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Wheat grain microfungi in the Polissya region

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Abstract

Quantitative and qualitative information on the micromycetes found in Polissya-grown wheat grain is presented in this article. Wheat grain samples from the Polissya area had an average of 2.88 104 3.62 103 colony forming units (CFU) per 1 g of grain throughout the investigation period. Twenty fungal species from nine different taxa were found in Polissya wheat kernels. The samples were infected with fungi from the genera Alternaria (92.5%), Mucor (92.5%), Aspergillus (83.1%), Penicillium (47.2%), Fusarium (60.4%), Phoma (15.1%), Mycelia (15.1%), Trichotecium (1.9%), and Monascus (1.9%). Among the Aspergillus species, the detection rates were lowest for Aspergillus niger (17.0%), Aspergillus candidus (9.4%), and Aspergillus terreus (1.9%). Species of Fusarium were found in 17.0% of the samples; they included Fusarium oxysporum, Fusarium moniliforme, Fusarium semitectum in (5.7%), and Fusarium culmorum (3.8%). The wheat grain's endophytic mycobiota consists of a very modest number of species of tiny fungus. Pure cultures were produced from F. sporotrichiella var. tricinctum isolate 1218/4 and F. sporotrichiella isolate 1218/5, both of which were isolated from the Polissya zone. The test culture of Candida pseudo-tropicalis strain 44 PC was resistant to the mycotoxins produced by these isolates, while the growth of F. sporotrichiella var. tricinctum 1218/4 was inhibited in a zone with a relative toxicity (Rf) of 0.05. This isolate also generated an unknown trichothecene mycotoxin (TTMT). Aspergillus flavus isolate 1219/3 was the first to create kojic and aspergillic acids, while Aspergillus flavus isolate 1221/1 was the second to synthesis penicillic and aspergillic acids, respectively, among the isolated fungus. Chickens of the meat and egg breed Adler Silver were given the sorbent "Mikosorb" to lessen the harmful effects of the mycotoxin deoxyniva- lenol on their bodies. Deoxynivalenol's toxic effects on the bodies of hens in the experimental group were found to be mitigated when the sorbent "Mikosorb" was added to the diet at a rate of 2.0% of the total feed weight. The 12.0% drop in avian deaths provided more confirmation of this.

Keywords: Microscopic fungi; wheat grain; mycotoxins; deoxynivalenol; sorbent “Mycosorb”; Adler silver cross chickens.

1. Introduction

Wheat is one of the main cereal grains used as human food and animal feed. The problem of contamination of wheat grain with microscopic fungi, and as a result, their secondary metabolites, is one of the main factors determin- ing the health of animals and humans. Therefore, it is essen- tial to study the contamination of wheat crops with micro- scopic fungi, especially pests, and diseases, in the case of long-term storage. Using various scientific approaches to improve grain quality, studying fungi that infect seeds dur- ing storage and grain cultivation and processing is a crucial task today (Stuper-Szablewska & Perkowski, 2014; Jangol,2018; Jaroshenko et al., 2018; Minooeianhaghighi et al.,2021).Micromycetes that infect wheat seeds are often capableof synthesizing toxic metabolites that cause poisoning in humans and animals and have carcinogenic and cumulative properties. To date, more than 250 microscopic fungi are known that can produce up to 500 secondary metabolites of different chemical nature, which are united by the common name “mycotoxins” (Vasjanovych et al., 2016; 2017; Iliff et al., 2022). The root causes of the increase in the distribution and harmfulness of toxin-forming micromycete species are several factors, among which the main ones are the change in the phytopathological situation in agrocenoses due to long-term systematic violation of the requirements of farm- ing systems, as well as highly favorable weather conditions for the development of micromycetes that have developed over the past 5–10 years (Tymoshchuk et al., 2014).

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Micromycetes are potential phytopathogenic agents that can infect plants both during the growing season and during storage of grain and grain fodder, reducing its nutritional value and, under appropriate conditions, accumulating my- cotoxins in it (Rozhkova et al., 2017; Felšöciová et al., 2021; Kolomiets et al., 2022).

The study of wheat grain micromycetes is devoted to the work of various scientists: (Hmel'nyc'kyj et al., 2012; Kotyk et al., 2013). The issues of mycotoxin content in cereals were studied by (Kotsyumbas et al., 2010; Dvorska, 2013).

The contamination of food and feed with mycotoxins is currently being studied worldwide. Mycotoxins in grain are currently detected in Europe, the USA, Africa, Asia, and Australia. Toxicological studies (Antonjak et al., 2010; Tsvilikhovskyi et al., 2010) have shown that almost 25–40 % of grain is contaminated with mycotoxins annually,and losses caused by fungal contamination can reach tens of billions of dollars per year (Golovchak, 2007; Voloshchuk etal., 2017). Most of the mycotoxins produced by Fusarium fungi are derivatives of 12,13-epoxytrichothec-9-ene. The chemical structure of trichothecene mycotoxins is based on a system of united rings called trichothecene (Godtfredsen et al., 1967). Natural trichothecenes contain a double bond at the C-9 - C-10 position and an epoxy group at the 12th and 13th carbon atoms. Trichothecene mycotoxins are highly chemically resistant and thermostable. Under natural condi- tions, they are practically unaffected by natural environmen- tal factors. One is deoxynivalenol (DON, vomitoxin) (Bamburg, 1983). Researchers studied the effects of 12,13-epoxytrichothecenes (8-acetylneosolaniol, diacetotox- yscirpenol, T-2 toxin, NT-2 toxin, neosolaniol, diacetyl-NT-2 toxin, and T-2 tetraol) on the organisms of day-old broiler chickens in Minnesota (Chi et al., 1979). They observed the appearance of symptoms such as appetite, asthenia, diarrhea,and coma in the experimental birds, which, in their opinion, indicated that the toxicity of mycotoxins depends on the modification of links in the structure of toxin molecules.

The scientist (Viczko, 2019) proved that at the highest dose of DON (5.0 μg/g egg weight), it reduced the viability of chicken embryos and increased absolute indicators: the relative weight of the liver and spleen caused simultaneous bile stagnation in the liver and spleen inflammation. There was a dose-dependent increase in granulopoiesis and lipid peroxidation in the liver. However, the mRNA expression ofgenes associated with immune and oxidative stress in chick- en embryos remained unchanged. His results indicate that the chicken embryo responds to the introduction of DON by affecting its immunity and oxidative status. Mycotoxins in poultry feed are an important factor in financial losses in poultry production. DON can cause toxicological and im- munotoxic effects in chickens. The main effects at low con- centrations are reduced feed intake, while higher doses cause severe weight loss and impaired resistance to infec- tion, particularly bacterial infection (Qu et al., 2019; Yao & Long, 2020; Sun et al., 2022; Hou et al., 2023). One crucial aspect of DON toxicity is damage to the gastrointestinal tract of animals. DON affects the digestive organs of chick- ens, especially the duodenum and cecum, as evidenced by their shorter and thinner villi. In addition, this toxin impairs intestinal function by reducing the absorption of nutrients (glucose and amino acids). There is evidence that DON impairs immune function in broiler chickens. Feeding grain contaminated with DON reduces serum antibody titers against Newcastle disease virus (NDV) and infectious bron- chitis virus (IBV) in laying hens and broilers.

Researchers (Girish & Smith, 2008) found that mycotox-ins negatively affect the intestinal barrier's function, reduc- ing the intestinal epithelium's integrity. Apoptosis, increased colonization of pathogenic microorganisms, cytotoxicity, oxidative stress, inhibition of protein synthesis, and lipid peroxidation are characteristic of the toxic effects of myco- toxins on the intestinal epithelium. They directly or indirect- ly affect the host's immune responses (Brezvyn et al., 2021).Such immunotoxic effects of mycotoxins make poultry susceptible to many infectious diseases. The administration of silver deoxynivalenol at a dose of 70 mg/kg body weight to Adler cross chickens caused a decrease in body weight, changes in the activity of alkaline phosphatase isozymes andthe metabolism of certain macronutrients in the blood se- rum, accompanied by pathological changes in the kidneys, heart, and liver (Ostrovs'kyj et al., 2014; Ostrovs'kyj, 2016).

At present, there is an urgent need to implement veteri- nary and sanitary, and preventive measures to develop and introduce into production new methods and means of pre- vention and treatment of mycotoxicosis in animals and poul-try based on the use of natural or artificial sorbents together with toxin-contaminated feed.

The work aimed to study the quantitative and qualitative composition of microscopic fungi of wheat grain grown in the Polissya region of Ukraine and their toxigenic potential when fed to Adler silver sorbent “Mikosorb” to chickens.

2. Materials and methods

As a result of mycological studies, 53 samples of wheat grain from the Polissya region were used. Samples for re- search were collected from agricultural enterprises of vari- ous forms of ownership, the private sector, elevators, breed- ing stations, and regional seed inspections of Ukraine ac- cording to the guidelines for sanitary and mycological as- sessment and improvement of feed quality (Andrijchuk etal., 2011), following GOST 13586, 3-83 and DSTU 3570-
97. For in-depth studies of the toxin-synthesizing capacity ofDON microbes, a wheat grain culture of var: “Remeslivna” from the Myronivsky Institute of Wheat named after
V. M. Remesla of the Ukrainian Academy of Agrarian Sci- ences and the Institute of Plant Physiology and Genetics of the National Academy of Sciences. This variety has been widely used in agricultural enterprises in all climatic zones of Ukraine (steppe, forest-steppe, and Polissya) since itsstate registration in 2004.

This wheat variety is a medium early variety with a pro- tein content of 14.3 %. The resistance to the lodging of this variety is 8–9 points, resistance to shattering – 8 points, resistance to root rot – 7–8 points, resistance to Septoria – 7–8 points, resistance to brown rust – 7–8 points, resistance to powdery mildew – 7–8 points. Since this variety is grown in different climatic conditions, we chose it as a generalized sample susceptible to micromycetes for further research.

The epiphytic mycorrhizal flora was studied by direct inoculation, for which wheat grains were placed in Petri dishes on the surface of Chapek's medium in 6–7 pieces. The material from each sample was sown in 4 Petri dishes, two cultivated at 24 °C and the other at 37 °C. Pure cultures were obtained by inoculating the fungi into tubes on Chapek's slant agar, and their cultural properties were taken into account, and microscopy was performed to determine the species. In order to determine the endophytic composi- tion of the mycobiota, the grain was treated with a 3 % for- malin solution for 3 min before sowing, after which the material was washed with sterile distilled water to neutralize the disinfectant, to which a 5 % ammonia solution was add- ed.

The method of serial dilutions was used to determine the degree of contamination of the material with micromycetes (the number of colony-forming units per 1 g of grain). The grain was ground in an electric mill, and serial dilutions were prepared. In order to perform this, a 10 g sample of crushed grain was poured into 100 cm^3 of sterile distilled water and shaken for 20 min, and serial dilutions of 1 : 100,1 : 1000, and 1 : 10000 were prepared. Subsequently, 1 cm^3 of the prepared dilutions was inoculated onto the surface of Chapek's medium at one dilution per two Petri dishes. Incu- bation was carried out at 24 and 37 °C, and colonies were counted on days 3 and 5 after sowing. The content of colo- ny-forming units per 1 g of wheat grain was calculated usingthe following formulas:

$$C = \frac{Km}{V} \times N$$

$$N = \frac{N_1 + N_2 + \dots Nm}{Km\left(\frac{1}{K_1} + \frac{1}{K_2} + \dots \frac{1}{Km}\right)}$$

3. Results and discussion

3.1. Results

During the research period, an average of 2.88-10^4 ±3.62-10^3 colony forming units (CFU) per 1 g of grain was found in wheat grain samples collected in the Polissya re- gion. The results of the studies described in Table 1 show that the most commonly isolated fungi among the epiphytic mycobiota were *Mucor* spp. and *Alternaria alternata*. Less frequently in the studied wheat samples, we observed the infection with *Aspergillus fumigatus*, *Aspergillus flavus*, *Penicillium* spp., and *Fusarium sporotrichiella*. All other fungi we identified were found only in individual samples inisolated cases.

We identified 20 species of microscopic fungi among the epiphytic mycobiota. Among mucoral fungi, *Absidia corymbifera* (32.1 %) and *Rhizopus oryizae* (24.5 %) were also detected in the samples.

Aspergillus niger (17.0 %), *Aspergillus candidus* (9.4 %), and *Aspergillus terreus* (1.9 %) were less common among the aspergilli. *Fusarium* spp. (17.0 %), *Fusarium oxysporum, Fusarium moniliforme, Fusarium semitectum* in (5.7 %), and *Fusarium culmorum* (3.8 %) of the samples were identified.

When performing mycological studies of endophytic mycobiota (Table 2), we often identified the following fun- gi: *Alternaria alternate,*

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Aspergillus flavus, and *Mycelia sterilia*. In the selected wheat samples, grain damage by fungi of the genus *Mucor* spp: *Mucor* spp. *Penicillium* spp. *Mycelia sterilia* and *Phoma exiqua*. This indicates that fewerspecies of microscopic fungi represent the endophytic my- Where C is the content of fungal diaspores in 1 g of the substrate; K_1, K_2, Km, etc. are the denominators of the max- imum and used dilutions, starting with the minimum; V is the volume of the sown mixture (cm^3); N_1, N_2, etc. is the number of diaspores, first the average, and then in each dilution (DSTU 8051:2015).

Identification of isolates to species and varieties was car- ried out using commonly accepted keywords (Domsch et al., 1980; Obrazhej et al., 1998; Ruhljada et al., 2009; Ostrovs'kyj et al., 2018). The sizes of conidia, mycelium,and other elements of fungi were determined by crushed drop microscopy using an eyepiece micrometer.

The toxicity of the isolated isolates was determined by microbiological and mycotoxicological methods using thin- layer chromatography. To study the effect of DON on Adler silver chickens when fed the sorbent “Mycosorb” in 2 % of the total feed weight, the birds were divided into three groups of 100 heads each according to the principle of ana- logs. Chickens were kept in deep litter, each group in a sepa-rate section. Birds in the control group were fed a complete diet that did not contain toxins, while experimental group No. 1 was fed wheat grain containing the toxin deoxyniva- lenol as part of the feed (“Experimental (T)”). Experimental group No. 2 “(M+T)” (toxin + mycosorb) – received com- plete feed with wheat grain containing deoxynivalenol and mycosorb in the amount of 2 % by weight of the feed. The birds were constantly monitored, weighed weekly, and blood was taken for biochemical studies. The research re- sults were statistically analyzed using the built-in statistical functions of MS Excel and Stat Soft “Statistica 10” soft- ware.cobiota of the wheat grain.

Among the isolates of micromycetes isolated from the Polissya region, pure cultures were obtained from *F. spo- rotrichiella var. tricinctum* isolate 1218/4, and *F. spo- rotrichiella* isolate 1218/5. Both isolates were atoxic against the test culture *Candida pseudotropicalis strain* 44 PC, but *F. sporotrichiella var. tricinctum* 1218/4 produced a growth retardation zone with Rf 0.05 and produced an unidentified trichothecene mycotoxin (TTMT). Among the isolated fun- gi, *Aspergillus flavus* isolate 1219/3 and *Aspergillus flavus* isolate 1221/1 were the first to produce kojic and aspergillic acids and the second to synthesize penicillic and aspergillic acids.

Microscopic fungi are permanent contaminants of grain crops. Under susceptible environmental conditions of tem- perature and humidity, they can synthesize secondary me- tabolites – mycotoxins. One of the most commonly detected mycotoxins in grain crops is deoxynivalenol (DON, vomi- toxin), produced by fungi of the genus Fusarium. In order to study the possibility of reducing the negative impact of the mycotoxin deoxynivalenol on the body of chickens of the meat and egg breed Adler Silver, the birds were fed the sorbent “Mikosorb”. The study was conducted at a poultry enterprise under the conditions of the Educational and Sci- entific Research Center (ESRC) of Bila Tserkva NAU. As a result of the experiment (Table 3), it was found that when added to the diet, the sorbent “Mikosorb” in the amount of 2.0 % of the total feed weight reduces the nega- tive effect of deoxynivalenol on the body of chickens of the experimental group. This was confirmed by a 12.0 % reduc- tion in poultry mortality. Feeding “Microsorb” in the amount of 2.0 % by weight of complete feed contributed to an increase in the average daily weight gain of poultry dur- ing the experiment by 5.43 % compared to the experimental group that consumed feed with DON toxin. During the ex- periment, the birds of the experimental group No. 2 con- sumed 28,91 kg of feed more than group No. 2. Feeding of “Mikosorb” to chickens of experimental group 2 contributedto an increase in the level of profitability of poultry rearing up to 12.0 % compared to poultry that consumed feed af- fected by DON toxin.

Table 1

Epiphytic mycobiota of wheat grain in the Polissya zone

Types of isolated micromycetes	Polissya region (53 samples)	
	Fact. (number of samples)	% of identified
Zygomycota, Zygomycetes, Mucorales, Mucoraceae		
Mucor spp.	49	92.5
Absidia corymbifera (Cohn) Jacc. Et A. Trotter	17	32.1
Rhizopus oryzae Went.	13	24.5
Together mucoral	44	83
Ascomycota, Plectomycetes, Eurotiales		
Monascus rubber van Tieghem	1	1.9
Mitosporic fungi, Coelomycetes, Sphaeropsidales, Sphaerioidaceae		
Phoma exiqua Desmazieres	8	15.1
Hyphomycetales, Dematiaceae		

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Alternaria alternata (Fr.) Keissl	49	92.5
Moniliaceae		
Aspergillus fumigatus Fres.	31	58.5
Aspergillus flavus Zr:Fr	28	52.8
Aspergillus niger van Tieghem	9	17
Aspergillus candidus Zk:Fr.	5	9.4
Aspergillus terreus Thom.	1	1.9
Together aspergillus	44	83
Penicillium spp.	25	47.2
Trichothecium roseum (Pers:Fr.) Zk.	1	1.9
Hyphomycetales, Agonomycetales, Agonomycetaceae		
Mycelia sterilia (Pink)	8	15.1
Tuberculariales, Tuberculariaceae		
Fusarium spp.	9	17
Fusarium sporotrichiella Bilai	16	30.2
Fusarium oxysporum (Schlecht.) Snyd. Et. Hans	3	5.7
Fusarium moniliforme Sheld.	3	5.7
Fusarium semitectum Berk. et. Rav	3	5.7
Fusarium culmorum (W.G.Sm.) Sacc.	2	3.8
Together fusariums	32	60.4

Table 2

Endophytic mycobiota of wheat grain in the Polissya zone

Types of isolated micromycetes	Polissya region (53 samples)	
	Fact. (number of samples)	% of identified
Zygomycota, Zygomycetes, Mucorales, Mucoraceae		
Mucor spp.	11	20.8
Together mucoral	11	20.8
Mitosporic fungi, Coelomycetes, Sphaeropsidales, Sphaerioidaceae		
Phoma exiqua Desmazieres	9	17
Hyphomycetales, Dematiaceae		
Alternaria alternata (Fr.) Keissl	52	98.1
Moniliaceae		
Aspergillus fumigatus Fres.	3	5.7
Aspergillus flavus Zr:Fr	22	41.5
Aspergillus niger van Tieghem	1	1.9
Together Aspergillus	25	47.2
Penicillium spp.	11	20.8
Trichothecium roseum (Pers: Fr.) Zk.	1	1.9
Hyphomycetales, Agonomycetales, Agonomycetaceae		
Mycelia sterilia (Pink)	12	22.6
Tuberculariales, Tuberculariaceae		
Fusarium spp.	3	5.7
Fusarium sporotrichiella Bilai	3	5.7
Fusarium oxysporum (Schlecht.) Snyd. Et. Hans	5	9.4
Fusarium moniliforme Sheld.	3	5.7
Together fusariums	13	24.5

able 3

Economic efficiency of feeding Mycosorb to Adler Silver chickens (M ± m, n = 100)

Indicators	Groups of poultry		
	Control	Experimental № 1 (T)	Experimental No. 2 (M+T)
The initial number of chickens, goal.	100	100	100
Number of livestock at the end of the experiment, goal.	91	74	86
Preservation, %.	91.0	74.0	86.0
The average number of livestock during the study period, heads.	95.5	87.0	93.0
Total weight of birds at the beginning of the experiment (4 weeks old) kg	31.81 ± 0.133	31.57 ± 0.129	31.35 ± 0.125
Average live weight of 1 head at the beginning of the experiment (age four weeks), g	318.1 ± 6.11	315.7 ± 7.19	313.5 ± 8.17
Total weight of poultry at the end of the experiment (7 weeks of age) kg	53.15 ± 0.143	42.00 ± 0.121	51.47 ± 0.132
Average live weight of 1 head at the end of the experiment (age seven weeks) g	584.1 ± 11.21	567.7 ± 14.19	598.5 ± 12.16
Feed consumption during the experiment (4-7 weeks), kg	86.24 ± 0.381	51.16 ± 0.279	80.07 ± 0.374

Cost of feed during the experiment (4-7 weeks), UAH	238.02	141.20	220.99
The cost of using Mycosorb during the experiment (4-7 weeks of 2% to feed), UAH	-	-	138.36
Electricity cost for the period of the experiment, UAH	197.26	197.26	197.26
The cost of dead poultry during the growing process, UAH	145.80	421.20	226.80
Other production costs, UAH	172.25	172.25	172.25
Gross live weight gain obtained during the experiment, kg	21.34 ± 0.229	10.43 ± 0.193	20.12 ± 0.215
The cost of raising poultry for the period of the experiment, UAH	753.33	931.91	800.66
Cost of live weight gain in poultry, UAH	950.70	464.86	896.74
Profit from live weight gain, for the period of research, UAH	197.37	-467.05	96.08
Profitability, %	26.2	-	12.0

3.1. Discussion

The contamination of wheat grain with micromycetes largely depends on the region's climatic conditions, the cultivation period, and compliance with storage conditions (Vasjanovych et al., 2016). Our results indicate that the Polissya zone is characterized by higher precipitation and, accordingly, higher humidity, contributing to increased wheat grain contamination with microscopic fungi. The increase in the number of isolated species of microscopic fungi causes the detection of a significant variety and con- centration of mycotoxins in the grain. A similar positive correlation between the number of fungal species and their toxicity was noted by other authors (Vasjanovych et al.,2017). The introduction of 2.0 % by weight of feed in-creased bird productivity by 5.43 %. A positive effect on increasing the productivity and safety of chickens with the use of sorbents in bird feeding was reported by other authors(Kotsyumbas et al., 2010).

4. Conclusions

Twenty microscopic fungi from 9 genera were isolated from wheat grain in the Polissya region. Among them were the genera *Alternaria* (92.5 %), *Mucor* (92.5 %), *Aspergillus*(83.0 %), *Penicillium* (47.2 %), *Fusarium* (60.4 %), *Phoma* (15.1 %), *Mycelia* (15.1 %), *Trichotecium* (1.9 %) and *Monascus* (1.9 %) of the samples. One of them was a pro- ducer of trichothecene mycotoxin; the other two synthesizedkojic, penicillic and aspergillic acids.

Introducing the silver sorbent "Mikosorb" in the amount of 2.0 % by weight of feed into the composition of mixed fodder for chickens of the meat and egg breed Adler con- tributed to an increase in bird productivity by 5.43 %. Using the sorbent "Mikosorb" increases the safety of meat and egg breed Adler silver chickens by 12.0 %. Feeding the sorbent "Mikosorb" in an amount of 2.0 % of the feed weight to chickens of the meat and egg breed Adler Silver contributes to an increase in gross weight gain by 9.69 kg during the experiment.After analyzing the scientific results from the literature and our research results, we concluded that the abovemen- tioned studies on wheat grain damage should be conducted throughout Ukraine during harvesting and storage in ware- houses or storages.

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Indo-American Journal of Agricultural and Veterinary Sciences

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