AGRICULTURAL BIOLOGY,
2018, V. 53, № 5, pp. 969-976
(SEL'SKOKHOZYAISTVENNAYA BIOLOGIYA)

ISSN 2412-0324 (English ed. Online)
ISSN 0131-6397 (Russian ed. Print)
ISSN 2313-4836 (Russian ed. Online)

UDC 633.854.78:632.4:615.9

doi: 10.15389/agrobiology.2018.5.969eng
doi: 10.15389/agrobiology.2018.5.969rus

REASONS OF CONTAMINATION OF PRODUCTION LOTS OF SUNFLOWER (*Helianthus annuus* L.) SEEDS BY MYCOTOXINS

A.A. BURKIN, M.I. USTYUZHANINA, E.V. ZOTOVA, G.P. KONONENKO

All-Russian Research Institute of Sanitary, Hygiene and Ecology — Branch of Federal Science Center Kovalenko All-Russian Research Institute of Experimental Veterinary RAS, Federal Agency of Scientific Organizations, 5, Zvenigorodskoe sh., Moscow, 123022 Russia, e-mail kononenkogp@mail.ru (✉ corresponding author), aaburkin@mail.ru, ustpuma@list.ru, ezotova63@gmail.com
ORCID:
Burkin A.A. orcid.org/0000-0002-5674-2818
Ustyuzhanina M.I. orcid.org/0000-0001-7405-7577
Zotova E.V. orcid.org/0000-0002-1479-8602
Kononenko G.P. orcid.org/0000-0002-9144-615X
The authors declare no conflict of interests
Acknowledgements:
The authors are grateful to Dr E.A. Piryazeva for help in assessing the composition of seed mycobiota and isolation of pure cultures of fungi
Received January 14, 2018

Abstract

In recent years, in our country there has been a clear trend towards sustainable growth in the production of sunflower oil. During long-term monitoring high risks of contamination with mycotoxins are established for the by-products of oil and oil-extracting industries, oil cakes and meals, which are traditionally in demand as valuable raw materials for mixed fodders (G.P. Kononenko et al., 2018). Among the possible reasons of contamination are the violations of the technology of production and storage of the final products, but the problem of the sanitary quality of oil seeds coming from the farms remains without attention. The purpose of our work was to survey the lots destined for the production of oil by comparing the contamination with mycotoxins of the main seeds and typical accompanying impurities. The average samples of sunflower oil seeds from the farms of the Belgorod, Voronezh, Kursk and Lipetsk regions of the 2016 year crop were fractionated into seeds and impurities (according to GOST 22391-2015) for separate study of their contamination. We also examined a batch of seeds stored after cleaning in two compartments, in one of which there was a shutdown of the ventilation system and, as a result, self-warming foci arose. In addition, sunflower growing plants that were collected in June-September 2016 and 2017 in the subsidiary farms of the Moscow, Tver, Voronezh and Rostov regions were analyzed for mycotoxins. Ground parts (n = 65) were cut at a height of 5 cm from the soil surface, dried in a shaded ventilated room and crushed whole. Another part of the plants (n = 29) was divided into leaves, stems and baskets before grinding. The mycotoxins group containing T-2 toxin (T-2), diacetoxycirpenol (DAS), deoxynivalenol (DON), zearalenone (ZEN), fumonisins (FUM), alternariol (AOL), aflatoxin B_1 (AB_1), sterigmatocystin (STE), cyclopiazonic acid (CPA), emodin (EMO), ochratoxin A (OA), citrinin (CIT), mycophenolic acid (MPA), PR toxin (PR) and ergot alkaloids (EA) were determined by the enzyme immunoassay. Our tests show that in seeds AOL is almost universally encountered, MPA and EMO are somewhat less frequent, the remaining mycotoxins are not detected. In contrast, in impurities, in addition to AOL, EMO and MPA, fusariotoxins (T-2, DAS, ZEN) are often enough, whereas CPA and CIT are more rare, and DON, STE, EA occur in a few samples. The ranges of AOL, EMO and MPA content are significantly wider and the average values for the samples are significantly higher than those found in the seeds. The newly discovered fact of multiple and intensive contamination of impurities with mycotoxins has a great practical importance, as it is an experimental justification for the need for thorough harvest cleaning for further processing. Additional experiments with vegetating sunflower plants show the maximum content of mycotoxins in leaves and pseudanthium (sunflower head) as compared to stems. A sharp increase in the mycotoxin accumulation both in seeds and in impurities occurs under self-warming conditions. The obtained results show that fungi of the genera *Alternaria* and *Penicillium* can cause damage to the harvested crop. We will elucidate their role in more detail in further studies.

Keywords: *Helianthus annuus*, sunflower, seeds, impurities, mycotoxins, *Fusarium*, *Alternaria*, *Aspergillus*, *Penicillium*, enzyme immunoassay

Sunflower (*Helianthus annuus* L.) is cultivated in many countries for oil

seeds widely used edible oil. At the same time, in different areas, based on the generalized data on the contamination of seeds and the products of their processing with mycotoxins, the real risks of the influence of these toxicants on a humans are confirmed [1-4]. Considering the frequent occurrence and high content of Alternaria toxins with genotoxic effect, seeds, and sunflower oil are classified as products that pose a serious threat to public health [5-7].

In Russia, where about 70% of the world's sunflower crops are located, sunflower oil production in recent years has shown a tendency to sustainable growth. For oil cakes and meals, the by-products of oil-pressing and oil-extracting industries, which are traditionally in demand as a valuable raw material for mixed fodders, a long-term monitoring has been established the significant mycotoxin contamination [4]. Among its possible causes, there are violations of technological schemes during seed processing, transportation and storage of final products at enterprises, but the problem of the sanitary quality of oil seeds, coming from agricultural producers, remains without attention.

In the present work, in mycotoxicological assessment of commercial batches of sunflower oil seeds, we determined for the first time the nature of the contamination and revealed an increased accumulation of mycotoxins in the impurities. A survey of vegetating sunflower plants before harvesting showed that the main contribution to the contamination is made by the fragments of baskets and leaves. The new data on a sharp increase in contamination of batches of seeds by mycotoxins that have undergone self-warming are of particular interest, since information about mycotoxin formation in such conditions is very limited.

The aim of the work was to compare the contamination by mycotoxins of seeds and impurities in the production batches, intended for the production of sunflower oil.

Techniques. The study was performed on samples from 19 batches, a bulk sample per each batch, of sunflower oil seeds (*Helianthus annuus* L.), produced at agricultural enterprises of Belgorod, Voronezh, Kursk and Lipetsk Regions in 2016. Before conducting the analysis, the samples from each batch were fractionated into main seeds and impurities, separating the small part (the entire passage through a sieve with holes of 3.0 mm in diameter) and organic impurities (husks, remains of leaves, stems, baskets) from the residue on the sieve [8].

While conducting the mycological analysis of the samples from the batch of seeds stored after harvesting in two compartments, in one of which the storage conditions were violated, superficially sterilized seeds and the grinding of unsterilized seeds were incubated on a nutrient medium followed by detachment and species identification of the fungi *Alternaria* and *Penicillium* by culture and morphological features [9, 10]. Toxin formation in typical strains was evaluated after culture on malt extract agar medium (MEA, Liofilchem, Italy) [11] in the dark at 25 °C for 7 days.

For the analysis of vegetative sunflower plants, they were selected in June-September 2016 and 2017 in the subsidiary farms of Moscow, Tver, Voronezh, and Rostov Regions. The ground parts (n = 65) were cut at a height of 5 cm from the soil surface, dried in a shaded ventilated room and crushed whole. Another part of the plants (n = *29*) before grinding was divided into leaves, stems, and baskets.

In the group of mycotoxins, determined by the enzyme immunoassay using commercial and certified research test systems [12, 13], there were T-2 toxin (T-2), diacetoxyscirpenol (DAS), deoxynivalenol (DON), zearalenone (ZEN), fumonisins (FUM), alternariol (AOL), aflatoxin B_1 (AB_1), sterigmatocystin (STE), cyclopiazonic acid (CPA), emodin (EMO), ochratoxin A (OA), citrinin (CIT), mycophenolic acid (MPA), PR toxin (PR) and ergot alkaloids (EA).

The quantitative results were statistically processed by the single-factor

analysis of variance [14] using the R version 3.4.3 program (https://cran.r-project.org/bin/windows/base/old/3.4.3/) [15]. The differences between the mean s(M) were estimated by the significance level p = 0.05.

Results. The samples from the production batches of the sunflower seeds were fractionated into the main seeds and impurities to study separately the nature of their contamination (Table 1).

1. Occurrence (n^+) and the content of mycotoxins (μg/kg) **in seeds and impurities of batches of sunflower (*Helianthus annuus* L.) seeds** (Belgorod, Voronezh, Kursk and Lipetsk Regions, 2016)

Mycotoxin	Main seeds (n = 19)	Impurities	
		organic impurities (n = 19)	small part (n = 18)
T-2	–	15 (3-11-100)[a]	18 (2-12-100)[a]
DON	–	1 (1000)	2 (79, 1000)
DAS	–	14 (145-250-395)[a]	15 (130-305-935)[a]
ZEN	1 (15)	15 (25-34-50)[a]	16 (25-37-63)[a]
FUM	1 (79)	–	–
EA	–	1 (6)	4 (2-7-16)
AOL	15 (21-450-3080) [a]	19 (40-2390-7940)[b]	18 (44-1800-5620)[b]
AB_1	–	–	–
STE	–	2 (9, 12)	1 (20)
CPA	1 (200)	9 (77-145-250)[a]	7 (64-135-250)[a]
EMO	8 (12-42-125)[a]	17 (130-825-1620)[b]	17 (50-415-795)[c]
OA	–	–	1 (9)
CIT	–	12 (25-44-94)	6 (21-32-43)
MPA	6 (12-28-53)[a]	16 (16-245-2400)[b]	15 (27-260-1050)[b]
PR	–	4 (230-360-645)	2 (235, 300)

N o t e. T-2 — T-2 toxin, DAS — diacetoxyscirpenol, DON — deoxynivalenol, ZEN — zearalenone, FUM — fumonisins, EA — ergot alkaloids, AOL — alternariol, AB_1 — aflatoxin B1, STE — sterigmatocystin, CPA — cyclopiazonic acid, EMO — emodin, OA — ochratoxin A, CIT — citrinin, MPA — mycophenolic acid, PR — PR-toxin; n — the number of examined samples. The number of positive samples n^+ is indicated before the brackets, the minimum-average-maximum mycotoxin content in the positive samples is shown in brackets. A dash means that no positive samples were found. In one line, for values with different superscript indices (a, b, c), the differences are statistically significant at p = 0.05.

2. Occurrence (n^+) and the content of mycotoxins (μg/kg) **in leaves, baskets, stems and whole sunflower (*Helianthus annuus* L.) plants** (Moscow, Tver, Voronezh and Rostov Regions, 2016 and 2017)

Mycotoxin	Whole plants (n = 65)	Parts of plants (n = 29)		
		leaves	baskets	stems
T-2	14 (2-6-20)	23 (2-3-6)[a]	16 (2-15-145)[a]	1 (2)
ДАС	24 (97-165-265)	25 (130-265-645)[a]	9 (130-205-315)[a]	–
ДОН	–	2 (76, 100)	–	–
ЗЕН	–	4 (28-34-39)[a]	5 (26-29-33)[a]	–
ФУМ	–	–	–	–
ЭА	52 (2-14-60)	22 (2-14-100)	–	2 (2, 3)
АОЛ	42 (14-32-91)	23 (12-40-100)[a]	14 (15-110-775)[a]	3 (20-200-415)
AB_1	–	1 (2)	1 (4)	–
СТЕ	17 (12-14-25)	11 (10-16-27)	1 (25)	–
ЦПК	64 (89-235-500)	29 (130-415-980)[a]	20 (50-150-400)[b]	4 (115-130-140)
ЭМО	27 (19-37-10)	26 (25-47-100)[a]	17 (20-61-225)[a]	2 (26, 30)
ОА	7 (4-5-7)	3 (5-5-6)	1 (6)	–
ЦИТ	12 (32-42-50)	16 (29-44-63)	4 (21-42-60)	–
МФК	15 (15-52-225)	11 (13-21-35)[a]	11 (13-42-215)[a]	2 (13, 36)
PR	9 (150-180-265)	20 (130-300-500)	4 (135-195-260)	–

N o t e. T-2 — T-2 toxin, DAS — diacetoxyscirpenol, DON — deoxynivalenol, ZEN — zearalenone, FUM — fumonisins, EA — ergot alkaloids, AOL — alternariol, AB_1 — aflatoxin B1, STE — sterigmatocystin, CPA — cyclopiazonic acid, EMO — emodin, OA — ochratoxin A, CIT — citrinin, MPA — mycophenolic acid, PR — PR-toxin; n — the number of examined samples. The number of positive samples n^+ is indicated before the brackets, the minimum-average-maximum mycotoxin content in the positive samples is shown in brackets. A dash means that no positive samples were found. In one line, for values with different superscript indices (a, b), the differences are significant at p = 0.05.

Out of the 15 studied mycotoxins, AOL was almost universal, MPA and EMO were less frequent, and ZEN, FUM, and CPA were found in single samples. On the contrary, along with AOL, EMO and MPA, the impurities quite often contained fusariotoxins (T-2, DAS, ZEN), more rarely CPA and CIT, in

few cases DON, STE, EA and PR. There were no significant differences in the nature of the contamination of the organic impurities and the small part in most mycotoxins. The content ranges of AOL, EMO, and especially MPA in impurities were significantly wider and, by average values, significantly exceeded the indicators of seeds. The fact of multiple and intense contamination of impurities, described for the first time, is of practical importance, since it experimentally substantiates the need for thorough cleaning of sunflower seeds supplied for further processing.

It was of interest to identify which parts of the plants that get into the seeds during harvesting make a major contribution to contamination. To do this, we studied the component composition of mycotoxins in the ground parts, as well as leaves, baskets, and stems of a vegetative sunflower (Table 2). The contamination of plants was multiple; most often there were CPA, EA, AOL, EMO, followed by STE, CIT, and MPA, less often OA and PR, out of fusariotoxins, T-2 and DAS were detected with a frequency of 14/65 and 24/65. The accumulation of T-2, DAS and AOL could be caused by phytopathogenic fungi *Fusarium* and *Alternaria*, whereas almost constant detection of CPA, EA, and EMO in the samples seems to be associated with the members of genera *Aspergillus* and *Penicillium* [16-18]. They often accompany the pathogens of fungal diseases of sunflower in very small quantities [19], but their role in the production of toxins is yet to be assessed.

In leaves and baskets, the complex of the main contaminants as a whole corresponded to the established one for whole plants, and toxic metabolites were found extremely rarely in the stems (see Table 2). These results are consistent with the previously published ones for a smaller sample [20]. The heterogeneous nature of the distribution of mycotoxins among the plant organs is seen as a manifestation of the complex associative connections of these organisms with microscopic fungi, mainly endophytes [21, 22]. According to the obtained data, the content of all toxins, except for ZEN and MPA, was less in the baskets in comparison with the leaves. At the same time, a statistically significant decrease in the content of CPA was observed. The extensive information, which has appeared in recent years, on the composition and content of fungal metabolites in lichen thalli [23], and now in sunflower, has become an important contribution to understanding the biochemical mechanisms of regulation of coenotic interactions. The advanced comprehensive studies of endogenous fungi and the metabolic profile, formed in plants with the participation of the entire associated community of organisms, seem to be especially promising.

We had the opportunity to analyze the samples from a batch cleaned and splinted for storage between two premises in one of which technical problems led to the appearance of self-warming foci. In the main seeds under unfavorable condition, a much larger amount of AOL and MPA was detected (Table 3). According to the results of the mycological analysis in the seeds of these compartments, there were both similarities and differences. The number of affected representatives of the genus *Alternaria* was equally extensive (85-95%), and the frequency of *Penicillium* spp. in the affected part was significantly higher (248×10^3 CFU/g vs. 7×10^3 CFU/g); moreover, with a frequency of 10 %, they were accompanied by one of the species of *Aspergillus glaucus* Gr. From morphological features, the species composition of *Alternaria* and *Penicillium* fungi in both parts was fairly uniform. The typical strains of *A. tenuissima* (Nees & T. Nees: Fr.) Wiltshire and P. *stoloniferum* Thom in the laboratory express tests were highly active and formed AOL and MPA of more than 10000 ng/ml of the medium. The ability of *A. tenuissima* to produce AOL was described previously [24], and for the species *P. stoloniferum*, subjected to multiple taxonomical movements [25] and

currently recognized as synonymous with *P. brevicompactum*, the possibility of MPA biosynthesis was repeatedly confirmed by foreign and Russian researchers [26-28]. In addition, *A. pseudoglaucus* Blochwitz from *Aspergillus glaucus* Gr. also belong to MPA producers [26]. Accounting a general trend of increased AOL and MPA amounts in sunflower seeds with changing external conditions, there were no effects of growth inhibition and suppression of specific toxins biosynthesis between these species. The reports on the mutual influence of toxigenic fungi of different genera coexisting on the same biological substrate are still few. Nevertheless, it was shown that the rate of colonization of wheat caryopsides with the fungus *A. tenuissima* and the amount of formed AOL significantly increased after the preliminary treatment of fusariotoxins DON or ZEN [29]. Recently, the relationship between aggressive trichothecene-producing species *Fusarium* and *Alternaria* fungi in oats has been characterized as symbiotic [30].

3. Mycotoxin contents (μg/kg) **in the main fraction and impurities of the same batch of sunflower (*Helianthus annuus* L.) seeds depending on storage conditions** (Kursk Region, 2016)

Mycotoxin	Main seeds	Impurities	
		organic impurities	small part
T-2	–/–	–/3	3/5
DAS	–/–	–/–	215/400
DON	–/–	–/–	–/–
ZEN	–/–	–/–	49/45
FUM	–/–	–/–	–/–
EA	–/–	–/–	–/–
AOL	26/240	955/245	480/390
AB_1	–/–	–/–	–/–
STE	–/–	–/–	–/15
CPA	–/–	–/–	–/250
EMO	–/41	735/600	355/1000
OA	–/–	–	–/–
CIT	–/–	26/-	–/–
MPA	53/2630	125/1000	345/6310
PR	–/–	–/–	–/–

N o t e. T-2 — T-2 toxin, DAS — diacetoxyscirpenol, DON — deoxynivalenol, ZEN — zearalenone, FUM — fumonisins, EA — ergot alkaloids, AOL — alternariol, AB_1 — aflatoxin B1, STE — sterigmatocystin, CPA — cyclopiazonic acid, EMO — emodin, OA — ochratoxin A, CIT — citrinin, MPA — mycophenolic acid, PR — PR-toxin. Estimates for due and violated storage condition (self-warming foci) are indicated through a slash. A dash means that no positive samples were found.

For organic residues in the impurities, as a result of self-warming, there was an increase in the contamination of MPA and the appearance of STE and CPA, but the same clear tendency towards AOL, EMO and fusariotoxins (T-2, DAS, ZEN) was not observed (see Table 3). It is probable that higher accumulation of MPA on the dead sunflower tissues is related to the fact that under these conditions *P. stoloniferum* gets a chance to realize the potential of a mycophilic fungus of physiological necrotrophs [27]. The fact regarding its habitat on the stroma of the phytopathogen *Helminthosporium sativum* Pam. and the inhibiting effect against the pathogens of common sunflower diseases *Botrytis cinerea* Fr. and *Verticillium dahliae* are described [31].

The sharp accumulation of mycotoxins, not only in seeds but also in the impurities, serves as another argument in favor of the increased attention to cleaning batches before storage and processing.

When examining the production batches of seeds, as well as the samples from self-warming foci, we did not note a single case of AB_1 detection given the sufficiently high sensitivity of the applied method (0.002 mg/kg). Nevertheless, AB_1 is officially recognized as the only indicator of safety (not more than 0.005 mg/kg) for food oilseeds (including sunflower), as well as for unrefined vegetable oils of all types and their products [32-34]. The apparent inconsistencies between the mycotoxicological criteria for the control of sunflower oil cakes and meals,

and the prevalence of real carriers of the threat to animal health we examined in detail earlier [4].

Thus, in sunflower oil seeds, the main contaminants of mycogenic origin are represented by alternariol, mycophenolic acid, and emodin. The impurities that get into the batch during harvesting and contain the remnants of the herbage of plants are characterized by a more intensive accumulation of these mycotoxins and the appearance of a number of others, i.e. fusariotoxins, as well as cyclopiazonic acid and citrinin. If the storage conditions are violated, higher humidity and temperature lead to sharply increased accumulation in seeds of alternariol and mycophenolic acid which have toxic effects, including genotoxicity and immunosuppressive activity. This first information on the nature of contamination by mycotoxins of commercial sunflower oil seed batches is a step towards the creation of a Russian replenishable database to improve the hygienic and sanitary requirements of food and fodder safety.

REFERENCES

1. Chulze S.N., Torres A.M., Dalcero A.M., Etcheverry M.G., Ramirez M.L., Farnochi M.C. *Alternaria* mycotoxins in sunflower seeds: incidence and distribution of the toxins in oil and meal. *J. Food Prod.*, 1995, 58: 1133-1135.
2. Suganthi M.P.F., Ravi R., Purushothaman M.R., Mohan B., Vasanthakumar P. Occurrence of ochratoxin A in sunflower cake and evaluation of detoxified sunflower cake in poultry. *Indian Journal of Poultry Science*, 2003, 38(1): 37-41.
3. Mmongoyo J.A., Wu F., Linz J.E., Nair M.G., Mugula J.K., Tempelman R.J., Strasburg G.M. Aflatoxin levels in sunflower seeds and cakes collected from micro- and small-scale sunflower oil processors in Tanzania. *PLoS ONE*, 2017, 12(4): 1-14 (doi: 10.1371/journal.pone.0175801).
4. Kononenko G.P., Ustyuzhanina M.I., Burkin A.A. The problem of safe sunflower (*Helianthus annuus* L.) use for food and fodder purposes (review). *Agricultural Biology*, 2018, 53(3): 485-498 (doi: 10.15389/agrobiology.2018.3.485eng).
5. Ostry V. *Alternaria* mycotoxins: an overview of chemical characterization, producers, toxicity, analysis and occurrence in foodstuffs. *World Mycotoxin J.*, 2008, 1(2): 175-188 (doi: 10.3920/WMJ2008.x013).
6. EFSA Panel on Contaminants in the Food Chain. Scientific Opinion on the risks for animal and public health related to the presence of *Alternaria* toxins in feed and food. *EFSA Journal*, 2011, 9(10): 2407 (doi: 10.2903/j.efsa.2011.2407).
7. Arcella D., Escola M., Ruiz J.A.G. Dietary exposure assessment to *Alternaria* toxins in the European population. *EFSA Journal*, 2016, 14(12): 4654 (doi: 10.2903/j.efsa.2016.4654).
8. *GOST 22391-2015. Podsolnechnik. Tekhnicheskie uslo*viya [RF State Standard 22391-2015. Sunflower]. Moscow, 2015 (in Russ.).
9. Raper K.B., Thom C., Fennell D.I. *A manual of the Penicillia*. Williams & Wilkins Company, USA, Baltimore, 1949.
10. Simmons E.G. *Alternaria. An identification manual*. CBS, Utrecht, 2007.
11. *Introduction to food- and airborne fungi*. 6th edition. R.A. Samson, E.S. Hoekstra, J.C. Frisvad, O. Filtenborg (eds.). CBS, Utrecht, 2000.
12. *GOST 31653-2012 Korma. Metod immunofermentnogo opredeleniya mikotoksinov* [RF State Standard. Feeds. Mucotoxin analysis by ELISA test]. Moscow, 2012 (in Russ.).
13. Burkin A.A., Kononenko G.P. Mycotoxin contamination of reindeer moss. *Russian Agricultural Science*, 2011, 37(2): 182-184.
14. McDonald J.H. *Handbook of biological statistics*. 3rd edition. Sparky House Publishing, USA, Baltimore, 2014. Available dostupa: http://www.biostathand-book.com. No date.
15. *The R project for statistical computing*. Available http://www.r-project.org. No date.
16. Chang P.-K., Erlich K.C., Fujii I. Cyclopiazonic acid biosynthesis of *Aspergillus flavus* and *Aspergillus oryzae*. *Toxins*, 2009, 1: 74-99 (doi: 10.3390/toxins1020074).
17. Gerhards N., Neubauer L., Tudzynski P., Li S.-M. Biosynthetic pathways of ergot alkaloids. *Toxins*, 2014, 6: 2381-2395 (doi: 10.3390/toxins6123281).
18. Izhaki I. Emodin — a secondary metabolite with multiple ecological functions in higher plants. *New Phytologist*, 2002, 155(2): 205-217 (doi: 10.1046/j.1469-8137.2002.00459.x).
19. Vypritskaya A.A. *Mikobiota podsolnechnika v Tambovskoi oblasti* [Mycobiota of sunflower plants in the Tambov region]. Tambov, 2015 (in Russ.).
20. Zotova E.V., Kononenko G.P., Burkin A.A. *Sovremennaya mikologiya v Rossii*, 2017, 7: 202-204 (in Russ.).

21. Rodriguez R.J., White J.F. Jr., Arnold A.E., Redman R.S. Fungal endophytes: diversity and functional roles. *New Phytol.*, 2009, 182(2): 314-330 (doi: 10.1111/j.1469-8137.2009.02773.x).
22. Zhang H.W., Song Y.C., Tan R.X. Biology and chemistry of endophytes. *Nat. Prod. Rep.*, 2006, 23(5): 753-771 (doi: 10.1039/b609472b).
23. Kononenko G.P., Burkin A.A. Distribution of mycotoxins and usnic acid in the thalli of epigeous lichens. *Biology Bull.*, 2015, 42(3): 213-219 (doi: 10.1134/S1062359015030036).
24. Tralamazza S.M., Piacentini K.C., Tadashi Iwase C.H., de Olivera Rocha L. Toxigenic *Alternaria* species: impact in cereals worldwide. *Current Opinion in Food Science*, 2018, 23: 57-63 (doi: 10.1016/j.cofs.2018.05.002).
25. Scott J.A., Wong B., Summerbell R.C., Untereiner W.A. A survey of *Penicillium brevicompactum* and *P. bialowiezense* from indoor environments, with commentary on the taxonomy of the *P. brevicompactum* group. *Botany*, 2008, 86(7): 732-741 (doi: 10.1139/B08-060).
26. Burkin A.A., Kononenko G.P. Producers of mycophenolic acid in ensiled and grain feeds. *Applied Biochemistry and Microbiology*, 2010, 46(5): 545-550 (doi: 10.1134/S0003683810050145).
27. Frisvad J.C., Filtenborg O. Terverticillate *Penicillia*: chemotaxonomy and mycotoxin production. *Mycologia*, 1989, 81(6): 837-861 (doi: 10.2307/3760103).
28. Overy D.P., Frisvad J.C. Mycotoxin production and postharvested storage rot of ginger (*Zingiber officinale*) by *Penicillium brevicompactum*. *J. Food Protect.*, 2005, 68(3): 607-609.
29. Müller M.E.H., Urban K., Köppen R., Siegel D., Korn U., Koch M. Mycotoxins as antagonistic or supporting agents in the interaction between phytopathogenic *Fusarium* and *Alternaria* fungi. *World Mycotoxin J.*, 2015, 8(3): 311-321 (doi: 10.3920/WMJ2014.1747).
30. Orina A.S., Gavrilova O.P., Gagkaeva T.Yu., Loskutov I.G. Symbiotic relationships between aggressive *Fusarium* and *Alternaria* fungi colonizing oat grain. *Agricultural Biology*, 2017, 52(5): 986-994 (doi: 10.15389/agrobiology.2017.5.986eng).
31. Rudakov O.L. *Mikofil'nye griby, ikh biologiya i prakticheskoe znachenie* [Mycophilic fungi, their biology and practical significance]. Moscow, 1981 (in Russ.).
32. *Gigienicheskie trebovaniya bezopasnosti i pishchevoi tsennosti pishchevykh produktov. Sanitarno-epidemiologicheskie pravila i normativy. SanPiN 2.3.2.1078-01* [Hygienic requirements for safety and nutritional value of food. Sanitary amd epidemiological rules and regulations. SanPiN 2.3.2.1078-01]. Moscow, 2008 (in Russ.).
33. *TR TS 021/2011 Tekhnicheskii reglament Tamozhennogo soyuza «O bezopasnosti pishchevoi produktsii»*. Utverzhden resheniem Komissii Tamozhennogo soyuza ot 9 dekabrya 2011 g. № 880 [TR TS 021/2011 Technical regulations of the Customs Union "On food safety" of December 9, 2011 No 880] (in Russ.).
34. *TR TS 015/2011 Tekhnicheskii reglament Tamozhennogo soyuza «O bezopasnosti zerna»*. Utverzhden resheniem Komissii Tamozhennogo soyuza ot 9 dekabrya 2011 g. № 874 [TR TS 015/2011 Technical Regulations of the Customs Union "On the safety of grain" of December 9, 2011 No 874] (in Russ.).