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Research article

STUDY OF CHILI PEPPERS AND PAPRIKA CONTAMINATION WITH *ALTERNARIA* TOXINS AS HEALTH RISK FACTORS

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At present, researchers show considerable interest in unregulated toxins of widespread fungi in nature, in particular, metabolites of microfungi of Alternaria genus. These toxins are potentially hazardous for human health and, under unfavorable conditions, capable of producing toxic metabolites, Alternaria toxins (AT), which have shown genotoxicity, mutagenicity and acute toxic effects. They are frequently detected not only in cereals and oilseeds, fruits, nuts, vegetables, but also in spices, especially dried red peppers.

The aim of the study was to investigate the occurrence of Alternaria toxins in paprika and chili peppers sold on the domestic market as well as to assess their intakes by humans through their consumption.

Concentration of 5 Alternaria toxins (alternatiol (AOH), alternariol monomethyl ether (AME), altenuene (ALT), tentoxin (TEN), tenuazonic acid (TeA)) was detected in 37 samples of dried red peppers marketed in Moscow and Moscow region in 2024 year including paprika (20 samples) and chili pepper (17 samples). The analysis of Alternaria toxins contamination was carried out by high-performance liquid chromatography coupled to tandem mass-spectrometric detection (HPLC-MS/MS).

TeA was detected in 84 % of red pepper samples in concentration from 43 to 3295 µg/kg and TEN – in 40 % cases, in low levels ranging from 1.0 to 11 µg/kg. The occurrence of AT in paprika was higher than in chili pepper. Combined (2 or more toxins) contamination with Alternaria toxins was found only in paprika samples (65 %; the predominant combination is TeA and TEN). Intakes of Alternaria toxins associated with consumption of chili and paprika did not exceed the reference values and ranged from 0.0003 % (for TEN) to 0.24 % (for TeA) of the threshold of toxicological concern (TTC).

Keywords: *Alternaria toxins, tenuazonic acid, tentoxin, paprika, chili pepper, contamination, HPLC-MS/MS, risk assessment.*

Among the most common contaminants leading to crop spoilage, mycotoxins (MTs), including *Alternaria* toxins (ATs) produced by such mold fungi of the genus *Alternaria* as *A. alternata, A. tenuissima, A. solani*, etc., are of interest [1−4]. Their accumulation can begin both at the pre-harvest stage and in the post-harvest period, during transportation and storage, and the possibility of their simultaneous contamination of products makes this

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problem extremely urgent [5, 6]. According to the literature data, not only food raw materials but also processed foods, in particular, red pepper, are exposed to AT contamination [2, 7, 8]. Among ATs, a real threat to public health is formed by alternariol (AOH), its methyl ester (AME), tentoxine (TEN), tenuazonic acid (TEA) and altenuane (ALT) [9−13]. According to the European Food Safety Agency (EFSA), AOH and TeA are the most widespread of them [8].

Consumption of foods contaminated with ATs can result in various diseases, for example, esophageal cancer in Henan, China, and Onyalai disease in African countries [3].

At present, there are no national or international regulations on the content of AT in food products. At the same time, European countries have introduced indicative levels for TEA, AOH and AME for a number of foodstuffs, exceeding which are grounds for additional research[1].

Chili pepper and paprika are the most susceptible to molds able to produce MTs, especially during prolong storage in inappropriate storage conditions. Spice processing methods can also influence fungal growth and subsequent accumulation of toxins [14]. According to the literature, MTs produced by mold fungi *Aspergillus* spp. and *Penicillium* spp. are priority contaminants in species [14−17]. Previous studies have shown that spices were most frequently contaminated with aflatoxins and ochratoxin A. As regards contaminated chili pepper and paprika, TEN was found in 41–57 % cases; AOH, 19 % cases; AME, 6–14 %; and ALT, 6–10 % [16].

The aim of this study was to investigate the occurrence of *Alternaria* toxins in paprika and chili peppers sold on the domestic market as well as to assess health risks associated with their consumption for the population of the Russian Federation.

Materials and methods. Dried red pepper samples were bought in retail outlets in Moscow and the Moscow region in 2024. Overall, 37 samples were examined including 20 paprika samples and 17 chili pepper samples produced in Uzbekistan (15), India (6), Spain (6), China (3), Armenia (2) and 5 samples of unknown origin.

Preparation of samples for screening of AT in spices was carried out in accordance with the developed methodology. A ground sample weighing 1 gram was put in a 50 cm^3 Falcon tube, added with 10 cm^3 of distilled water, shaken until wetting was complete and then left to swell in ultrasonic cleaner Elmasonic S15H (Elma, Germany) for 10 minutes. Next, the sample was added with 10 cm^3 of acetonitrile acidified with 1 %-solution of acetic acid, shaken for 10 minutes and then again treated with ultrasound for 10 minutes. The sample was added with 1 gram of NaCl and 4 grams of anhydrous $MgSO_4$, and mixed intensively after each addition, either by hand or using a vortex. The extract was centrifuged for 10 minutes at 10,000 rpm (Hettich, Rotina 38). Five cm^3 of supernatant were put into a 15 cm^3 falcon tube, added with 3 cm^3 of hexane saturated with acetonitrile and mixed in a shaker for 20 minutes. Next, the sample was centrifuged for 1 minute at not less than 4000 rpm (Hettich, Rotina 38); 3 cm^3 of defatted acetonitrile layer were taken and vapored until dry in a rotor evaporator (BioChromato, Japan). The rest was again dissolved in 0.1 cm^3 of methanol and added with 0.4 cm^3 of water. The resulting solution was put into a 1.5 cm^3 Eppendorf tube and centrifuged for 10 minutes at 15,000 rpm in a SL 16R centrifuge (Thermo Scientific, USA). 0.4 cm^3 of supernatant were put into a chromatographic vial.

ATs were identified using a HPLC system Agilent Technologies 1100 consisting of

[1] Commission recommendation (EU) 2022/553 of 5 April 2022 on monitoring the presence of *Alternaria* toxins in food: recommendations. *Official Journal of the European Union*, 2022. Available at: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32022H0553&from=EN (May 14, 2024).

the gradient pump, column thermostat and autosampler, which was connected with a triple quadrupole mass spectrometric detector (Triple Quad 6400). Reversed phase high performance liquid chromatography coupled to tandem mass spectrometry (HPLC-MS/MS) in the positive electrospray mode at atmospheric pressure and multiple reaction monitoring (MRM) was used to identify ATs.

Analytes were divided on a column filled with silica gel with added octadecylsilane groups (Zorbax SB-C18, 150 × 4.6 mm, 3.5 μm, pore size is 80Å, Agilent). The mixture of water : acetonitrile : formic acid (95 : 5 : 0.1 % vol.) was used as the mobile phase A; acetonitrile : formic acid (100 : 0.1), the mobile phase B. The gradient scheme was as follows: the start at 0 % B; the 7th minute, linear growth to 75 % B; the 10th minute, linear growth to 100 % B; the column equilibration from 17th to 19th minute at 100 % B; linear decline to 0 % during 0.5 minute; the 25th minute, the column equilibration at 0 % B. The eluent flow rate was 0.4 cm^3/min; the column temperature, 30 °C; the injection volume, 20 mm^3. Each sample was analyzed twice. The column temperature was 30 °C. Calibrations with a relevant 'clean' matrix were used for quantification. The MT recovery varied between 70 and 91 %; limits of quantification (LOQ) for the method amounted to 1, 4, 6, 8 and 20 µg/kg for TEN, AME, AOH, ALT and TeA respectively.

The following standards were used: AOH (99.3 %), AME (99.77 %), ALT (98 %), and TEN (99.84 %) (Fermentek, Israel). Stock solutions of the toxins were prepared in methanol in the concentration of 200 µg/cm^3. The TeA standard, concentration of 100.6 µg/cm^3 was bought from Romer Company, Biopure, Austria. All stock solutions were stored at -18 °C.

Results and discussion. The study of frequency and levels of contamination in 37 dried red chili samples established TeA (84 %) and TEN (40 %) to be the priority contaminants in these products (Table 1).

Table 1

Contamination of dried red pepper samples with *Alternaria* toxins

Toxins	The number of contaminated samples		Contamination range, µg/kg	Average MT contents in contaminated samples, µg/kg	MT contents in total samples, µg/kg	
	abs.	%			average	90 %
Paprika (n = 20)						
TeA	20	100	42.5–3295.4	1746.9	1746.9	2849.40
TEN	12	60	1.0–10.5	4.4	2.7	8.1
AOH	1	5	41.4	41.4	2.1	0
AME	1	5	8.2	8.2	0.4	0
ALT	0	0	< 8.0			
Chili pepper (n = 17)						
TeA	11	65	143.6–2481.5	640.4	943.3	1492.2
AOH	0	0	< 6.0			
AME	0	0	< 4.0			
TEN	0	0	< 1.0			
ALT	0	0	< 8.0			
Dried red pepper (n = 37)						
TeA	31	84	42.5–3295	1225	1462	2604
TEN	12	40	1.0–10.5	2.7	1.4	5.6
AOH	1	3	41.4	41.4	1.12	< LOQ
AME	1	3	8.20	8.20	0.22	< LOQ
ALT	0	0	< 8.0			

Chili pepper samples were less contaminated with ATs; only TeA was detected in them with frequency of 65 %. The levels of contamination varied between 143.6 and 2481.5 μg/kg, 640.4 μg/kg on average. Four out of 5 analyzed ATs were found in paprika samples: TeA was found in 100 % cases in concentrations between 143.6 and 2481.5 μg/kg, 1746.9 μg/kg on average; TEN was found in 60 % in low concentrations up to 10.5 μg/kg. AOH and AME were detected in single cases in concentrations of 41.4 and 8.2 μg/kg.

In contrast to our study findings, C. Mujahid et al. found four ATs (TeA, AOH, AME and TEN) in chili pepper samples with much higher TeA concentrations varying between 4510 and 20,478 μg/kg [18]. As regards paprika samples, all analyzed samples were contaminated with TeA, both in our study and in [18, 19]; however, according to their data, contamination levels were significantly higher and reached 18,856 and 37,300 μg/kg respectively.

More detailed analysis of TeA occurrence in dried red pepper samples established that the highest frequency of contamination was in samples obtained from Spain and Uzbekistan. TeA was founded less frequently and in lower concentrations in spice samples delivered from China (33 %, the average concentration was 740 μg/kg) and Armenia (50 %, 674.2 μg/kg) (Table 2). The highest average and maximum contamination levels were found in samples produced in Spain, India and Uzbekistan. Four ATs were simultaneously found in only one sample from Uzbekistan.

Sixty percent of the analyzed paprika samples were contaminated with two and more ATs. TeA and TEN were found in 11 samples; simultaneously 4 MTs were found in one sample: TeA, 1234.8 μg/kg; AOH, 41.4 μg/kg; AME, 8.2 μg/kg; and TEN, 7.9 μg/kg. According to [18], simultaneous contamination with three and more ATs was more frequent and amounted to 88 % and the levels of contamination were also higher. Simultaneous exposure to several ATs can have a significant adverse effect on the general toxicity in comparison with individual exposures [13, 20].

The contributions made by the analyzed spices into ATs intake were assessed considering the data on dried red pepper consumption provided by the Federal Customs Service of the Russian Federation and the levels of ATs contamination found in its samples in this study (Table 3). In Russia, average consumption of chili pepper and paprika is 0.206 gram per person[2]. An average body weight of a person is 70 kg. ATs burden was

Table 2

Occurrence of tenuazonic acid in dried red pepper samples depending on a country of origin

Country of origin	The number of analyzed samples	The number of contaminated samples		Contamination range, μg/kg	Average TeA contents in total samples, μg/kg
		abs.	%		
Uzbekistan	15	14	93	160.1−2284.3	1549.8
Spain	6	6	100	1588.4−2910.9	2103.0
India	6	4	67	42.5−3295.0	1026.4
China	3	1	33	2221.3	740.4
Armenia	2	1	50	1348.4	674.2
Unknown	5	5	100	389.0−2842.6	1508.6

[2] Federal'naya Tamozhennaya sluzhba. Tamozhennaya statistika vneshnei torgovli RF [Federal Customs Service. Customs Statistics of Foreign Trade of the Russian Federation]. Available at: http://stat.customs.gov.ru (May 27, 2024) (in Russian).

Table 3

Calculated average daily intake of *Alternaria* toxins for the population due to consumed chili pepper and paprika

Toxin	Calculated average daily intake of *Alternaria* toxins		Threshold of toxicological concern (TTC) for a toxin, ng/kg of body weight per day
	ng/kg of body weight	% of TTC	
TeA	3.6	0.24	1500
AOH	0.003	0.13	2.5
AME	0.0005	0.03	2.5
TEN	0.004	0.0003	1500

assessed by comparing the calculated average daily consumption with a relative value of threshold of toxicological concern (TTC) (1500 ng/kg of body weight for TeA and TEN, 2.5 ng/kg of body weight for AOH and AME) [2, 8].

According to the study results, ATs intake for the population calculated for daily consumption of dried red pepper is considerably below their TTC and does not create any significant health risks for the RF population.

Conclusions:

1. *Alternaria* toxins are established to contaminate dried red pepper; tenuazonic acid is the priority contaminant with its occurrence reaching 100 % in paprika (42.5–3295.4 μg/kg), and 60 % in chili pepper (143.6–2481.5 μg/kg). Combined contamination of paprika with tenuazonic acid and tentoxin was found in 65 % cases. Frequency of AOH and AME contamination in paprika was low.

2. Estimated intake of *Alternaria* toxins with paprika and chili pepper give evidence of their low exposure and the absence of any serious hazards for population health.

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Competing interests. The authors declare no competing interests.

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