The most frequent were *P. aurantiogriseum* (7.55%), *P. brevicompactum* (3.55%) and *P. expansum* (3.55%). *Penicillium* species widely occur in all food com-

modities and environments. Some of them are markedly xerophilic organisms (*P. brevicompactum, P. implicatum, P. chrysogenum*). Some species (*P. digitatum, P. expansum, P. italicum*) have been implicated in patogenesis of fruit diseases. Psychrotrophic species can develop at refrigeration temperatures. Some of the species isolated from the samples of fresh salads (*P. aurantiogriseum, P. chrysogenum*) have been recognised as potential producers of mycotoxins (1, 17, 18). *P. aurantiogriseum* is one of the species with the highest incidence in food and feed originating from our environment (28). It grows in a wide temperature range, from –2 to 30ºC, with the optimal temperature at 23ºC and minimal $a_w$ of 0.81 (17).

Species of the genus *Aspergillus* accounted for 14.67% of the total isolated mycopopulation (Table 1). The most frequently was *A. niger*. It occurs mainly as a storage mould, however, it can be found in fields, too. It is considered more likely in warmer climates as its optimum growth conditions range from 35 to 37ºC. This species is frequently isolated from dried commodities as it can grow at $a_w$ of 0.77. Its presence has been well documented in numerous foodstuffs: fresh fruits, vegetables, spices, nuts, cereals, meat products, dried fish, cheese (1, 17, 19). Conidia of *A. niger* are resistant to microwave and solar heating and UV irradiation. Some isolates demonstrated ability to produce OA (19). Besides *A. niger*, other *Aspergillus* species contaminated the tested samples: *A. glaucus* (sexual form *E. herbariorum*), *A. restrictus, A. versicolor* and *A. wentii*. *A. versicolor* is a potential producer of STC. This species has been reported in baby foods, cereals, nuts, foodstuffs obtained in health food stores, frozen and fermented meat products, dried sardine (1, 17).

The genus *Alternaria* is field moulds that frequently contaminate cereals, fruits and vegetables. One of the isolated species *A. alternata* grows over a wide temperature range from -5 ºC to +36ºC, the optimum temperature being 25ºC and minimum $a_w$ of 0.88 (17). It is a producer of many toxic metabolites.

Species of the genus *Fusarium* are field moulds and mostly contaminate cereals and vegetables. *F. proliferatum* was the most frequently isolated species (1.78%) from the *Fusarium* population (3.11%) (Table 1). It has been recognised as a pathogen in many plants (13). It grows at $a_w$ and temperatures ranging from 0.97 to 0.92 and from 20 to 30ºC, respectively (17). It is known as a fumonisin producer (13, 16, 17). Other potential mycotoxin producers of the genus *Fusarium* (*F. oxysporum* and *F. subglutinans*) were also isolated from the samples.

*Eurotium* species were less prevalent in the mycopopulation of fresh salads (2.22% frequency) (Table 1). *E. amstelodami* (1.33% frequency) was predominant *Eurotium* species. Similarly to *P. aurantiogriseum*, it is capable of decomposing cellulose and lignin, therefore it is important in the biodegradation of plant materials and deterioration of plant food (29). In the *Eurotium* population of fresh salads, three more species were isolated: *E. chevalieri, E. herbariorum* and *E. rubrum*. They all are xerophilic (minimal $a_w$ of 0.70). They have been isolated from cereals, nuts, dried fruits and vegetables, cheese, dried meat and fish (1, 17). *E. herbariorum* is a potential producer of STC.

Genera *Botrytis, Geotrichum, Emericella, Mucor, Paecilomyces, Phialophora, Phoma, Trichoderma* and *Xeromyces* were found in the lowest percentage of the tested samples (frequency 3.99%), represented with one species of each (Table 1).

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**Presence of potentially toxigenic species in samples of fresh salads**

According to literature data, 22 out of 41 identified species are potentially toxigenic, (Table 2), which accounts for 46.18% of the total mycopopulation of fresh salads. *Penicillium* species showed the highest frequency (19.52%) among tested samples. Secondary metabolites of *Penicillium* spp. differ in nature and many of them have been listed as mycotoxins. The most important toxins are ochratoxin A – OA (carcinogenic and nephrotoxic), citrinin (nephrotoxic), xantomegnin, viomellein and vioxantin (nephro- and heaptotoxic), nephrotoxic glycopeptides, verrucosidin (neurotoxin), patulin and penicillic acid (general mycotoxins) and penitrem A (neurotoxin) (1). Some secondary metabolites with unknown toxicity towards vertebrates can be used as indicators of toxigenic species. For example, anicin and verucin A have been produced by only four *Penicillium* species, which are known to produce nephrotoxins (OA, nephrotoxic glycopeptides): *P. nordicum, P. verrucosum, P. polonicum* and *P. aurantiogriseum* (30). Anicin and verucin A (31, 32) are easily identified by HPLC as opposed to nephrotoxic toxins whose structure remains unknown.

Potentially toxigenic *Aspergillus* species accounted for 12.0% of the total isolated mycopopulation. Within this genus, producers of OA (*A. niger*) and STC (*A. versicolor*) prevailed (Table 2).

*A. alternata,* the only isolated *Alternaria* species with a relatively high frequency (6.89%) in tested samples (Table 2), is known to produce several mycotoxins, of which the most important is tenuazoic acid. Toxins of *A. alternate* (AAT) are highly toxic metabolites with a structure similar to fumonisins. The production of one or more of the toxins was reported in tomato, wheat, barley, maize, Chinese sugar cane, rape seed, olives and spices (17). A maximal production of alternariol, its monomethylether and altenuene, was established at 25ºC and 0.98 $a_w$ (33), and tenuazonic acid at 0.90 $a_w$ and 25ºC (34).

Potentially toxigenic *Fusarium* species were much less present (3.11%). They are potential producers of moniliformin and fumonisins (Table 2).

Out of four *Eurotium* species, three were potential producers of toxic metabolites which constituted 2.09% of the mycopopulation of salads. In this genus, the potential producers of echunilin and STC were dominant (Table 2). Another species, producers of STC, were the species of the genera *Emericella* and *E. nidulans* with 0.22% frequency. Among toxigenic species, *Paecilomyces variotii* (0.22%), a producer of patulin and viriditoxin, was also isolated (Table 2).

Generally, potential producers of OA were present in the highest percentage (17.77%). Producers of moniliformin, fumonisins and STC consisted 3.11%, 2.67% and 2.44% of the mycopopulation, respectively. In tested samples, aflatoxigenic species were not detected.

**Table 2**. Frequency of potentially toxigenic species isolated from samples of fresh salads and the list of their toxins (1, 13, 16, 17, 18, 19)

| Species | Toxin | Mould frequency (%) |
|---|---|---|
| *Alternaria alternata* | alternariol, alternariol monomethyilether, alterotoxin I and II, altenuene, tenuazonic acid, | 6.89 |
| *Aspergillus niger* | naphtho- 4-pyrones, malphormins, ochratoxin A (few isolates) | 10.22 |
| *A. versicolor* | sterigmatocystin, nidulotoxin | 1.78 |
| *A. wentii* | emodin, ventilacton | 0.89 |
| *Eurotium amstelodami* | echinulin, physcion | 1.33 |
| *E. chevalieri* | echinulin, neoechinulin, physcion (according to some authors) | 0.22 |
| *E. herbariorum* | echinulin, physcion, sterigmatocystin | 0.44 |
| *Emericella nidulans* | sterigmatocystin, emestrin | 0.22 |
| *Fusarium oxysporum* | moniliformin, zearalenone, beauovericins, enijatine, fusarin C, wortmannin, nivalenol, fusarenone X, sambutoxin, fusaric acid, naphthoquinone pigments, nectriafurone, gibepyrones | 0.44 |
| *F. proliferatum* | fumonisins B1, B2, B3, beauovericins, fusaroproliferin, fusaric acid, fusarin C, moniliforme, naphthoquinone pigments, fusapyrone | 1.78 |
| *F. subglutinans* | moniliforme, fumonisin B1, fusaric acid, fusaproliferin, hlamidosporol, beauovericins, naphthoquinone pigments | 0.89 |
| *Paecilomyces variotii* | patulin, viriditoxin | 0.22 |
| *Penicillium aurantiogriseum* | penicillic acid, verrucosidin, nephrotoxic glycopeptides, anacine, auranthine, aurantiomine, ochratoxin A | 7.55 |
| *P. brevicompactum* | botryodiploidin, mycophenolic acid, Raistrick phenols, brevianamide A | 3.55 |
| *P. chrysogenum* | roquefortine C, meleagrin, chrysogine, penicilline, ochratoxin A | 0.44 |
| *P. citrinum* | citrinin, tanzawaic acid A | 0.89 |
| *P. commune* | cyclopiazonic acid, rugulovasine A i B, cyclopaldic acid | 0.44 |
| *P. expansum* | roquefortine C, patulin, citrinin, communesins, chaetoglobosin C | 3.55 |
| *P. glabrum* | citromycetin | 2.67 |
| *P. janthinellum* | janthitrem | 0.44 |
| *P. rugulosum* | rugolosin | 0.22 |
| *P. solitum* | cyclopenin, cyclopenp. compactins | 1.11 |

**Biosynthesis of OA and STC by mould originating from fresh salads**

Although ochratoxigenic species were present in the highest percentage, some of the examined isolates did not show OA biosynthesis capabilities (Table 3). Abarca et al. (35)

mentioned impaired ochratoxin production by *A. niger* (two out of 19 isolates). *P. aurantiogriseum* and *P. chrysogenum* have been frequently reported as producers of penicillic acid and roquefortin C (1, 17). These findings are in conformity with the results obtained in the study of Dimić (1999). Tested isolates of *P. aurantiogriseum* (5) and *P. chrysogenum* (6) did not show capability to produce OA. However, ochratoxin producing activity of *P. aurantiogriseum* was reported by Škrinjar et al. (36). Thirty eight percent of isolates were found to be able to synthesise this toxin on sterilised wheat kernels in concentrations ranging form 40.0 to 65.0 μg $kg^{-1}$.

A small increase in the pH (6.6) in comparison to the initial value was observed in two isolates of *A. niger*, whereas it slightly dropped in the isolates of *Penicillium* spp. (Table 3). The differences in mycelia weights were negligible.

**Table 3.** Biosynthesis of ochratoxin A by *Aspergillus niger* and *Penicillium* spp. originating from fresh salads of various vegetables

| Species (isolates) | pH | Dry matter (g 100 $mL^{-1}$) | Ochratoxin A (ng $mL^{-1}$) |
|---|---|---|---|
| *A. niger* (S1) | 4.80 | 4.69 | nd |
| *A. niger* (S2) | 6.62 | 4.64 | nd |
| *A. niger* (S3) | 7.25 | 4.13 | nd |
| *A. niger* (S4) | 2.61 | 4.94 | nd |
| *A. niger* (S5) | 6.60 | 4.32 | nd |
| *A. niger* (S6) | 7.13 | 4.29 | nd |
| *A. niger* (S7) | 4.30 | 4.42 | nd |
| *A. niger* (S8) | 3.80 | 4.51 | nd |
| *P. aurantiogriseum* (S1) | 3.25 | 2.87 | nd |
| *P. aurantiogriseum* (S2) | 3.73 | 3.58 | nd |
| *P. aurantiogriseum* (S3) | 4.07 | 3.90 | nd |
| *P. aurantiogriseum* (S4) | 3.45 | 3.42 | nd |
| *P. aurantiogriseum* (S5) | 4.00 | 2.56 | nd |
| *P. aurantiogriseum* (S6) | 4.15 | 3.87 | nd |
| *P. aurantiogriseum* (S7) | 4.20 | 4.03 | nd |
| *P. aurantiogriseum* (S8) | 3.75 | 3.45 | nd |
| *P. aurantiogriseum* (S9) | 4.34 | 4.18 | nd |
| *P. aurantiogriseum* (S10) | 4.78 | 3.70 | nd |
| *P. chrysogenum* (S1) | 5.10 | 3.35 | nd |
| *P. chrysogenum* (S2) | 4.78 | 3.15 | nd |
| *P. chrysogenum* (S3) | 5.60 | 3.47 | nd |

nd – not detected

STC was synthesised by isolates of *A. vesicolor*, whereas other potential producers did not exhibit this ability (Table 4). Current studies implied to frequent occurrence of STC positive strains of *A. versicolor*. Eighteen out of total 58 tested isolates, showed the ability to synthesise the toxin (37), whereas Mills and Abramson (38) found that 30 iso-

lates of total 32 were toxigenic. A high toxigenic potential of this species was also confirmed by Halls and Ayres (39). Škrinjar and Ač (36) found STC in seven isolates out of total nine. Dimić (4) reported that 90% of ten *A. versicolor* strains produced STC. In relation to the initial pH (6.6), a small drop of its value was observed in all strains. Similarly to the OA synthesis, the differences between the mycelial weights were minor.

**Table 4.** Biosynthesis of sterigmatocystin by *Aspergillus* spp. and *Eurotium* spp. originating from fresh salads of different vegetables

| Mould isolates | pH | Dry matter (g 100 $mL^{-1}$) | Sterigmatocystin (ng $mL^{-1}$) |
|---|---|---|---|
| *A. versicolor* S1 | 5.43 | 3.52 | 109.2 |
| *A. versicolor* S2 | 5.11 | 3.05 | 56.3 |
| *E. nidulans* S1 | 5.7 | 2.85 | nd |
| *E. herbariorum* S1 | 5.25 | 3.48 | nd |
| *E. herbariorum* S2 | 4.93 | 4.07 | nd |

## CONCLUSION

Moulds were detected in all salad samples. Considering that isolated species were potentially toxigenic, while isolates of *A. versicolor* also biosynthesised STS, consummation of such salads could endanger public health. A successful programme aimed at subduing their growth should include actions that prevent spore germination and their spread in the environment. The formation of mycotoxins is in relation with the mycelial growth. Upgrading the hygienic conditions during processing, lowering the humidity, pH, temperature, addition of natural and synthetic preservatives, are all means by which the mould growth and consequently, the mycotoxin production, in raw materials and food commodities could be decreased.

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# ПОЈАВА ПОТЕНЦИЈАЛНО ТОКСИГЕНИХ ВРСТА ПЛЕСНИ У САЛАТАМА ОД РАЗЛИЧИТИХ ВРСТА ПОВРЋА СПРЕМНИХ ЗА КОНЗУМИРЊЕ

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У узорцима свежих салата од различитих врста поврћа спремних за конзумирање одређен је укупан број плесни, извршена њихова изолација и идентификација, са посебним освртом на потенцијално токсигене врсте. Одабрани потенцијални продуценти охратоксина А (ОА) и стеригматоцистина (STC) су испитани на биосинтезу ових микотоксина.

Укупан број плесни у испитиваним узорцима се кретао од 10,0 до $4,7\times10^2$ cfu $g^{-1}$. У укупној микопопулацији доминирали су родови *Cladosporium* (42,89%), *Penicillium* (25,78%), *Aspergillus* (14,67%) и *Alternaria* (6,89%). Доминантна врста је била *C. cladosporioides* (40,44%), затим следе *A. niger* (10,22%), *P. aurantiogriseum* (7,55%), *A. alternata* (6,89%) и *Fusarium* spp. (3,11%). Остале врсте су биле заступљене са 2,22% (*Eurotium* spp.), 1,56% (*Botrytis* spp.), 0,67% (*Phoma* spp.), 0,44% (*Geotrichum* spp., *Mucor* spp., *Phialophora* spp.) и 0,22% (*Emericella* spp., *Paecilomyces* spp., *Trichoderma* spp., *Xeromyces* spp.)

Од 41 идентификоване врсте плесни 22 су према литературним подацима биле потенцијално токсигене, што је чинило 46,18% укупно изоловане микопопулације. У највећем проценту су били заступљени потенцијални продуценати ОА (17,77%). Потенцијани продуценти монилиформина су били заступљени са 3,11%, фумонизина са 2,67% и STC са 2,44%.

Испитивани изолати потенцијалних продуцената ОА нису показали способност синтезе овог микотоксина, док су изолати *A. versicolor* биосинтетисали STC у концентрацијама од 56,3 и 109,2 ng $ml^{-1}$.

Добијени резултати показују да овакви производи могу представљати опасност по здравље људи, с обзиром да су изоловане потенцијално токсигене врсте, а изолати *A. versicolor* су и биосинтетисали STC.

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