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Research article

MYCOTOXIN CONTAMINATION OF FRESH BERRIES AND FRUITS MARKETED IN THE CENTRAL REGION OF RUSSIA

I.B. Sedova[1], Z.A. Chalyy[1], N.R. Efimochkina[1], I.E. Sokolov[1], V.A. Koltsov[2], T.V. Zhidekhina[2], S.A. Sheveleva[1], V.A. Tutelyan[1,3]

[1]Federal Research Centre of Nutrition, Biotechnology and Food Safety, 2/14 Ustinskii proezd, Moscow, 109240, Russian Federation
[2]I.V. Michurin Federal Research Centre, 30 Michurin Str., Michurinsk, Tambov region, 393774, Russian Federation
[3]I.M. Sechenov First Moscow State Medical University of the Ministry of Health of the Russian Federation, 8 Trubetskaya Str., bldg 2, Moscow, 119991, Russian Federation

New emerging strains of toxigenic molds in agricultural areas and insufficient data on levels of their toxic metabolites occurring in domestic horticultural fruits and berries require risk assessment of MT contamination for this plant group of mass consumer products.

This study concentrated on samples of fresh fruits and berries sold on the consumer market (185 samples, including 127 intact and 58 with signs of deformation and molding). We applied our own developed technique for quantification of mycotoxins based on HPLC-MS/MS.

In this study, we were the first in the RF to examine contamination of garden strawberries, raspberries, currants, huckleberries, blueberries, gooseberries, dogwood, plums, blackthorn, apples, pears) with 27 MT including poorly studied emergent MT (EMT), produced by Aspergillus, Penicillium, Fusarium and Alternaria.

Strawberries, gooseberries, black currants and raspberries turned out to be the most contaminated with MT; red currants, apples and pears were less contaminated. The greatest variety of MT and EMT species was found in strawberries (20 MT), gooseberries (8 MT), black currants (7 MT) and raspberries (6 MT).

Among the regulated MT, fumonisins B1 and B2, deoxynivalenol, zearalenone, T-2 toxin, ochratoxin A and aflatoxin B1 were detected in intact strawberries; patulin, in raspberries; deoxynivalenol and zearalenone, in black currant. As for damaged and moldy berries and fruits, the list of detectable toxins was expanded, primarily due to the detection of several types of unregulated EMTs. EMT tenuazonic acid was mainly detected in moldy berries; its levels increased manifold in almost all species, except for strawberries in which penicillic acid prevailed.

These new data on MT contamination in fruits and berries indicate the necessity to perform in-depth hygienic assessment of such products sold on the Russian market to identify MT, EMT and their producers. The obtained results will be used to identify hazards at the first stage in risk assessment with its focus on MT and EMT contamination of fresh fruits and berries.

Keywords: *mycotoxins, emergent mycotoxins, strawberry, raspberry, contamination, HPLC-MS/MS.*

Irina B. Sedova – Candidate of Biological Sciences, Senior Researcher at the Laboratory for Enzymology of Nutrition (e-mail: isedova@ion.ru; tel.: +7 (495) 698-53-65; ORCID: https://orcid.org/0000-0002-6011-4515).

Zakhar A. Chalyy – Junior Researcher at the Laboratory for Enzymology of Nutrition (e-mail: brew@ion.ru; tel.: +7 (495) 698-53-65; ORCID: https://orcid.org/0000-0002-9371-8163).

Natalia R. Efimochkina – Doctor of Biological Sciences, Leading Researcher at the Laboratory of Biosafety and Nutrimicrobiome Analysis (e-mail: karlikanova@ion.ru; tel.: +7 (495) 698-53-83; ORCID: https://orcid.org/0000-0002-9071-0326).

Ilya E. Sokolov – Junior Researcher at the Laboratory of Food Toxicology and Nanotechnology Safety Assessment (e-mail: sokolov.iliya1993@gmail.com; tel.: +7 (495) 698-53-68; ORCID: http://orcid.org/0000-0003-2819-6001).

Vladimir A. Koltsov – Candidate of Agricultural Sciences, Senior Researcher at the Laboratory of Advanced Post-Harvest Technologies (e-mail: kolcov.mich@mail.ru; tel.: +7 (47545) 2-07-61; ORCID: https://orcid.org/0000-0002-2841-6126).

Tatiana V. Zhidekhina – Candidate of Agricultural Sciences, Head of the Berry Crops Department (e-mail: berrys-m@mail.ru; tel.: +7 (47545) 2-07-61; ORCID: https://orcid.org/0000-0001-9543-7069).

Svetlana A. Sheveleva – Doctor of Medical Sciences, Head of the Laboratory of Biosafety and Nutrimicrobiome Analysis (e-mail: sheveleva@ion.ru; tel.: +7 (905) 521-97-21; ORCID: https://orcid.org/0000-0001-5647-9709).

Victor A. Tutelyan – Academician of the Russian Academy of Sciences, Doctor of Medical Sciences, Professor, Head of the Laboratory for Enzymology of Nutrition; research supervisor (e-mail: tutelyan@ion.ru; tel.: +7 (495) 698-53-46; ORCID: https://orcid.org/0000-0002-4164-8992).

The Russian market for fresh fruits and berries has been growing quite steadily. According to the Russian Federal State Statistics Service (Rosstat), this year harvests of fruits and berries are higher by 8.1 % than last year ones and have reached 2.6 million tons[1]. Development of intensive gardening and implementation of technologies that allow long-term storage of harvests have enabled Russian producers to reduce dependence on imports on the Russian market. Increasing the availability of fruits and berries cultivated in our country has led to a substantial growth in their consumption.

Fruits and berries are a significant component in human diets. They are an important source of vitamins, microelements, and biologically active substances, including antioxidants; the latter protect the human body from oxidative stresses and prevent ageing. Based on the principles of healthy nutrition and the need to increase the consumption of fresh fruits and berries, the Order of the Russian Ministry of Healthcare approved the norms for the consumption of fresh fruits per person in the amount of 100 kg per year[2].

Cultivated fruits and berries, like other agricultural plants, are known to be susceptible to microbial infection and spoilage during vegetation, harvesting and storage. The major role in these processes belongs to fungi from *Fusarium*, *Penicillium*, *Alternaria*, *Aspergillus*, *Geotrichum*, *Rhizopus*, *Botrytis*, *Cladosporium*, *Sclerotinia*, *Colletotrichum*, and *Phytophthon* genus; many of them produce dangerous mycotoxins (MTs) [1–4]. Identified fungi species can vary depending on farming or storing conditions and this involves changes in an MT spectrum [5]. In addition to well-known and controlled MTs, it is quite possible to identify toxic fungi metabolites that are currently unregulated. These emergent MTs [6–8] should be investigated and hazards posed by their occurrence in foods should be assessed. Occurrence of new and poorly explored mold strains in agricultural areas [9–11] and insufficient data on MT contamination in domestically grown fruits and berries justify the necessity to explore the nature and levels of MT and EMT contamination in this plant group of mass consumer products.

The greatest health risk caused by MTs is associated with their chronic intake with consumed foods. MT occurrence in a food chain is a key concern due to their ability to produce adverse toxic effects even when contamination levels are rather low. The sanitary legislation in the EAEU set contents of only one MT, patulin (PAT); its levels in fruits and berries (apples, tomatoes, sea-buckthorn, viburnum berries and products made of them) are regulated in the Customs Union Technical Regulation 'On food safety'[3] (CU TR 021/2011).

Growing consumption of fresh fruits and berries by the RF population can be directly linked to a growing MT burden that has not been estimated before due to, among other things, absence of available highly sensitive methods for their identification. The authors of the present study have developed methods for quantitative analysis of a wide MT range [12]; these methods made it possible to estimate MT occurrence in food grains and in some non-grain plant products including dried fruits, coffee, cocoa, tea, spices, etc., given different threshold values at levels below the limit of detection.

People are exposed to various MTs consumed with foods. It is known that MTs could

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[1] Valovoi sbor plodov, yagod, vinograda, chainogo lista i khmelya po Rossiiskoi Federatsii (po kategoriyam khozyaistv) [The gross harvest of berries, grapes, tealeaves and hops in the Russian Federation (as per categories of farms)]. *Rosstat*, 2022. Available at: https://rosstat.gov.ru/search (June 06, 2022) (in Russian).

[2] Ob utverzhdenii Rekomendatsii po ratsional'nym normam potrebleniya pishchevykh produktov, otvechayushchikh sovremennym trebovaniyam zdorovogo pitaniya: prikaz Ministerstva zdravookhraneniya Rossiiskoi Federatsii ot 19 avgusta 2016 g. № 614 (s izmeneniyami na 1 dekabrya 2020 goda) [On Approval of the Recommendations on rational standards of food products consumption that conform to the up-to-date requirements of healthy diet: the Order by the RF Public Healthcare Ministry issued on August 19, 2016 No. 614 (last edited on December 1, 2020)]. *KODEKS: electronic fund for legal and reference documentation*. Available at: https://docs.cntd.ru/document/420374878 (June 06, 2022) (in Russian).

[3] TR TS 021/2011. O bezopasnosti pishchevoi produktsii: Tekhnicheskii reglament Tamozhennogo soyuza [CU TR 021/2011. On food safety: the Customs Union Technical Regulation]. *The Eurasian Economic Commission*. Available at: http://www.eurasiancommission.org/ru/act/texnreg/deptexreg/tr/Pages/PischevayaProd.aspx (October 12, 2022) (in Russian).

produce a variety of adverse combine health effects [13–15]. Thus it is essential to carry out mycotoxins survey in fresh fruits, berries and vegetables considering all data, including low detectable levels.

In this study, we aimed to examine the nature and levels of contamination with regulated MTs, their derivatives and emergent MTs in domestically grown fruits and berries that are sold on the consumer market in the RF.

Materials and methods. Samples of fresh fruits and berries were brought from the Tambov region (Michurinskii and Morshanskii districts); some samples were bought in retail outlets in Moscow and the Moscow region. A total of 185 samples were examined (apples, pears, strawberries, raspberries, black and red currant, dogwood, gooseberries, plums, blackthorn, huckleberries and blueberries). The research objects were the sorts of fruits and berries that were the most resistant to non-quarantine diseases typical for plants cultivated in the Tambov region.

MT levels were identified with high performance liquid chromatography - tandem mass spectrometry (HPLC-MS/MS). MTs identified in fruits and berries included those regulated in plant foods such as ochratoxin A (OTA), patulin (PAT), deoxynivalenol (DON), fumonisins B1 and B2 (FB1, FB2), T-2 toxin, aflatoxin B1 (AFL B1), zearalenone (ZEN); their derivatives and structural analogues (aflatoxins B2, G1, G2, HT-2 toxin, β-zearalenol (β-ZEL). We also identified occurrence of MTs that are not currently regulated such as penicillic acid (PA), nivalenol (NIV) and emergent MTs such as sterigmatocystin (STC), mycophenolic acid (MPA), moniliformin (MO), citrinin (CIT), enniatins A and B (ENN A and ENN B), beauvericin (BEA), tenuazonic acid (TeA), tentoxin (TEN), alternariol (AOH), its methyl ether (AME) and altenuene (ALT).

Sample preparation. A sample was first powdered and mixed thoroughly; a portion weighing 10 grams was then put into a 50-ml centrifuge tube and added 10 ml of acetonitrile/water mix (80/20 % vol.) with acetic acid solution (1 % of volume). Extraction was performed during 30 minutes; first in a shaker (10 minutes), then in an ultrasound bath (10 minutes), and again in a shaker (10 minutes).

A resulting extract was centrifuged for 5 minutes at 7000 rpm. 1 cm^3 of the extract was put into an eppendorf tube and added 1 cm^3 of methanol, mixed, centrifuged again and then the supernatant was poured into chromatography vials for analysis. Each sample was analyzed in two replicates.

MTs were quantified with Waters Xevo liquid chromatography tandem mass spectrometry system with triple quadrupole mass spectrometer detector (electrospray ionization-ion trap) with the heated source. The device was controlled with MassLynx V4.2 software. The source had the following parameters: capillary voltage was 0.5 kV; cone voltage, 3 V; the source temperature, 500 ºC; desolvation temperature, 500 ºC; gas flow in the cone, 150 L/Hr; desolvation gas flow, 1000 L/Hr; collision gas flow, 0.15 cm^3/min; nebulizer pressure, 7 Bar. Analytes were divided onto a column filled with silica gel that used octadecylsilane as its stationary phase (Zorbax SB-C18, 150 × 4.6 mm, 3.5 μm, pore size was 80 Å, and carbon share was 10 %, Agilent). The column temperature was 30 °C; the autosampler temperature, 4 °C. The eluent flow rate was 0.5 cm^3/min. The injection volume was 10 mm^3. Table 1 provides data on parameters of MT detection within Selected Reaction Monitoring (parent and daughter ions, fragmentor voltage and collision energy).

The following mobile phases were used in the analysis: (A), water/methanol (95/5 % vol.); (B), methanol/water (5/95 % vol.); both phases were modified with 10 мM of ammonium acetate. The gradient scheme in positive polarity was as follows: the start at 10 % B; the 7th minute, 75 % B; the 17th–19th minutes, 100 % B; the column equilibration from 19.5th to 24th minute at 10 % B. The gradient scheme in negative polarity was as follows: the start at 0 % B; 1 minute, 0 % B; the 7th minute, 70 % B; the 15th–17th minute, 100 % B; the column equilibration from 19.5 to 22nd minute at 0 % B.

Table 1

MT detection parameters for HPLC-MS/MS

Analyte	Parent ion, m/z	Daughter ions*, m/z	Fragmentor voltage, V	Collision energy, V	Retention time, min
Negative polarity					
AME	271.112 [M-H]$^-$	**255.974**	74	22	15.7
		182.992	74	48	
		255.974	74	22	
PA	169.062 [M-H]$^-$	**109.982**	14	8	8.82
		92.951	14	14	
		125.059	14	8	
ZEN	317.445 [M-H]$^-$	**130.915**	82	28	14.75
		174.979	82	24	
		149.045	82	22	
CIT	281.180 [M+CH_3OH-H]$^-$	**249.073**	2	18	14.43
		205.077	2	26	
		130.063	2	36	
β-ZEL	313.232 [M-H]$^-$	**174.120**	90	24	13.17
		187.902	90	26	
		130.06	90	34	
TeA	196.217 [M-H]$^-$	**138.996**	54	18	13.73
		111.951	54	24	
MO	96.998 [M-H]$^-$	**40.913**	4	26	3.00
		68.938	4	24	
AOH	257.273 [M-H]$^-$	**146.978**	2	32	12.79
		212.993	2	22	
		185.006	2	28	
PAT	153.117 [M-H]$^-$	**80.941**	16	12	7.43
		52.976	16	14	
		108.968	16	10	
TEN	413.609 [M-H]$^-$	**271.176**	2	16	13.00
		141.038	2	20	
		108.968	2	22	
NIV	371.268 [M-CH_3COO]$^-$	**281.153**	12	14	7.17
		311.167	12	10	
		191.033	12	24	
Positive polarity					
PA	170.955 [M+H]$^+$	**125.000**	12	12	7.51
		97.050	12	16	
CIT	251.111 [M+H]$^+$	**233.129**	52	16	9.58
		205.060	52	26	
		191.025	52	24	
AME	273.070 [M+H]$^+$	**184.162**	44	36	15.16
		258.123	44	26	
ALT	293.202 [M+H]$^+$	**239.124**	2	20	10.24
		257.126	2	14	
DON	297.174 [M+H]$^+$	**249.138**	16	10	6.92
		231.135	16	12	
		203.126	16	42	
AFL B1	313.047 [M+H]$^+$	**241.080**	56	36	10.44
		284.948	56	22	
		213.132	56	42	
AFL B2	315.130 [M+H]$^+$	**287.104**	80	24	10.23
		259.039	80	28	
		243.120	80	36	

End of the table 1

Analyte	Parent ion, m/z	Daughter ions*, m/z	Fragmentor voltage, V	Collision energy, V	Retention time, min
MPA	321.306 $[M+H]^+$	**207.129**	4	24	10.65
		159.046	4	32	
		102.965	4	42	
STC	325.140 $[M+H]^+$	**310.033**	82	22	15.05
		253.122	82	42	
		196.927	82	50	
AFL G1	329.110 $[M+H]^+$	**243.058**	72	24	9.72
		199.793	72	40	
		283.014	72	24	
AFL G2	331.1304 $[M+H]^+$	**189.067**	22	38	9.53
		245.075	22	30	
		217.009	22	34	
OTA	404.1298 $[M+H]^+$	**189.067**	2	22	10.40
		245.075	2	62	
		217.008	2	34	
TEN	415.4022 $[M+H]^+$	**256.194**	60	28	12.06
		302.257	60	12	
		132.081	60	40	
HT-2	442.3404 $[M+NH_4]^+$	**215.114**	16	12	11.56
		302.257	16	6	
		263.188	16	12	
T-2	484.396 $[M+NH_4]^+$	**305.216**	54	12	12.54
		245.191	54	10	
		215.113	54	18	
ENN B	640.666 $[M+H]^+$	**196.140**	70	24	19.76
		214.200	70	24	
		186.200	70	38	
ENN A	682.730 $[M+H]^+$	**210.175**	90	22	21.91
		228.171	90	24	
		200.171	90	46	
FB1	722.634 $[M+H]^+$	**352.430**	18	34	10.03
		334.374	18	40	
FB2	706.638 $[M+H]^+$	**336.447**	10	36	12.29
		318.391	10	38	
		354.440	10	32	
BEA	784.730 $[M+H]^+$	**134.089**	82	62	20.31
		244.217	82	28	
		262.213	82	22	

Note: * means that daughter ions used for MT quantification are given in **bold**; the rest are used for qualitative confirmation.

Standard solutions of 27 MTs were prepared from dried standards (Sigma-Aldrich; Fermentek, Jerusalem, Israel). Stock standards were prepared in acetonitrile (AFL, STC, CIT, trichothecenes of groups A and B, ZEN and its analogues, OTA, PA, PAT), methanol (*Alternaria* toxins, ENN A, ENN B, BEA, MO, MPA) or in an 'acetonitrile/water' mixture (50/50 % vol.) as it was the case with FB1, FB2 with a concentration equal to 100 or 500 µg/mm^3. Standard solutions were used to make a multi-standard and calibration solutions. All solutions were stored at -18 °C.

To quantify MTs, external calibrations on a 'clean' matrix were applied. 'Positive' samples were divided into two sub-groups; the first one included samples with MTs levels being higher than the limit of detection (LOD) but lower than the limit of quantification (LOQ); the second one was made of samples that contained MTs in concentrations being higher than LOQ. LOD and LOQ were calculated as per 3-σ and 10-σ criteria. MT recovery varied between 60 and 120 %.

The obtained data were statistically analyzed with IBM SPSS Statistics 23 (Statistical package for social sciences, USA) and Microsoft Office Excel 2007 (Microsoft Corp., USA). Data on MT content in total samples were given as arithmetic mean (mean) and 90-th percentile (90 %); contamination levels lower than the limit of quantification were taken as equal to 0. Data on MT levels in contaminated samples were given as a range of MT levels (range) and arithmetic mean (mean).

Results and discussion. We examined MT occurrence and levels of MT contamination in 185 samples of fresh fruits and berries. The structure and levels of identified MT contamination varied significantly depending on a culture. Strawberries, black currant and raspberries turned out to be the most contaminated with MTs among berries.

Table 2 provides data on MT occurrence and levels in the examined fruits and berries. Twenty three out of 27 analyzed MTs were identified in **strawberries**; some of them (CIT, ENN A and ZEN) were detected in trace quantities. PA prevailed in strawberries (53 % of the cases), followed by FB2 (43 %), AFL G2 (30 %) and NIV (25 %). Average levels in samples contaminated with these MTs reached 28–69 μg/kg (for PA and NIV). FB1 (18 % of the cases), AME (7.5 %) and BEA (5.0 %) were identified less frequently. Some samples were contaminated with fusariotoxins DON, ZEN, β-ZEL, ENN A and B and *Alternaria* toxins ALT, TEN and AOH though contamination levels were not high for them. TeA was not identified in any strawberry samples. C. Juan et al. [16] reported low levels of contamination with toxins produced by *Alternaria* fungi in strawberries.

MTs were less frequently identified in **raspberries** as compared with strawberries. PAT was the most frequent contaminant; 23 % out of 13 analyzed samples contained PAT in quantities between 5.82 and 7.29 μg/kg (Table 2) and this was by several times lower than the maximum permissible level (MPL) established for this toxin regarding several sorts of berries and fruits. Two raspberry samples (15 %) were contaminated with TEN in low quantities; NIV was identified with the same frequency (its average level was 5.4 μg/kg). *Alternaria* toxins AOH, ALT and TeA were identified in some samples; the latter has the highest acute toxicity in comparison with other *Alternaria* toxins [17, 18].

Table 2

MT occurrence and levels in fresh fruits and berries

Toxin	MT occurrence, %		MT content in total samples, μg/kg		MT levels in contaminated samples, μg/kg	
	total, including	higher than LOQ	average	90 %	range	average
Strawberries (40 samples)						
PA	52.5	50.0	13.89	42.09	1.31–131.81	27.78
FB2	42.5	32.5	0.77	2.10	1.50–5.10	2.37
AFL G2	30.0	30.0	0.76	1.28	0.25–20.0	2.54
NIV	25.0	25.0	17.24	62.74	28.67–200.90	68.98
FB1	17.5	7.5	2.12	0	4.60–66.00	21.24
AME	17.5	7.5	0.17	0	1.81–2.61	2.28
BEA	17.5	5.0	0.10	0	0.53–3.51	2.02
T-2	15.0	15.0	0.55	1.42	0.97–7.46	3.64
HT-2	12.5	12.5	0.649	0.91	2.25–19.92	5.16
ENN B	12.5	2.5	0.01	0	0.52	0.52
ENN A	12.5	0	< 0.5	< 0.5	< 0.5	< 0.5
ALT	7.5	7.5	0.32	0	0.72–10.71	4.24

End of the table 2

Toxin	MT occurrence, %		MT content in total samples, µg/kg		MT levels in contaminated samples, µg/kg	
	total, including	higher than LOQ	average	90 %	range	average
DON	5.0	5.0	2.51	0	2.44–98.09	50.26
AFL B2	5.0	5.0	0.33	0	0.23–12.91	6.57
MPA	5.0	2.5	0.01	0	0.36	0.36
TEN	5.0	5.0	0.25	0	0.57–9.44	5.01
OTA	5.0	2.5	0.16	0	6.55	6.55
AOH	2.5	2.5	0.03	0	1.37	1.37
β-ZEL	2.5	2.5	0.25	0	9.83	9.83
AFL G1	2.5	2.5	0.14	0	5.69	5.69
AFL B1	2.5	2.5	0.08	0	3.37	3.37
CIT	2.5	0	< 0.5	< 0.5	< 0.5	< 0.5
ZEN	2.5	0	< 1.0	< 1.0	< 1.0	< 1.0
Raspberries (13 samples)						
PAT	23.1	23.1	1.46	5.90	5.82–7.29	6.34
TEN	15.4	15.4	0.03	0.16	0.16–0.21	0.18
NIV	15.0	9.0	0.83	< 3.0	3.00–7.85	5.42
TeA	7.7	7.7	1.66	< 12.5	21.59	21.59
ALT	7.7	7.7	0.08	< 0.5	1.08	1.08
AOH	7.7	0	< 0.2	< 0.2	< 0.2	< 0.2
Black currant (14 samples)						
DON	100	100	22.02	58.9	0.53–92.5	22.02
TEN	28.5	21.4	0.49	0.64	0.45–5.70	2.26
AOH	14.3	14.3	0.26	1.10	1.10–2.60	1.85
TeA	7.1	7.1	0.94	< 5.0	13.12	13.12
ZEN	7.1	7.1	0.15	< 0.3	0.9	0.9
HT-2	7.1	0	< 0.5	< 0.5	< 0.5	< 0.5
MPA	7.1	0	< 0.2	< 0.2	< 0.2	< 0.2
Red currant (12 samples)						
HT-2	16.6	8.3	0.26	1.59	3.17	3.17
AOH	8.3	8.3	0.10	< 0.4	1.15	1.15
TeA	16.6	8.3	4.02	< 4.0	48.2	48.2
STC	8.3	0	< 0.2	< 0.2	< 0.2	< 0.2
Gooseberries (13 samples)						
TEN	30.8	30.8	0.09	0.38	0.16–0.42	0.31
DON	30.8	23.1	2.63	10.29	8.25–15.64	11.39
HT-2	15.4	15.4	0.34	1.78	1.78–2.63	2.21
AOH	7.7	7.7	0.07	< 0.5	0.93	0.93
T-2	15.4	0	< 1.0	< 1.0	< 1.0	< 1.0
FB2	7.7	0	< 7.0	< 7.0	< 7.0	< 7.0
AFL G1	7.7	0	< 0.2	< 0.2	< 0.2	< 0.2
TeA	7.7	0	< 25.0	< 25.0	< 25.0	< 25.0
Blueberries (2 samples)						
AOH	100	100	12.1	22.5	1.70–22.50	12.1
AME	50	50	1.75	1.75	3.50	3.50
Dogwood (6 samples)						
NIV	50	17	55.70	< 20.0	305.09	305.09
AME	17	17	0.7	< 0.7	2.29	2.29
Plum and blackthorn (4 samples)						
MO	100	100	105.31	149.9	79.50–149.95	105.31
Apples (17 samples)						
TEN	12	12	0.07	< 0.5	0.56–0.69	0.62

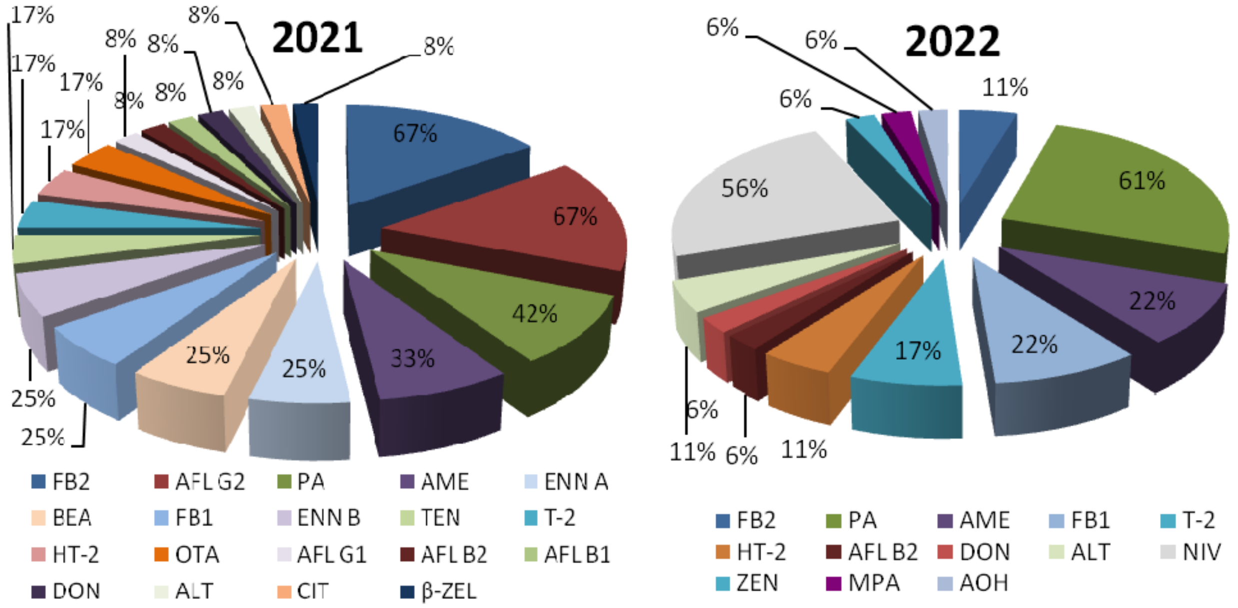

Figure 1. MT occurrence in strawberries grown in 2021 and 2022

Currant and dogwood samples were mostly contaminated with metabolites of *Alternaria* and *Fusarium* fungi. All the **black currant** samples contained DON though its levels were not high and varied between 0.5 and 92.5 µg/kg; still, this toxin is known to be able to damage genome even in the lowest concentrations. Emergent TEN, AOH and TeA were identified in half of the analyzed samples. **Red currant**, as opposed to black one, was contaminated with MTs much less frequently. Some samples were contaminated with HT-2, AOH and TeA. NIV was identified in 3 out of 6 **dogwood** samples (in one case its level was higher than 300 µg/kg); AME was another toxin identified in these berries. Eight out of 27 analyzed MTs were identified in **gooseberry** samples. Among them, TEN and DON occurred most frequently as they were identified in 31 % of the cases. **Blueberry** samples contained *Alternaria* toxins AOH and AME. MO was identified in all the **plum** and **blackthorn** samples with its average level being 100 µg/kg.

This study revealed that MT contamination was the lowest in apples and pears. Only TEN was identified in 12 % of **apple** samples and its levels were low; MTs were not detected in the analyzed **pear** samples.

We estimated a spectrum of MTs identified in strawberries. The results indicate that the greatest contribution to contamination in these products is made by toxicogenic microfungi from *Fusarium* genus and, to a lesser extent, by *Aspergillus* sp. and *Penicillium* sp. We analyzed taxonomy of molds identified in fruits and berries and compared the data with profiles of identified MTs. The results indicate there is high prevalence of MT producers from *Alternaria* genus in raspberry, gooseberry, currant, and plums (unpublished data).

We comparatively analyzed contamination of strawberries harvested in 2021 and 2022 to estimate influence exerted by climatic and seasonal factors on MT accumulation in berries. Strawberries harvested in 2021 contained 19 MTs (Figure 1); MT produced by ‘storage fungi’ prevailed among the identified ones, first of all, AFL G2, as well as some other AFL, OTA, emergent fusariotoxins ENN A and B and BEA. Only 13 MTs were identified in samples harvested in 2022; PA occurred by 20 % more frequently than in the previous year and in contrast AFL occurred much less frequently and no emergent fusariotoxins were identified. NIV was identified the most frequently among other fusariotoxins. Less diverse MTs were likely due to warmer weather (as compared with the previous year) in the Central region in Russia during a period when berries grew and ripened, harvesting included.

A significant number of the analyzed fruit and berry samples were simultaneously contaminated with several MTs: 75 % of strawberry samples, 50 % of black currant samples, 38 % of gooseberry samples, 23 % of raspberry samples and 8 % of currant samples contained more than one MT.

AFL G2+PA+FB2, PA+FB2, PA+FB2 and PA+AFL G2 were identified in **strawberry** samples. The remaining combinations included one or several of the above-listed MTs and fusariotoxins FB1, T-2, HT-2, NIV, ENN and BEA. *Alternaria* toxins TEN, AME and ALT occurred much less frequently. We should note that one of 40 analyzed strawberry samples contained 13 MTs including AFL B1 and OTA (6.55 µg/kg, which was higher than the hygienic standard established for OTA in other types of plant products) as well as several fusariotoxins including DON and FB1+FB2 (98.09 µg/kg and 66.00 µg/kg accordingly), MPA and CIT (in trace quantities). AFL contamination in strawberries was also reported by T. Klapec et al., 2022, who identified several AFL (except AFL B1) in 70 % of the analyzed samples; the maximum contamination level reached 3.185 µg/kg [19].

High levels of contamination with fusariotoxins in intact strawberry samples can partially occur due to the fact that phytopathogenic molds, while damaging the root system, are still able to penetrate other parts of a plant. As a result, MTs synthesized by them can be identified in berries and the process does not always involve any obvious spoilage. Other researchers also described cases when MTs were identified in berries, fruits and vegetables without any visible spoilage signs [20]. The study that focused on asparagus revealed that MTs were able to migrate from soils into edible parts of a plant through the root system [21].

PAT, NIV and TeA, metabolites of *Penicillium sp.*, *Fusarium sp.* and *Alternaria sp.*, were simultaneously identified in 23 % of **raspberry** samples. **Currant** samples were contaminated with various combinations of *Alternaria* toxins and fusariotoxins in 50 % of the cases. DON+TEN with AOH or without it were identified in black currant samples (29 %); DON+AOH and DON+TeA also occurred in these berries (7 %).

In addition, we compared MT contamination in visually intact berries and fruits and in the same sorts with visible damage and spoilage. The analyzed samples were divided into two groups: without visible damage and spoilage (127 samples) and with some signs of mechanical damage and/or molding (58 samples). When analyzing strawberries, we estimated an additional sub-group from which we had preliminarily excluded berried with visible spoilage in addition to a sub-group that contained intact berries (Table 3).

Higher levels of MT contamination in moldy and damaged samples against intact ones were typical for all the analyzed berries and fruits (Figure 2).

Fusariotoxins prevailed in intact strawberries; basic contaminants in this culture included NIV (46 % of the samples, the average level in contaminated samples was 68.98 µg/kg), FB2 (18 %, 1.75 µg/kg), T-2 and FB1 (14 %, 2.03 and 9.18 µg/kg accordingly), HT-2 (9 %, 1.10 µg/kg) and DON (5 %, 2.44 µg/kg). The samples were also contaminated

Table 3

Analyzed berries and fruits

Berries and fruits	Intact samples	With visible spoilage	Berries and fruits	Intact samples	With visible spoilage
Strawberries	22 + 17*	18	Raspberries	13	4
Apples	17	3	Dogwood	6	3
Black currant	14	6	Pears	5	3
Red currant	12	6	Plums, blackthorn	4	6
Gooseberries	13	9	Blueberries and huckleberries	3	0

N o t e : * means mixed samples with preliminarily removed damaged and moldy berries.

Figure 2. MT contamination in intact and damaged berries and fruits

with MTs produced by 'storage fungi' *Penicillium* and *Aspergillus*: PA (50 %, 40.34 µg/kg), AFL G2 (14 %, 1.08 µg/kg), AFL B2 (5 %, 0.23 µg/kg) and MPA (5 %, 0.36 µg/kg); metabolites of *Alternaria* were less frequent: we identified AME (14 %, 2.51 µg/kg) and ALT (5 %, 1.18 µg/kg). The ratio between these three MT groups was as follows: 53 % (fusariotoxins) : 37 % (MT produced by 'storage fungi') : 10 % (*Alternaria* toxins).

An extended sampling was made of samples without visible damage but also included samples that contacted damaged and moldy berries (39 samples overall). We identified up to 20 different MTs in it. Additionally, OTA and STC were detected as well as such emergent MTs as ENN A and B, BEA, CIT, AOH and TEN. FB1 and FB2 prevailed among fusariotoxins (40 % and 18 % accordingly), followed by NIV (25 %) and BEA (15 %). It is noteworthy that NIV and DON occurred only in intact berries whereas EMTs (ENN A, ENN B and BEA), on the contrary, were identified in samples that contained berried of heterogeneous quality. The ratio between three MT groups remained practically the same: 54 % (fusariotoxins) : 36 % (MT produced by 'storage fungi') : 10 % (*Alternaria* toxins).

Twenty MTs were also identified in damaged and moldy strawberries (18 samples) including ZEN that did not occur in other subgroups of strawberry samples. Among other fusariotoxins, we identified greater frequency and higher levels of contamination with fumonisins, T-2 toxin, HT-2 toxin, BEA, ENN A and B. There was an increase in occurrence and levels identified for all the MTs produced by 'storage fungi' as well as a number of samples that were contaminated with *Alternaria* toxins.

It is noteworthy that tenuazonic acid (TeA), an emergent MT produced by *Alternaria* spp., was identified practically in all spoilt berries and fruits, excluding strawberry where penicillic acid (PA) occurred more frequently (in 72 % of the samples). The highest TeA levels were identified in moldy currant; all the currant samples were contaminated with this MT. The average TeA level equaled 1031 µg/kg in red currant (by 20 times higher than in intact samples); the level grew by more than 5 times in moldy raspberry and gooseberry and by 2.5 times in moldy black currant as opposed to intact samples (up to 350 µg/kg). Occurrence of TeA reached 50–100 % in these product groups whereas there were very few cases when it was identified in intact berries and fruits.

PAT detection in raspberry, plum and blackthorn, CIT in dogwood, AOH, AFL G2 and MPA in black currant also indicate the products were spoilt. T-2 toxin, HT-2 toxin and ENN A more frequently occurred in moldy raspberry samples; there was also more frequent contamination with *Alternaria* toxins TEN and AOH (together with TeA) whereas its levels remains relatively low. Quality of berries had practically no effects on occurrence and levels of contamination with PAT and NIV. We identified DON and emergent TEN, AOH, TeA and CIT in damaged and moldy black currant; the latter was identified only in poor quality berries.

Moldy and damaged gooseberry samples contained EMT much more frequently: AOH levels grew by 7 times and we also identified AME and MPA. NIV, CIT, TEN and TeA were identified in damaged and moldy dogwood berries. NIV occurred in such samples more frequently than in intact ones. Only poor quality dogwood turned out to be contaminated with CIT and TeA in quantities 8.0 and 11.1 µg/kg accordingly.

All the plum samples, both intact and moldy ones, contained EMT MO; AOH, PAT and TeA were identified only in moldy plums in quantities 1.06, 5.15 and 277.6 µg/kg accordingly. The data on PAT detection in plums which we obtained in this study are in line with the results obtained by N.H. Aziz et al., who found this toxin in 4 out of 10 analyzed samples at levels between 180 and 200 µg/kg [22]. It is worth mentioning that T-2 toxin was identified in one out of three moldy apple samples in quantity 134 µg/kg. This is higher than the hygienic standards for this toxin contents in some plant products.

Conclusions:

1. HPLC-MS/MS method for detection of mycotoxins (MTs and EMTs) in fruits and berries was developed. Conditions for chroma-

tographic separation of 27 analytes including poorly explored TeA and PA were optimized. Sample preparation procedure provided sufficient recoveries (over 60 %). Limits of detection and quantification were estimated.

2. This study was the first in the Russian Federation to concentrate on examining most popular fruits and berries among the mass consumer foods to identify a wide range of 27 MTs in them. Strawberries, gooseberries, black currants and raspberries turned out to be the most contaminated with MT; red currants, apples and pears were less contaminated. Each type of berries and fruits had its own typical contaminants; thus, PA, AFL G2 and FB1 prevailed in strawberries, MO in plums and blackthorn and DON in black currant.

3. Data obtained in this research indicate occurrence of not only regulated MTs in fruits and berries (T-2, FB1, FB2, DON, ZEN, OTA and AFL B1 in strawberry, PAT in raspberry, DON in gooseberry and black currant) but also their derivatives (AFL B2, G1, G2, STC in strawberry; NIV in dogwood and raspberry; HT-2 in gooseberry and red currant) and poorly explored EMTs (PA, ALT, TEN, MPA, ENN B, BEA and AOH in strawberry; TeA and TEN in black currant, raspberry and gooseberry AOH and AME in blueberry; MO in plums).

4. From MTs, TeA prevailed in the most of berries and fruits with visible spoilage excluding strawberry, apples and pears; PA prevailed in spoilt strawberry. The contamination levels of MT were by several times higher in spoilt and damaged berries and fruits against intact ones. The detection of PAT in plums and raspberry and CIT in black currant and dogwood as well as STC, OTA, ZEN and AFL B2 in strawberries also confirmed their fungal invasion.

5. The data obtained on the nature and levels of contamination of MT and EMT in fresh fruits and berries indicate the necessity to perform hygienic assessment of fruits and berries sold on the Russian market. This assessment should cover not only regulated PAT and AFL but also emergent mycotoxins and their producers. To calculate contribution made to intake with food by the most typical MTs and EMTs in such products, it is advisable to perform in-depth survey of penicillic acid, aflatoxins and fumonisin B1 contamination of strawberry and tenuazonic acid in raspberries, current, gooseberries and plums.

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