

## TOXIGENIC FUNGI ON CEREAL CROPS IN RUSSIA

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Toxigenic fungi are pathogenic microorganisms that produce mycotoxins and cause mycoses and mycotoxicoses. According to FAO, 25% of the world's grain production is contaminated with mycotoxins. In developing countries, up to 36% of all diseases are directly or indirectly related to fungal mycotoxins. The review considers the situation with infestation of grain crops in different regions of Russia by toxigenic fungi of the genera *Claviceps*, *Fusarium*, *Alternaria*, *Aspergillus* and *Penicillium* and accumulation of mycotoxins dangerous for humans and animals. *Claviceps* fungi are widespread on cereals, especially harmful on rye. They contain toxic alkaloids with nerve agent action. The toxins produced by *Fusarium* are harmful to human and animal health. Different *Fusarium* species can produce a wide range of mycotoxins. Fungi of the genus *Alternaria* are widely distributed on agricultural crops. Diseases caused by *Alternaria* affect usually the grain of all cereals. The main danger of grain contamination by *Alternaria* species is the presence of secondary metabolites toxic to plants, animals and humans in agricultural products. Fungi of the genus *Aspergillus* affect grain of wheat, barley, corn and other crops. Species of the genus *Aspergillus* produce toxins harmful to humans and animals. They have carcinogenic, mutagenic, teratogenic and immunosuppressive properties. Fungi of the genus *Penicillium* mainly cause seed mold. Seed mold causes reduced germination and often seed death. Fungi of the genus *Penicillium* produce a large number of mycotoxins. They have nephrotoxic, carcinogenic and mutagenic properties. Disease development and mycotoxin production are influenced by climatic conditions.

**Keywords:** fungal species, mycotoxicoses, mycotoxins, monitoring of fungi, toxigenic fungi

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### INTRODUCTION

Toxigenic fungi and mycotoxins began to be studied in Russia in the 30–40s of the last century. The term mycotoxicosis was first introduced into scientific literature by Prof. A. H. Sarkisov. This term was understood as alimentary diseases of non-infectious nature, in which the reproduction of microbe in the organism was not established. Mycotoxicosis occurred under the influence of toxic products released by the fungal cell. Fungal infections of cereal crops caused by toxigenic fungi cause huge economic losses in agricultural industries (Levitin, 2004).

Toxigenic fungi are studied in many countries (Logrieco, Visconti, 2004). A number of review articles have been devoted to this problem (Fulgueira, Borghi, 2000; Pitt, 2000; Kononenko et al., 2021; Alkuwari et al., 2022). In this review we tried to highlight the situation with toxigenic fungi on grain crops in Russia.

### Occurrence of toxigenic *Claviceps purpurea* on cereals

*Claviceps purpurea* fungus causes ergot of cereals. Large-scale development of ergot was first noted only during the Second World War and post-war years. Currently, this disease is widespread everywhere, but to a greater extent in the Northwestern region and central areas of the Non-Black Earth zone. Affection of production crops of winter rye in Kirov Region averaged from 0.02 to 1.7% depended on varieties, in 2017, the spread of the disease in some fields reached 5%. The level of ergot damage depends more on the number and size of sclerotia in the ear than on its distribution in the crop (Shchekleina, Sheshegova, 2018).

The disease is generally considered a disease of rye, but it is also found on wheat, triticale, barley, oats, millet and other grains. When infected by the fungus, black and purple sclerotia form in the ear of the plant contained ergoalkaloids that are toxic to humans and animals. Sclerotia-contaminated flour products can cause epileptic convulsions in humans.

Among the alkaloids, ergotamine is the best known, which has a strong nerve agent. Ergot alkaloids affect the nervous, circulatory, reproductive and immune systems, leading to increased or decreased blood pressure, muscle contractions, decreased fertility, reduced immune response, hallucinations and dry gangrene of the gastrointestinal tract and extremities. Eating bread made from rye or wheat contaminated with spores of the fungus causes poisoning in humans, sometimes to epidemic proportions (Sarkisov, 2000).

### Occurrence of toxigenic *Fusarium* species on grain crops

It is known that *Fusarium* species cause considerable damage of cereal. Invasion of the kernel by *Fusarium* destroys the starch granules, storage proteins and cell walls, resulting in a poor quality products. As a result a *Fusarium* epidemic can decrease yield to up to 30%. Various *Fusarium* species are capable of producing mycotoxins in crops, which cause severe poisoning, damage blood-forming organs and immunity, and decrease productivity in animals.

The first information about the toxigenicity of fungi of the genus *Fusarium* became known during the Second World War as a result of an expedition led by A.H. Sarkisov to Altai. The expedition was aimed at deciphering the cause of a deadly and widespread disease of humans and animals. The causative agents of the disease turned out to be toxin-forming species of the genus *Fusarium* (Sarkisov, 1954).

In the last 10–15 years *Fusarium* head blight (FHB) of cereals has been very widely spread in Russia. Only in the Krasnodar Region (North Caucasus) there were three large epidemics of FHB. The loss of wheat crop reached 25–50% and the contamination of cereal grains by mycotoxins increased more than 25 times (Levitin et al., 1994). In 25–80% of wheat samples the concentration of deoxynivalenol (DON) exceeded the permissible level. During 1989–1992 on average about 23% samples of cereals (wheat, barley, rye) in Russia were contaminated by DON. Amongst them, 9% of samples contained DON in concentrations exceeding the permissible level. In 0.4% of samples of bread and groats products concentrations of mycotoxins exceeded hygienic standards (Tutelyan, 1995). During 1989–1992 on average about 23% samples of cereals (wheat, barley, rye) in Russia were contaminated by DON. Amongst them, 9% of samples contained DON in concentrations exceeding the permissible level.

Different *Fusarium* species can produce a wide range of secondary metabolites. Strains of *Fusarium graminearum*, *F. culmorum*, and *F. roseum* are known to produce trichothecene mycotoxins of group B and belong to two chemotypes differing in their ability to produce deoxynivalenol (DON) or nivalenol (NIV). Deoxynivalenol can be produced by *F. graminearum*, *F. crookwellense*, and *F. culmorum*

(Logrieco et al., 2003). These species and additionally *F. equiseti* and some strains of *F. oxysporum* are also zearalenol (ZON)-producers. Fumonisin (FUM) are produced by the typical maize pathogens *F. fujikuroi*, *F. proliferatum*, and *F. oxysporum*. The European Commission passed threshold values for DON, ZON and FUM in unprocessed cereals and food. *F. roseum*, *F. graminearum*, and *F. culmorum* share a gene cluster responsible for the biosynthesis of trichothecene mycotoxins. The toxins produced by these fungi are harmful to human and animal health (Chandler et al., 2003).

It is known that mycotoxins of fungi of the genus *Fusarium* (deoxynivalenol, nivalenol, T-2 toxin, diacetoxyscirpenol), in addition to affecting the gastrointestinal tract, cardiovascular and nervous systems, have mutagenic effects, induce chromosomal rearrangements, affect protein biosynthesis. Some species, such as *F. verticillioides* and *F. proliferatum* have hepatotoxic, nephrotoxic, neurotoxic and carcinogenic effects. The species *F. avenaceum* produces mycotoxins moniliformin and fusarin C. The first is an immunosuppressor, suppresses protein biosynthesis, and causes pathological changes in cardiac muscle. The second one has a carcinogenic and mutagenic effect on cells of warm-blooded organisms. Fusariotoxins are very persistent, not destroyed by boiling and cooking food products (Kononenko, Burkin, 2003).

The problem of cereal grain contamination by mycotoxins is very important and actual for Russia. The content of mycotoxins in grain crops may vary in different regions of Russia. In our researches significant differences in toxin production between populations of *F. graminearum* from North Caucasus and the Far East were observed. The number of isolates producing DON and ZON in concentration 1.0 and 0.3 mg/g respectively in the North Caucasus population was 3 times higher than in the Far East population. The strains of *F. sporotrichioides* isolated from infected cereal grain in Siberia produced the T-2 toxin at a very high concentration (3000 mg/kg), while the European strains synthesised this toxin at a level of 100–300 mg/kg (Gagkaeva, Levitin, 1997).

The ability of fungal isolates, selected in the same region, to produce mycotoxins can vary widely. For example, *F. culmorum* isolates, selected from wheat seeds in the Moscow district (Central Russia) produced DON in amounts of 1.9–1850.0 mg/kg. Some of them can also produce other mycotoxins: 3-acetyl-deoxynivalenol (3AcDON) at levels of 1.8–21.9 mg/kg and moniliformin (MON) at levels of 0.7–3.7 mg/kg. Some isolates of *F. equiseti* produced toxin fusarochromanone at levels of 13.0–527.9 mg/kg. Isolates of *F. avenaceum* produced only MON at levels of 2.9–9.0 mg/kg. Eighty two percent of the *F. avenaceum* isolates from fusariosis grain produced MON at levels of 191.1 mg/kg, some of them produced butenolide

5-actamido-2(5H)-furanone simultaneously. The isolates of *F. culmorum* from infected grain produced DON at an average level of 24.5 mg/kg. Moreover, some isolates also produced 3AcDON at levels of 250.5 mg/kg (Kononenko et al., 1999).

The mycotoxin contamination of cereal grain in Asian part of Russia was studied in the laboratory of mycotoxicology in All-Russian Research Institute of Veterinary Sanitation, Hygiene and Ecology during 1995–2002. Among 1545 of samples 537 (34.8%) were contaminated by Fusarium toxins. The frequency of T-2 toxin detection was 69.4% in Ural district, 86.9% – in West Siberia, 29.6% – in Far East. The average concentration of T-2 toxin in grain was 10–100 µ/kg in Asian part of Russia. In 24 samples (5.4%) mainly from Far East concentration of T-2 toxin exceeded the permissible level (110.0–625.5 µ/kg). In Far East besides T-2 toxin in wheat samples the group including 8-oxotrichotecene (4 deoxynivalenol and its analogies) and zearalenone (ZEA) was detected. In 2001 the quantity of grain samples contaminated by 8-oxotrichotecene and zearalenone (ZEA) was 52.9%; in 2002–90.7%. 23.5% of the contaminated samples contained 8-oxotrichotecene in concentration 1000 µ/kg, indicating a difficult mycotoxicological situation in this region of Russia. The monitoring for Fusarium species composition was also carried out in the Ural (Pirayeva, 2001). The infection of cereal seeds was 3.5%; the barley and oats seeds were infected very strongly. From infected seed samples 11 *Fusarium* species were isolated. *F. poae* and *F. avenaceum* were dominant species (Kononenko, Burkin, 2003).

In the laboratory of Mycology and Phytopathology of All-Russian institute of Plant Protection analysis of the species composition of fungi of the genus *Fusarium* and their mycotoxins in the Asian part of Russia revealed 16 species, of which *F. sporotrichioides*, *F. avenaceum*, *F. poae*, and *F. anguioides* were predominant. Toxins have been detected: deoxynivalenol, fumonisins, T-2 and HT-2 toxins, nivalenol, moniliformin, and beauvericin (Gavrilova et al., 2023).

Thus, the studies have established a very high variability of *Fusarium* species in Russia and a wide spread of contamination of grain crops with mycotoxins. Similar studies are necessary to assess the pathogenic and toxigenic potential of *Fusarium* species in different geographical zones of Russia.

#### Occurrence of toxigenic *Alternaria* species on cereals

Fungi of the genus *Alternaria* are widely distributed on agricultural crops. Diseases caused by *Alternaria* affect usually the grain all cereals. The samples from different regions of Russia were characterized by high degree of infection caused by *Alternaria* fungi. The degree of seed infection by *Alternaria* was an average 10%. During germination of

wheat seeds diseased with *Alternaria*, deformation of the seedling, appearance of gray spider mycelium, darkening of the root neck and stem base are observed. Seed infestation can occur during plant development, resulting in the symptom of black seed germ. Flour obtained from grain affected by black germ has black flecks, which significantly reduces its value in the production of bread and pasta products (Gannibal, 2008).

Grain infected with *Alternaria* species may contain the secondary metabolites Alternariol, Alternariol monomethyl ester and tenuazonic acid (Gannibal, 2007). They pose a danger to humans and animals. Russia, both highly toxigenic *Alternaria alternata* and *A. tenuissima* and non-toxic species *A. infectoria* are found in grain, and their ratio in different regions can vary greatly. Species *A. tenuissima*, *A. alternata*, *A. arborescens* affect seeds (including grain), vegetables, fruits, nuts and produce alternaria, alt毒素, tentoxin, tenuazonic acid. Analysis of wheat, barley and oat grain from seven regions of Russia showed extensive distribution and high levels of mycotoxin *Alternaria* in grain (Kononenko, Burkin, Zotova, 2020). Analysis of wheat, barley and oat grain samples obtained from the Ural and West Siberian regions of Russia in 2017–2019 showed high contamination with *Alternaria* species and the presence of four mycotoxins – alternariol (AOH), *Alternaria* monomethyl ester (AME), tentoxin (TEN), and tenuazonic acid (TeA) (Orina et al., 2021).

In medical terms, fungi of the genus *Alternaria* are considered primarily as fungi that cause allergic reactions in humans. However, fungi of the genus *Alternaria* are also known to frequently cause fungal lesions of the maxillary sinuses. They are the causative agents of nail and skin infections in humans and also cause abscesses on the cornea of the eye.

#### Occurrence of toxigenic *Aspergillus* and *Penicillium* species on cereals

Fungi of the genus *Aspergillus* affect grain of wheat, barley, corn and other crops. In the field, species of this genus develop during ripening and harvesting at high air humidity and on weakened plants. Affected grain becomes unsuitable for use in the food industry and for animal feed. *Aspergillus* colonies are found on a variety of products, mainly of plant origin. Grain damaged by insects is particularly susceptible to aspergillus infestation and increased concentration of mycotoxins (Medina et al., 2014; Monastyrsky, 2014). As a result of mycotoxin contamination of grain, the annual global economic damage reaches 16 billion dollars (Afonyushkin et al., 2005). In recent years, the amount of grain contaminated with mycotoxins has increased tenfold in Russia (Dzhavakhia et al., 2017).

Species of the genus *Aspergillus* produce aflatoxins, ochratoxin A, patulin, citrinin. Mycotoxins are particularly



dangerous for humans and animals. Aflatoxins have hepatotropic ability, with the liver being severely intoxicated. In addition, they have carcinogenic, mutagenic, teratogenic and immunosuppressive properties. The well-known disease aspergillosis is an opportunistic infection that usually affects the lower respiratory tract (Pfliegler et al., 2020).

The most dangerous species for humans and animals are: *Aspergillus fumigatus*, *A. terreus*, *A. niger*, *A. parasiticus*, and *A. flavus*. The species *A. fumigatus* is the cause of invasive pulmonary disease, *A. terreus* produces aflatoxin G2, causing acute renal failure, *A. niger* often cause otomycosis, *A. flavus* most often causes invasive extrapulmonary infection. According to foreign researchers, fungi of the genus *Aspergillus* were isolated in 72 recipients of hematopoietic stem cells, including species of *A. fumigatus* (56%), *A. flavus* (19%), *A. terreus* (16%), *A. niger* (8%), and *A. versicolor* (1%) (<https://applied-research.ru/ru/article/view?id=7716>).

In practice, it is not uncommon for seeds with high viability to have low germination rates. Biological analysis revealed that these seeds were heavily infected with *Penicillium*. Fungi of the genus *Penicillium* are widespread. Species of *Penicillium viridicatum*, *P. verrucosum*, *P. citrinum*, *P. digitatum*, etc. may occur on grain. They mainly cause seed mold. Seed molding leads to reduced germination and often to seed death. Especially often seed molding occurs at high humidity in closed damp rooms and warehouses. Fungi of the genus *Penicillium* produce a large number of mycotoxins. These include citrinin, patulin, rubratoxin, citreoviridin, ochratoxin, rugulosin, islandin and others. Some species such as *P. roqueforti*, *P. brevicompactum*, and *P. chrysogenum* produce PR-toxin and mycophenolic acid (Kononenko et al., 2021). Toxins of *P. citrinum* and *P. digitatum* possess pronounced hepatotoxicity, whereas toxin of *P. patulum* patulin possesses carcinogenic and mutagenic properties. Toxins of *P. citrinum* and *P. digitatum* possess pronounced hepatotoxicity. The most toxic are ochratoxins. They primarily affect the kidneys. Nephrotoxic effect is manifested in the development of toxic nephropathy, have teratogenic, embryotoxic, carcinogenic effect.

### Toxigenic fungi and mycotoxins in a climate change

The climate of our planet has been changing rapidly. The changes of climate can undoubtedly affect the spread and development of plant diseases and their relationship with the host. Climate represents the key factor in driving the fungal community structure and mycotoxin contamination levels pre- and post-harvest. Thus, there is significant interest in understanding the impact of interacting climate change-related abiotic factors (Perrone et al., 2020).

In Russia, from 1990 to 2000, the air temperature has increased by 0.4°C. Global warming manifests itself throughout Russia, but the Northwest Russia and Siberia. Over the past 10 years, the specialists of the Mycology and Phytopathology Laboratory, of All-Russian Research Institute of Plant Protection, having monitored the spread of cereal crop diseases in the Northwest Russia, identified some new diseases in the region. In the late 1980s and early 1990s, the heaviest wheat ear *Fusarium* epiphytoty broke out in the North Caucasus. *Fusarium graminearum* was the main pathogen. Since 2003, *F. graminearum* has been encountered in the Northwest Russia (Gavrilova, Gagkaeva, 2010). An average number of *Fusarium*-infected samples in the Northwest Region made 93.3% in 2007 and 87.3% in 2008. Disease distribution in the northern areas of the country is well explained by global warming and changes in air composition. It is evidenced by the articles of our colleagues from the Nordic countries. In recent years, *F. graminearum* has become the dominant species in cereals in the Netherlands (Waalwijk et al., 2003), England (Jennings et al., 2004), Northern Germany (Miedaner et al., 2008) and Finland (Yli-Mattila, Gagkaeva, 2010). Climate change is expected to change the species composition of *Fusarium* in northern Europe by 2050 (Parikka et al., 2012). And already in 2019, a typical southern species, *F. verticillioides* was discovered on winter wheat in southwestern Finland (Gagkaeva, Yli-Mattila, 2020).

A change of air temperature can actually change the dominant species. The situation in Northern Italy (Magan et al., 2011) can serve as an example. In this region, *F. verticillioides* prevailed in maize. The optimum growth factor of this species is the temperature of 25–30°C. In 2003–2004, the summer was hot and dry. *Aspergillus flavus* which is tolerant to the temperature of 35°C, has become the dominant species.

### CONCLUSION

In recent years, mycotoxin contamination of grain has increased more than 25 times (Levitin, Dzhavakhia, 2020). In 25–80% of wheat samples the concentration of deoxynivalenol (DON) exceeded the permissible level. On average for 1989–1992 in Russia, about 23% of samples of cereal crops (wheat, barley, rye) were contaminated with the mycotoxin deoxynivalenol (DON). Of these, 9% of samples contained DON in concentrations exceeding the permissible level. Mycotoxin concentrations in 0.4% of bread and cereal samples exceeded hygienic standards. The problem of cereal grain contamination by mycotoxins is very important and actual for Russia. Mycotoxicological studies have shown that 43 to 48% of grain samples contain mycotoxins in amounts exceeding the minimum permissible level, and 32% are contaminated with two or more toxins (Soldatenko et al., 2020). The authors of these studies

summarized some suggestions for predicting and preventing mycotoxin-related risks, as well as future perspectives and research needs to better understand the effects of climate change scenarios. Same mycotoxin can be produced by different fungi and the same fungus can produce different mycotoxins. A large number of toxigenic *Fusarium* species have been isolated from blood, cerebrospinal fluid, leg wounds, abdominal cavity, brain and lungs of sick people (Sugiura et al., 1999). Mycotoxins in animal feed are a serious concern. Generalization of the results demonstrated domination of fusarium toxins in the contamination of all types of feed grains and increased occurrence of T-2 toxin and ochratoxin A in barley. The maize grains demonstrated the whole complex of the tested fusarium toxins with the prevalence of T-2 toxin, deoxynivalenol, zearalenone and fumonisins (Kononenko et al., 2020).

Toxigenic fungi are actively studied in the Laboratory of Mycology and Phytopathology of the All-Russian Research Institute of Plant Protection (St. Petersburg) and in the Laboratory of Mycotoxicology of the All-Russian Research Institute of Veterinary Sanitation, Hygiene and Ecology (Moscow). Similar studies are needed to assess the pathogenic and toxigenic potential of fungi in different geographical zones of Russia.

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## Токсигенные грибы на зерновых культурах в России

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Токсигенные грибы — патогенные микроорганизмы, продуцирующие микотоксины и вызывающие микозы и микотоксикозы. По данным ФАО, 25% мирового производства зерна загрязнено микотоксинами. В развивающихся странах до 36% всех заболеваний напрямую или косвенно связаны с грибными микотоксинами. В обзоре рассматривается ситуация с зараженностью зерновых культур в различных регионах России токсигенными грибами родов *Claviceps*, *Fusarium*, *Alternaria*, *Aspergillus*, *Penicillium* и накоплением микотоксинов, опасных для человека и животных. Грибы рода *Claviceps* широко распространены на зерновых культурах, особенно вредоносны на ржи. Они содержат токсичные алкалоиды нервнопаралитического действия. Токсины, продуцируемые *Fusarium*, вредны для здоровья человека и животных. Различные виды рода *Fusarium* могут продуцировать широкий спектр микотоксинов. Грибы рода *Alternaria* широко распространены на сельскохозяйственных культурах. Заболевания, вызываемые *Alternaria*, поражают, как правило, зерно всех злаковых культур. Основная опасность заражения зерна видами рода *Alternaria* заключается в наличии в сельскохозяйственной продукции вторичных метаболитов, токсичных для растений, животных и человека. Грибы рода *Aspergillus* поражают зерно пшеницы, ячменя, кукурузы и других культур. Виды рода *Aspergillus* продуцируют токсины, вредные для человека и животных. Они обладают канцерогенными, мутагенными, тератогенными и иммунодепрессивными свойствами. Грибы рода *Penicillium* в основном вызывают плесневение семян, снижающую их всхожесть и часто вызывающую их гибель. Однако грибы рода *Penicillium* продуцируют и большое количество микотоксинов. Они обладают нефротоксическими, канцерогенными и мутагенными свойствами. Развитие заболеваний и продукция грибами микотоксинов зависят от климатических условий.

**Ключевые слова:** грибы, микотоксикозы, микотоксины, мониторинг грибов, токсигенные грибы