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THE PROBLEM OF SAFE SUNFLOWER (*Helianthus annuus* L.) USE FOR FOOD AND FODDER PURPOSES

(review)

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Abstract

Risks associated with the contamination of agricultural products with mycotoxins have been and still remain under the close attention of the world's biological science. In recent decades, special concern was related to the state of the grain harvest, intended for food and feed purposes. In most grain-producing countries, significant progress has been made in the identification of toxin-forming micromycetes and assessing the risk caused by the spread of mycotoxins (T.Yu. Gagkaeva et al., 2004; G.P. Kononenko et al., 2008, 2009; P.M. Scott et al., 2012). For the second most important group of agricultural plants, the oilseeds represented mainly by sunflower, soybean, peanut, rapeseed and cotton, there is a significant lag in such studies, which only has to be overcome. *Helianthus annuus* L. is cultivated everywhere, practically in all regions of the world, suitable for agriculture, and the area of its commercial cultivation is also extremely wide. The group of world leaders in the production of sunflower seeds includes the Russian Federation, Ukraine, Argentina, India and China, but this crop is also cultivated on a significant scale in other countries. The purpose of this review was to systematize and compare the world data on the composition of mycobiota and the nature of contamination by mycotoxins of sunflower (*Helianthus annuus* L.) seeds and the products of their processing. In surveys conducted in the Middle East, Africa, South and South-East Asia, the mycobiota of the seeds revealed the dominance of fungi of the genus *Aspergillu*s with the typical species *A. flavus* and the frequent detection of *A. niger*, and for less common fungi *Penicillium*, *Alternaria* and *Fusarium* common regularities were not traced. In India, Pakistan, Tanzania, Malaysia, Iran and Egypt, the contamination of seeds by aflatoxins was assessed as very high, and the seed processing products for oil retained the same type of contamination, but with an increased detection rate and a more intensive accumulation of mycotoxins (S. Dawar et al., 1991; S.K. Abdullah et al., 2010; H.R. Beheshti et al., 2013; J.A. Mmongoyo et al., 2017). In a number of works, experimental confirmation was obtained that, when storing seeds, especially in conditions of high humidity and temperature, the accumulation of aflatoxins sharply increases (H.H. Casper et al., 1982; P. Jeswal et al., 2013). In the countries of South America (Argentina, Brazil), fungi of the genera *Alternaria*, *Fusarium* and alterniotoxins predominated in sunflower seeds (C.R. Pozzi et al., 2005). In European countries, the fungi of the genera *Alternaria* and *Fusarium* are also classified as the main components of the mycobiota of sunflower seeds, but the data on the species composition of these micromycetes and the contamination of the seeds with mycotoxins are very limited. Long-term studies performed in the Russian Federation show widespread distribution of fungi of the genus *Alternaria* on the vegetative plants and sunflower seeds, most often of small-spore unspecialized species *A. tenuissima*, *A. alternata* and '*A. infectoria'* complex (M.V. Ivebor et al., 2012). *Fusarium* infection in the European area of cultivation is shown annually, while the species diversity is very significant (A.A. Vypritskaya, 2015). Nevertheless, until now, mycotoxicological evaluation of the yield of sunflower seeds in the main areas of commercial cultivation of the crop in our country has not been carried out. During monitoring surveys of sunflower oil cakes and meals, multiple combined contamination by mycotoxins was established with dominance of alternariol and ochratoxin A and a significant contribution of T-2 toxin, as well as citrinin, emodin, mycophenolic acid and cyclopiazonic acid (E.V. Zotova et al., 2017). The nature of the contamination of this feed raw material in the Russian Federation is fundamentally different from that described in the countries of the Middle East, Africa, South and South-East Asia, primarily due to the absence of aflatoxin B_1 and the significant occurrence of ochratoxin A, often in conjunction with citrinin. The generalization and comparative analy-

sis of the broad database of scientific data, undertaken in this paper, allow us to identify ways of eliminating shortcomings in restricting the standardization of mycotoxins and to outline the most relevant areas for future research.

Keywords: *Helianthus annuus* L., sunflower, seeds, sunflower meal, oilcake, micromycetes, fungal diseases, mycotoxins

Risks associated with the contamination of agricultural products with mycotoxins have been and remain at the center of close attention of the world's agricultural science. In recent decades, the state of the grain harvest produced for food and feed purposes has been of special concern. In most grain-producing countries, significant progress has already been made in identifying the main toxin-forming micromycetes and in assessing the prevalence of mycotoxins in grain products [1-3]. The main result of mycological and mycotoxicological examinations performed in the areas where fusariosis of grain and corn is registered is the understanding that this global problem is associated with a region, and its success requires special approaches, taking into account the places of growth [4-6].

For the second most important group of agricultural plants, the oilseeds, mainly represented by sunflower, soybean, peanut, rapeseed and cotton, there is a significant lag in these studies, which only has to be overcome, and the existing database needs to be generalized and analyzed.

The purpose of this review is to systematize and compare the world data on the composition of the mycobiota and the nature of contamination of sunflower (*Helianthus annuus* L.) seeds with mycotoxins while harvesting and storing the harvest, as well as in the processed products used for animal feed.

Sunflower is widespread virtually in all regions of the world, suitable for agriculture. The area of its industrial cultivation is also extremely wide. Seeds of this plant are a valuable food product and raw material for the confectionery and fat-and-oil processing industries, and waste from the production of sunflower oil (oil cake and meal) are in demand as feed raw materials. Green mass of tall varieties, rich in protein, is considered suitable for preservation (silage and haylage). Livestock eagerly eats heads of plants harvested during flowering; the remains of mature plants after harvesting (stems, leaves and heads) are also used for animal feed [7].

Concerns about the negative effects of sunflower consumption are mainly related to the long-established fact that plants have predisposition to diseases caused by imperfect mycelial, pycnidial and sclerotial fungi, as well as basidial, lower and marsupial fungi [7]. The main mycotoxins, having a sanitary importance, for most phytopathogens are not included in the number of physiologically active metabolites. However, the causative agents of Alternaria blight (*Alternaria* spp.), seed spotting syndrome (*Alternaria alternata*, *Cladosporium* sp.), tracheomycotic wilting and pink rot of heads (*Fusarium* spp.) are of interest from a toxicological point of view, and for *Penicillium* sp., *Aspergillus* sp., *Trichoderma* sp. and *Cladosporium* sp. fungi, which cause widespread seed molding, the ability to toxin production is known [8, 9]. Many of them are found in the composition of mycopopulations, accompanying the pathogenesis processes.

The group of world leaders in the production of sunflower seeds includes the Russian Federation, Ukraine, Argentina, India and China, but this crop is also cultivated on a significant scale in other countries. Soil-climatic, ecological and agro-technical conditions are known to have a decisive significance for the formation of plant mycobiota [10]. In connection with this, the risks of accumulation of mycotoxins in agricultural products in areas should be assessed by the main taxa of fungi, identifying potentially toxicogenic species and confirming the possibilities for the realization of their genetically determined abilities.

Describing the diversity of the mycobiotic composition of sunflower seeds before harvesting, during the post-harvest period and during storage, researchers in the countries of the Middle East, Africa, South and South-East Asia emphasized the explicit dominance of some genera and species (Table 1). *Aspergillus* fungi were constantly detected, their typical representative was *A. flavus*, *A. niger* was also often found, *A. terreus* and *A. fumigatus* were less common. By the species composition of less common fungi belonging to the genera *Penicillium*, *Alternaria* and *Fusarium*, the general patterns were not traced. The results of the examination of the seed harvest in India [11, 12, 18], Pakistan [13, 14] and the data of local national projects, carried out in different years in Iran [19], Nigeria [20], Sudan [21], the Republic of South Africa [17], Colombia [22], Iraq [15], Malaysia [16] and Tanzania [23], showed that the fungi of the genus *Aspergillus* are characterized by the highest occurrence and intensity of infection in comparison with representatives of other genera.

1. Main representatives of mycobiota of sunflower seeds (*Helianthus annuus* L.) in the countries of the Middle East, Africa, South and South-East Asia

Region	Taxon (genus)	Species	Reference
India (State of Tamil Nadu)	*Aspergillus*	*A. flavus*	[11]
India (State of Bihar)	*Aspergillus*	*A. flavus*	[12]
	Penicillium	*P. citrinum*, *P. verrucosum*	
	Fusarium	*F. moniliforme*	
Pakistan	*Aspergillus*	*A. flavus*, *A. niger*	[13]
	Alternaria	*A. alternata*, *A. tenuissima*	
	Fusarium	*F. moniliforme*, *F. solani*	
	Aspergillus	*A. flavus*, *A. niger*, *A. terreus*	[14]
	Alternaria	*A. alternata*	
	Fusarium	*F. moniliforme*, *F. pallidoroseum*	
Iraq	*Aspergillus*	*A. flavus*, *A. niger*, *A. fumigatus*, *A. terreus*	[15]
	Penicillium	*P. expansum*, *P. brevicompactum*	
	Alternaria	*A. alternata*	
	Fusarium	*F. oxysporum*, *F. solani*	
Malaysia	*Aspergillus*	*A. flavus*, *A. niger*	[16]
Republic of South Africa (Province of KwaZulu-Natal)	*Aspergillus*	*A. flavus*	[17]

The species *Aspergillus*, *Penicillium*, *Fusarium* and *Alternaria*, identified in sunflower seeds in these areas, are known as toxin-forming ones [24]. The strains of *Aspergillus flavus*, isolated from seeds in India, produced aflatoxin B_1 [25]. For isolates of the same species in Pakistan, the ability to biosynthesize aflatoxins was experimentally confirmed: 29 strains out of 41 produced aflatoxin B_1, and 8 also aflatoxin B_2 [26]. To simulate conditions that are close to the natural ones, the toxin formation of fungi was studied on sunflower seeds. However, whether the use of the same substrate for the correct approach to assessing fungi involvement in the contamination of biological entities by toxic metabolites is fundamental, is still to be determined. For two strains of *A. flavus*, it was found that the process of accumulation of aflatoxins on seeds was decelerated, compared to grain substrates, and the largest amounts of aflatoxins were formed only after 12 and 18 days [27].

In India, Pakistan, Tanzania, Malaysia and Iran, the contamination of seeds with aflatoxins was assessed as very high (Table 2). In India, along with aflatoxins, ochratoxin A, citrinin and zearalenone [12] were detected in seeds, as well as cyclopiazonic acid, which is a toxin, characteristic of *Aspergillus* fungi [29]. The by-products of seed processing for oil in these countries retained the same type of contamination that was inherent in seeds, but the detection rate and the accumulation of mycotoxins increased. In India, in the study of oil

cakes, aflatoxins were detected in all examined samples in amounts exceeding 30 μg/kg [30], cyclopiazonic acid in 10 samples (300-29000 μg/kg) [28], ochratoxin A in 48 and 76% of samples in the summer and winter periods in amounts of 31.84 and 76.52 μg/kg, respectively [31]. In Tanzania, according to the data of 2014-2015, 80.4% of the cake samples (n = 92) from local oil producers contained aflatoxins in amounts ranging from 1.4 to 598.4 μg/kg [23], which exceeded the detection rate in the seed crop with the same contamination (Table 2). For the oil cake sample from the eastern part of the Republic of South Africa, contamination with aflatoxins (84 μg/kg) was established, severely contaminated by the species *A. flavus* and *A. tamarii* [17]. Recently, aflatoxins B_1 (37.8 μg/kg), G_1 (57.8 μg/kg) and B_2 (7.0 μg/kg) [32] have been found in the consignment of meal imported to Malaysia.

2. Mycotoxins found in sunflower seeds in the countries of South America, the Middle East, Africa, South and South-East Asia

Region of cultivation, n	Mycotoxin	Detection rate, amount, μg/kg	Reference
India (State of Bihar), seeds before and after harvesting (n = 240)	Aflatoxin B_1	30 % (43-1070)	[12]
	Aflatoxin G_1	20 % (17-247)	
	Ochratoxin A	17 % (49-248)	
	Citrinin	15 % (23-433)	
	Zearalenone	3 % (111-125)	
Pakistan, seeds from different provinces (n = 24)	Aflatoxin B_1	54 % (≤ 437)	[26]
	Aflatoxins B_1 + B_2	21 % (≤ 14)	
Tanzania, seeds from different places, harvest 2014, 2015 (n = 90)	Aflatoxins	59 % (1,4-662,7)	[23]
South Africa (KwaZulu-Natal province) (n = 1)	Aflatoxins	5,6	[17]
Malaysia	Aflatoxin B_1	82,1 % (0,54-5,33)	[16]
Iran (Khorasan region), various stores (n = 50)	Aflatoxin B_1	13 % (≤ 168)	[28]
	Aflatoxin B_2	8 % (≤12,8)	

N o t e. n — the number of samples examined.

In Egypt, regional differences in the composition of seeds mycobiota were found. In 36 samples of the 1985 harvest selected in the country's markets, 63 species and 3 varieties of fungi, belonging to 18 genera, were identified [33]. Among them, according to the detection rate, representatives of the genera *Aspergillus* (100%) and *Penicillium* (88.9%) dominated, followed by fungi belonging to the genus *Fusarium* (36.1%). Contamination of seeds with *Aspergillus* spp. was the most intensive, and it was considerably less intensive for *Penicillium* and *Fusarium*. Fungi of the genus *Aspergillus* were represented by the species *A. niger*, *A. flavus*, *A. fumigatus*, *A. terreus*, *A. flavus* var. *columnaris*, *A. nidulans*, *A. sydowi*, *A. tamari*; among *Penicillium*, *P. chrysogenum* and *P. corylophilum* were found, among *Fusarium* — *F. oxysporum*, *F. moniliforme*, *F equiseti*, *F. semitectum* (first two more often), the species *Alternaria* spp. were extremely rare. In another study, in 16 seed samples collected at the experimental stations of the universities of Alexandria, Nobariya and Minya there were *Alternaria alternata*, *F. proliferatum*, *F. semitectum* and *F. semitectum* var. *majus* [34]. In 10 samples from Asyut Governorate, *A. niger* (16.5%), *A. flavus* (13.8%) and *Alternaria alternata* (15.3%), prevailed, and *P. digitatum* and *F. oxysporum*, *F. moniliforme*, *F. semitectum* [35] were less frequent.

These results completely correspond to the nature of seed contamination with mycotoxins. In the 1985 harvest [33], 26 samples (72%) were positive, with aflatoxins found in more than half of them (15 of 26). The group of aflatoxins in 10 positive samples was represented by all four metabolites, in 5 samples by only two ones (aflatoxins B_1 and B_2). Fusariotoxins, i.e. diacetoxyscirpenol (5/26), T-2 toxin (4/26), zearalenone (2/26), as well as sterigmatocystin (3/26) and ochratoxin A (1/26) were less common, but, unfortunately, quantitative measurements were not carried out. Later, it was reported that zearalenone and alternariol were

found in seeds in the same region [36].

In the United States, information on the detection of aflatoxin B_1 in sunflower seeds was first obtained by scientists from the University of North Dakota: while analyzing 11 samples of the 1978 crop, aflatoxin B_1 was found in 9 samples in amounts ranging from 10 to 225 μg/kg [37, 38]. This fact was the reason for more thorough research, during which the important role of storage conditions for the accumulation of aflatoxin B_1 was demonstrated. For example, in 1979-1981, in consignments from different manufacturers providing half of the oil production in this state, the contamination with aflatoxin B_1 did not exceed the allowable level (20 μg/kg); however, in the samples from storage facilities where molding and caking were observed, its content reached 100-1100 μg/kg [37, 38]. All this indicates that the key factor in the growth of contamination is post-harvest storage. When examining food and forage, imported into the United States during 1982-1986, aflatoxins in the amount of 179 μg/kg were found in one sample of sunflower seeds [39]. Subsequently, it was stated that in a sample from a consignment of moldy sunflower seeds that caused intoxication of pigs, cyclopiazonic acid was detected in an extremely high concentration, the 10000 μg/kg [40]. Apparently, under certain conditions, a sharp intensification of the growth of individual highly competitive toxigenic species from the number of mold-inducing seeds is possible.

Increased accumulation of mycotoxins during seed storage, especially in conditions of high humidity and temperature, is confirmed in other countries. According to a researcher from India, the content of aflatoxins in freshly harvested and stored sunflower seeds was 1000 and 2200 μg/kg, respectively [30]. When storing a batch of sunflower seeds in a specialized metal hangar at 25-32 °C, after 7 months the amount of aflatoxin B_1 increased more than 2-fold (from 205 μg/kg to 520 μg/kg) [41]. The increase in seed contamination during post-harvest storage is also indicated by the recent data of Indian authors obtained in the state of Bihar [12]. Not only the detection rate of four mycotoxins increased, but also the degree of contamination: aflatoxin B_1 (16 of 120, 43-355 μg/kg), aflatoxin G_1 (11/120, 17-85 μg/kg), ochratoxin A (14/120, 1-3 μg/kg) and citrinin (11/120, 23-65 g/kg) were identified before harvesting, aflatoxin B_1 (56/120, 463-1070 μg/kg), aflatoxin G_1 (36/120, 129-338 g/kg), ochratoxin A (27/120, 121-415 ⌈g/kg), citrinin (26/120, 65-433 μg/kg) and zearalenone (7/120, 111-125 μg/kg) were found after harvesting.

Judging by the published data, in the countries of South America the mycotoxicological situation with sunflower differs radically from the described one for other regions. Here, seed mycobiota is represented mainly by the fungi of the genera *Alternaria* and *Fusarium* [42]. In Brazil, in seeds harvested in one of the states, the contamination with *Fusarium verticillioides* was 70%, *Alternaria alternata* — 46%, *Cladosporium* spp. — 18%, and the rest (*A. flavus*, *Penicillium* spp., *Scopulariopsis* spp.) — from 2 to 10% [42]. In Argentina, seeds were often contained by alternariotoxins, the alternariol and its methyl ether (in 76% of 50 samples) [43]. In Brazil (Sao Paulo, Nova Odessa), contamination with these toxins was 18% and 10%, respectively, with a content of 24.9-170.9 and 14.1-108.6 μg/kg [41]. In cakes, among the most frequent contaminants there were alternariol (35-792 μg/kg) and its methyl ether (9-630 μg/kg); tenuazonic acid was also found [44].

In European countries, fungi of the genera *Alternaria* and *Fusarium* are often mentioned among the main components of mycobiota of sunflower seeds, but there is little data on the species composition. In Serbia (Province of Vojvodina), fungi belonging to 8 genera and 13 species were isolated from seeds, kernels and husk: *Alternaria alternata*, *Arthrinium phaeospermum*, *Aspergillus can-*

didus, *A. flavus*, *A. niger*, *A. ochraceus*, *A. versicolor*, *A. wentii*, *Cladosporium cladosporioides*, *Eurotium herbariorum*, *Penicillium aurantiogriseum*, *Rhizopus stolonifer* and *Trichoderma harzianum* [45]. In Finland, 20 species of fungi were distinguished in affected plant organs, among which there were 4 species of *Fusarium*, i.e. *F. avenaceum* (Fr.) Sacc., *F. equiseti* (Corda) Sacc., *F. oxysporum* Schlecht. and *F. sambucinum* Fuckel [46]. Assessment of the role of toxin-forming fungi *Fusarium* and *Alternaria* is difficult not due to the complexity of the composition of the represented complex of species, but because of the problems with species identification. The contribution of these fungi to the contamination of seeds, green mass and products of processing for food has been little studied so far. The ability to form toxins is known only for a few species of *Fusarium*, found in seeds and contaminated terrestrial organs of these plants. Many species of the genus *Alternaria* Nees are also classified as potentially toxicogenic ones. However, the authors did not find any information on the production of mycotoxins by isolates from sunflower seeds belonging to specific species of *Fusarium*, *Penicillium*, *Cladosporium*, *Scopulariopsis* and *Aspergillus* (*A. niger*, *A. fumigatus*, *A. terreus*, *A. candidus*, *A. ochraceus*, *A. versicolor*, *A. wentii*, *A. nidulans*, *A. sydowii* and *A. tamari*).

Reports on the occurrence of fusario- and alternariotoxins in seeds and products of their processing are still very few. Thus, in southern Italy, in one seed sample, infected with *A. alternata,* alternariol (360 μg/kg) and its methyl ether (130 μg/kg) were detected [47, 48]. In Hungary, contamination by T-2 toxins with a frequency of 13.6% and high accumulation (237-500 μg/kg, in average 230 μg/kg) was established for 22 samples of oil cake. Deoxynivalenol was detected less frequently and in smaller quantities (4.5%, 150 μg/kg), and zearalenone was absent [49].

The results of the annual food monitoring since 1983 in the US and EU countries, show that sunflower seeds supplied to the food markets of these countries are generally poorly contaminated with mycotoxins and do not pose a threat to the population. Nevertheless, in Italy, when the imported seeds were inspected, the content of aflatoxin B_1 in two samples was 50 and 90 μg/kg [50]. Recently, aflatoxins in an amount of less than 20 μg/kg were found in three samples of mixtures prepared with the addition of sunflower seeds, and seeds contained ochratoxin A (20 μg/kg) [51]. In 11 samples of seeds tested in January-February 2013 in a trade network of Uppsala (Sweden) and having the confirmation of Chinese production or without any indication of their origin, aflatoxins and ochratoxin A were not detected, but in the mycobiota everywhere there were fungi of the genus *Penicillium* represented by the toxicogenic species *P. expansum*, *P. chrysogenum*, *P. verrucosum*, *P. crustosum*, *P. albocoremium*, *P. brevicompactum*, *P. citrinum*, *P. rugulosum* and *P. polonicum* [52]. A study in the Netherlands in 2013-2014 showed that the contamination of food products with alternariotoxins (seeds, oil and paste prepared by means of its hydrogenation) is stable and the seeds are more often contaminated by tenuazonic acid (8/10, 240 μg/kg), rather than alternariol (1/10, 5.4 μg/kg) and its methyl ether (1/10, 1.1 μg/kg) [53, 54], but the modified forms of these toxins (sulfates and sulfoglycosides) were not detected.

The first reports regarding the detection of mycotoxins in the products of processing seeds for oil in the European market appeared when little was known about the problem of seed contamination [50]. In Germany, aflatoxin B_1 (17 μg/kg) was found in one of the four samples of oil cake imported in 1972-1973. In Hungary, 9.6% of the 73 consignments, imported in 1975, contained aflatoxins. In all 22 samples of oil cake imported into the UK from Argentina, India and the EU countries, contamination with alternariol was found

(180 μg/kg), its methyl ether (100 μg/kg) and tenuazonic acid (1900 μg/kg) [56]. In 2011, the European Commission based on research data, including those obtained from Russian scientists, raised the issue of contamination of food and forage with the toxins of *Alternaria* fungi and concluded that the risks of contamination of seeds, oil cakes and meal from sunflower seeds are especially high [57].

In the Russian Federation, sunflower accounts for up to 70% of the sown areas of oilseeds, which are concentrated mainly in the Southern, Central and Volga Federal Districts. The study of causative agents of diseases and fungi associated with this plant has a rich history, significant successes in the present and future development prospects. Long-term studies have shown a wide distribution of fungi of the genus *Alternaria* in vegetative plants and sunflower seeds, and in recent years it has been possible to specify their species composition. According to the phytoexamination data, in most of the 62 consignments of seeds from the Krasnodar Territory, Voronezh and Volgograd regions harvested in 2010-2011, there were often small-spore unspecialized species: *A. tenuissima* (52%), the complex of *A. infectoria* (25%) and *A. alternata* (14%) [58]. The results of a 25-year monitoring in the Krasnodar Territory showed significant damage caused by *Alternaria* to roots, stems, leaves, heads and seeds [59]. In the Tambov region, in all the surveyed areas in 1992-2015, the fungi of the genus *Alternaria* were found in 4.3-100% of vegetating plants (most often on heads and seeds) given the insignificant intensity of contamination [60]. A detailed description of the species composition of alternariosis pathogens in different regions of the country is given in the work of F.B. Gannibal [61].

In recent decades, more attention has been paid to fusariosis of sunflower in the European range of cultivation of this crop [62]. The prevalence and species composition of pathogens in the southern regions of Russia was studied [63-65]. In the Tambov region, the dominance of *F. oxysporum* (24.1%) and *F. verticillioides* (20.4%) was shown on plants, *F. oxysporum* (21.8%) and *F. oxysporum* var. *orthoceras* (20.0%) on seeds [66]. Fusariosis manifested itself annually to varying degrees (from single cases of diseases to spreading to 13.3% of plants and more), while the species diversity was quite significant, up to 20 species [60].

Domestic researchers attribute such fungi as *Aspergillus* spp., *Aureobasidium pullulans*, *Cladosporium* spp., *Epicoccum* spp., *Monilia sitophila*, *Mucor* spp., *Penicillium* spp., *Stachybotrys* spp., *Oedocephalum* spp. and *Trichothecium* spp. to accompanying ones, since they are found, as a rule, together with other causative agents and with a frequency of less than 1% [58-60].

Inspection of forage products from sunflower seeds for contamination with mycotoxins commenced in our country in 2002 [67]. For 42 samples of oil cake and meal, the contamination with mycotoxins proved to be quite high (54%) and in half of the samples was provided mainly by ochratoxin A. T-2 toxin was found in 16% (7.5-39.5 μg/kg), zearalenone only in one sample (77.5 μg/kg), aflatoxin B_1 and sterigmatocystin were not detected. Subsequently, significant contamination of oil cakes and meals with ochratoxin A was confirmed, 45.5% and 58.8% respectively, with a content of more than 50 μg/kg in 6.6% of the samples from the positive ones and 4-48 μg/kg in the rest [68].

In 2003-2006, during the analysis of production consignments of oil cakes and meals in 28.4% of 116 samples, another nephrotoxin was detected, the citrinin (from 14 to 300 μg/kg), often together with ochratoxin A (30 out of 33 positive samples) [69]. Among the contaminants, the toxin of the anthraquinone series emodin (in 4 samples out of 7) was found for the first time in an amount of 20-30 μg/kg [70], and in two samples of oil cake out of 58 there was cyclopiazonic acid (50 and 63 μg/kg) [71]. According to generalized data, in 2004-2009

the frequency of occurrence of ochratoxin A and citrinin was 50% (58/116) (as a rule, jointly present). Combined contamination was accompanied by a general quantitative pattern in their ratio: the citrinin content was higher than the one of ochratoxin A, although coincidences or similar values were also observed [72]. Taking into account that citrinin is known as bioactivator of ochratoxin A [73], the importance of this fact for the assessment of the risk of animals exposure to these toxins should be recognized. The obtained results permitted to state the hypothesis of probable sources of contamination which are the species *Aspergillus* and/or *Penicillium*, capable of producing these toxins separately or jointly.

3. Frequency of occurrence (%) and content of mycotoxins (minimum-average-maximal, μg/kg) **in sunflower cakes and meals in Russia [74]**

Mycotoxin	Meals, oil cakes, n = 334 (1997-2016)	By types of raw materials (2009-2016)	
		meals, n = 57	cakes, n = 45
T-2 toxin	17 (4-16-93)	23 (4-9-16)	36 (5-13-25)
Diacetoxyscirpenol	–	–	–
Deoxynivalenol	4 (40-92-375)	2 (375)	–
Zearalenone	0,6 (66-72-78)	2 (66)	–
Fumonisins	–	–	–
Ergoalkaloids	3 (5-17-40)	2 (11)	7 (5-19-40)
Alternariol	77 (19-262-1990)	77 (19-315-1990)	78 (20-205-955)
Roridine A	–	–	–
Aflatoxin B_1	0,3 (3)	–	–
Sterigmatocystin	6 (4-7-12)	9 (4-7-12)	20 (4-6-11)
Cyclopiazonic acid	21 (50-80-142)	18 (50-79-125)	44 (50-82-140)
Emodin	26 (20-217-5000)	32 (20-93-280)	31 (20-440-5000)
Ochratoxin A	59 (4-19-200)	82 (4-15-93)	69 (4-15-62)
Citrinin	33 (19-85-1020)	46 (20-93-1020)	20 (50-88-125)
Mycophenolic acid	25 (20-93-379)	35 (25-95-380)	16 (20-93-335)
PR-toxin	–	–	–

N o t e. n — the number of examined samples. A dash indicates that the samples containing mycotoxin are not found.

In 2008-2010, the first cases of detecting alternariol, mycophenolic acid, T-2 toxin, deoxynivalenol, sterigmatocystin and cyclopiazonic acid in cakes and meals were recorded.

Based on the results obtained for the entire monitoring period from 1997 to 2016 (Table 3), a large combined contaminant with the most frequent detection of alternariol and ochratoxin A and a slightly smaller spread of citrinin, emodin, cyclopiazonic acid, T-2 toxin and mycophenolic acid is characteristic for oil cakes and meals. The remaining mycotoxins (deoxynivalenol, zearalenone, ergoalkaloids and sterigmatocystin) were found in single cases or were not found at all (diacetoxyscirpenol, fumonisins, roridine A and PR-toxin). Both types of raw materials (see Table 3) had a similar type of contamination: the same set of major contaminants and similar accumulation rates, but for the oil cakes there was a slightly higher incidence of ergoalkaloids, sterigmatocystin and cyclopiazonic acid. In one sample, an abnormally high accumulation of emodin (up to 5000 μg/kg) was observed [74].

The long-term examination of oil cakes and meal on a large sample (334 samples) permitted to establish that the type of their contamination in Russia is fundamentally different from the one, described many times in other countries, primarily due to the absence of aflatoxin B_1 and a high rate of occurrence of ochratoxin A, often jointly with citrinin. The presence of ochratoxin A in meals was previously found in Hungary (18.2% of 22 samples, 100-260 μg/kg at an average of 160 μg/kg) [49] and in Yugoslavia [75].

The study of production consignments of raw materials (seeds intended for oil production) in our country has just begun, but the first assessments indicate a lesser degree of contamination compared to the one, established for processing products. In 2017, the first results of the analysis of mycotoxins in sun-

flower seeds proposed for sale to the population were obtained. Contamination with mycotoxins was weakly expressed. Out of 27 seed samples selected in Moscow farmers' markets in 2015-2016, only 6 were positive: two samples had alternariol (42 and 48 μg/kg) and emodin (58 and 208 μg/kg), in single cases T-2 toxin (158 μg/kg) and mycophenolic acid (250 μg/kg) [74]. In connection with this, attempts to discuss the role of fungi of the genus *Aspergillus*, capable of producing aflatoxins, which are actively undertaken in the domestic literature in response to the growing demands for the ecological safety of sunflower products in our country [76], do not have any grounds.

Undoubtedly, in the future, for drawing reasonable conclusions about the mycotoxicological status of this raw material, intended for consumption by the population and processing, it is necessary to continue monitoring sanitary-relevant indicators on a whole scale. However, even now, based on the results of assessing the contamination of seeds and oil cake/meal, it can be concluded that the post-harvest period, storage and processing are key stages for the possible increase in contamination. The need for long-term seed maintenance prior to implementation (the ripening phase, raw material stocks), as well as long processing and storage periods, creates favorable conditions for the dynamic development of mycobiota with a change in composition, in which growth activation and enhancement of metabolic activity of competitive species are possible. In this regard, it is extremely important to identify and control those factors, which are significant for ensuring the mycotoxicological safety of seed stocks, technological intermediates at processing plants and in ready-to-use products.

One should also take into account such a feature of post-harvest seed treatment as rapid self-warming of the wet threshed heap, which can (in case of large content of impurities) significantly affect their quality. Due to the uneven nature of seeds maturation, the moisture of consignments is not uniform. In those cases, when activation of mycobiota occurs with the advantage of competitive toxigenic species, a real threat of focal accumulation of mycotoxins is considered probable.

In our country, to ensure the safe use of sunflower seeds for food and fodder purposes, it is necessary to revise the approaches to the regulation of mycotoxins content and introduce reasonable control criteria. Obviously, the current indicators do not correspond to the real situation. In seeds, supplied for food purposes, the content of aflatoxin B_1 (5 μg/kg, not more) [77-79] is limited, and for feeding purposes there are limits of aflatoxin B_1 (20 μg/kg), ochratoxin A (50 μg/kg), T-2 toxin (100 μg/kg), deoxynivalenol (1000 μg/kg) and zearalenone (1,000 μg/kg) [79]. In sunflower meal, mycotoxins are not normalized [80], and T-2 toxin (not more than 100 μg/kg), deoxynivalenol (not more than 1000 μg/kg) and zearalenone (not more than 1,000 g/kg) [81] are regulated for cake.

Modern science has accumulated a significant amount of information on the extent of occurrence of micromycetes and mycotoxins in other important agricultural plants (soybean and peanut), which also requires generalization and critical analysis. Efforts are made to implement a similar approach for rapeseed, the individual segment of which is constantly growing in the modern market for agricultural products [82]. With regard to less commonly used oilseed and textile crops (castor plant, sesame, safflower, mustard, false flax, colewort, lallemantia, cotton plant, flax and kenaph), such studies have not yet been carried out.

The actual data on mycotoxins in vegetative sunflower plants is not sufficient to discuss the problem. However, the first experimental data indicate that at the initial phase of growth, before the heads are formed, multiple contamination occurs, and during the ripening period, mycotoxins are distributed unevenly

across leaves, stems, heads and seeds [74]. As compared to green parts of mature plants (leaves and heads), the contamination of achenes proved to be very moderate, which may be a general phenomenon associated with the action of protective mechanisms of generative organs from bio-damage in plants, which is of scientific interest and deserves a more detailed consideration. Until now, the processes associated with the emergence of metabolism products of microscopic fungi in sunflower plants from the beginning to the end of vegetation, as well as their localization in the plant, have not yet been studied. However, in recent years, the work has commenced to identify low-molecular compounds in glandular sunflower trichomes [83], which are supposed to be involved in protecting the organism from pathogens.

Thus, the comparison of scientific facts on the contamination of sunflower seeds by toxigenic fungi and mycotoxins, undertaken in the present paper, gives convincing confirmation of the urgency of the problem under discussion and its extreme complexity. Differences in the composition of mycobiota and components of the toxins complex are quite contrasting in the areas of cultivation of this crop. With prolonged seed storage before processing, the probability of a rapid and unpredictable exacerbation of the situation is very high due to the emergence of competitive advantages among highly active producers. To guarantee the safe use of sunflower for food and fodder purposes, it is necessary to continue research for grounded approach to mycotoxicological control of seed raw material and products of its processing. The consideration of known information and accumulation of new data on the peculiarities of seed contamination with toxin-forming fungi and mycotoxins in the main areas of commercial sunflower cultivation in the future will permit to recommend more effective preventive measures to reduce or prevent the threats of mycogenic intoxications of people and animals.

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