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EDITED BY S. STANKEVYCH, O. MANDYCH

Tallinn Teadmus, 2022

MODERN TRENDS IN THE DEVELOPMENT OF AGRICULTURAL PRODUCTION: PROBLEMS AND PERSPECTIVES

Edited by S. Stankevych, O. Mandych

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The monograph presented for review is a collection of the results of actual achievements of domestic agricultural scientists, obtained directly in real conditions. The authors are recognized experts in their fields, as well as young scientists and postgraduate students of Ukraine. Research is conceptually grouped into 5 sections: modern technologies in crop production and fodder production; economy of the agro-industrial complex; breeding and breeding in the 21th century; protection and quarantine of plants; agrochemistry and soil science. The monograph will be interesting for experts in plant breeding, economics, plant protection, selection, agrochemistry, soil science, scientific workers, teachers, graduate students and students of agricultural specialties of higher education institutions, and for all those who are interested in increasing the quantity and quality of agricultural products.

Keywords: modern technologies, crop production, fodder production, plant protection, quarantine, agrochemistry, soil science, economy of agroindustrial complex.

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REGULATION OF WINTER WHEAT SEEDS MYCOBIOTA BY SPRAYING WITH FUNGICIDES

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Some representatives of mycobiota of winter wheat seeds appear in it from the moment of flowering to harvest. Therefore, spraying plants at the beginning of flowering and later should significantly affect the grain mycocomplex. During 2018–2020, a study of the effect of spraying on the formation of the mycoflora of winter wheat seeds in the conditions of the North-Eastern Forest Steppe of Ukraine was conducted. The following preparations were involved in the study: Falcon, Imunotsytofit, Trykhofit, Haupsyn, and Chitosan. Mycocomplex analysis was performed on potatoglucose agar. The effect of fungicides on the mass of 1000 seeds was also studied. Analysis of the germination of the obtained seeds by controlling the length of the seedlings showed a decrease in this indicator when using a chemicals and an increase when spraying with biological agents, compared to the control.

Keywords: winter wheat, seed mycobiota, spraying, chemicals, biofungicides

Spraying plants in the second half of the wheat growing season affects the composition of seed fungi. For this, preparations of various origins are used, for example, chemical and biological fungicides, resistance inducers.

Today, chemicals are used more compared to others. They help prevent

crop losses from disease under intensive technologies (Ons et al., 2020). Thanks to the combination of various active substances in modern fungicides, the frequency of treatments and the pesticide load on wheat phytocenosis is reduced. Along with the advantages, farmers do not always have a high effect of chemical preparations, especially in the regulation of field seed infection caused by phytopathogenic fungi capable of producing mycotoxins. The most studied issue is the protection of wheat from Fusarium head blight, when spraying is carried out at the beginning of flowering to regulate it. The highest effectiveness of fungicides for the first decade of the 21st century was estimated at the level of 60-70% reduction of visual symptoms in the field (Gagkaeva et al., 2011). But some researchers in the conditions of creating an artificial infection have high results in limiting the development of Fusarium sp. The study of fungicides (Akanto Plus SC, 0.6 l/ha; Amistar Extra SC, 1 l/ha; Zantara, e.c., 1 l/ha) against Fusarium head blight with artificial infection of Fusarium avenaceum (Fr.) Sacc. culture had significant indicators of the effectiveness of their use - from 94 to 100% on 14 and 30 days after spraying (Dubrovskaya, 2020). There is not enough information regarding the restriction of other mycobiota fungi, which are also capable of producing mycotoxins, for example, Alternaria sp., which prevail in the North-Eastern Forest-Steppe of Ukraine (Rozhkova & Karpenko, 2016). For the most part, the effect of fungicide spraying on the content of these secondary metabolites is studied, rather than the number of Alternaria sp. (Scarpino et al., 2015).

Greening of grain production is possible due to the use of biological preparations based on various microorganisms against diseases. Biological control mechanisms are considered very carefully today, as fungicides negatively affect other non-target objects (Köhl et al., 2019). For example, bacteria from the genus Pseudomonas (rhizosphere microorganisms) have defense against phytopathogens: mechanisms several competition/antagonism with the production of secondary metabolites, induction of systemic plant resistance (Çakmakçı et al., 2017). P. aureofaciens are capable of synthesizing phenazine-type antibiotics, effectively suppressing the growth of a number of phytopathogenic fungi and bacteria, and stimulating plant growth due to the production of phytohormones (Burova et al., 2012).

Trichoderma sp. have several biological control mechanisms: antagonism, production of secondary metabolites (antibiotics), and hyperparasitism (Sood et al., 2020). Worldwide, more than 60% of effective biofungicides are obtained from it (Abbey et al., 2019). Species of this genus are well known to compete for nutrients, biological niches, or infection sites with pathogens in the plant rhizosphere (Ahluwalia et al., 2015). More than 180 secondary metabolites indicating different classes of chemicals have been isolated from *Trichoderma* sp. For example, *T. lignorum* (Tode) Harz. is able to produce coninins, viridin, dermadin, trichoviridin, lignorene and konigic acid, as well as gliotoxin and gliovirin (Masi et al., 2018). As hyperparasites *Trichoderma* sp. produce specialized enzymes that destroy the cell wall of target fungi (glucanases, chitinases and proteases), and also activate enzymes of the latter (Harman et al., 2004).

The use of biotic elicitors, which are also called resistance inducers, to activate non-specific resistance of plants allows reducing environmental pollution with pesticides (Zhuk et al., 2019). Elicitors belong to different classes of chemical compounds. Most of those described are carbohydrates, peptides, lipids, glycoproteins, and glycolipids (Rozhkova, 2016).

Chitosan is derived from chitin, which is the most abundant natural polymer after cellulose (Younes et al., 2015). It has some advantages over other biocontrol agents, not only in its potential to suppress disease development, but also in increasing plant resistance (Yin et al., 2010), as well as expanding biodiversity in the plant rhizosphere (Park & Chang, 2012; Hassan & Chang, 2017). Chitosan in agriculture can be used to control fungi (Chowdappa et al., 2014), bacteria (Yang et al., 2014), viruses (Jia et al., 2016), phytonematodes (El-Sayed & Mahdy, 2015) and in qualities of a food additive (Zhang et al., 2011). By comparing the effectiveness against the development of fusarium wilt of wheat and barley of biochemical chitosan and the bacteria *Pseudomonas fluorescens* MKB 158, the higher efficiency of the former was established in reducing the development of Fusarium head blight and preventing a decrease in the weight of 1000 grains (Khan & Doohan, 2009).

Preparations based on arachidonic acid (imunotsytofit) include mechanisms of resistance to pathogens of various etiologies, stimulate plant genes responsible for growth processes and the formation of phytohormones (Shapoval et al., 2014). The use of imunotsytofit on peaches to reduce the development of pathogens of leaf diseases led to the activation of enzymes of the antioxidant defense system (catalase, general peroxidase) (Mykhaylova et al., 2018).

The mechanisms of regulating the mycoflora of winter wheat seeds using chemical preparations and biofungicides by spraying plants in field conditions were investigated. The study of the effectiveness of winter wheat spraying was conducted on the experimental field of the Educational and Scientific Production Complex of the Sumy National Agrarian University (SNAU). A small-scale experiment was laid. Winter wheat plants (Bohdana variety) were sprayed with a manual sprayer in the evening (Trybel et al., 2001). The seed mycobiota was determined by a biological method in laboratory conditions (Naumova, 1970) using potato-glucose agar. The seeds were treated with a 1% solution of potassium permanganate for 1–2 minutes to study the internal complex of seed fungi. Fungi were identified by the structure of mycelium and sporulation (Watanabe, 2012).

Chemical protection is considered the most effective. Therefore, research on determining the effective regulation of seed mycobiota was started in 2018 with a detailed study of spraying with fungicides. The first treatment with drugs was carried out at the end of earing at the beginning of winter wheat flowering, the second - at the end of flowering. It was assumed that fungicide treatment will lead to an increase in the mass of 1000 grains, and double application will show even better results.

First, a macroanalysis of the grown seeds was carried out, which showed quite unexpected results. The smallest amount of black point, wrinkled, and small seeds was obtained from the variant with a one-time application of Falcon, c.e. A good result was obtained with Trykhofit spraying, but this biological preparation for some reason increased the number of black point. Many wrinkled seeds were noted in the variant with the simultaneous use of Falcon, EC and Imunotsytofit, tb. The use of preparations led to a decrease in the weight of 1000 grains, which was maximal in the variant with two-time spraying with Falcon (Table 1).

Spraying with fungicides led to significant changes in the mycobiota of winter wheat seeds: not only the amount of fungal isolation, but also their composition changed. They reduced the number of dominant *Alternaria* sp. and caused the appearance of *Mucor* sp., especially in the variant with the simultaneous use of Falcon and Imunotsytofit. *Trichoderma* sp. and *Tr. roseum* with a significant percentage of release appeared in the mycobiota after treatment with a chemical fungicide. Two-time spraying by Falcon caused the appearance of *Curvularia* sp., which is rare for the mycoflora of our zone, as well as a significant percentage of the isolation of *N. oryzae*. In the variant with the use of Trykhofit, the isolation of *Chaetomium* sp. was noted.

Table 1

Effect of spraying on mycoflora of winter wheat seeds and the weight of 1000 grains (SNAU, 2018)

r	of 1000 grains (SNAU, 2018	, 	
Variant	Isolation of colonies, %	Weight of 1000 grains,	
		g	
Control	Alternaria sp. 76.8	52.9	
	Melanospora sp. 10.2		
	Nigrospora oryzae 5.6		
	Cladosporium sp. 1.9		
	Aureobasidium pullulans		
	1.9		
	Penicillium sp. 1.9		
	Other species of fungi 1.7		
Falcon, EC	Alternaria sp. 31.5	48.66	
(one spraying)	<i>Mucor</i> sp. 17.5		
	Trichothecium roseum 8.4		
	<i>Trichoderma</i> sp. 7		
	Cladosporium sp. 4.2		
	Fusarium poae 2.8		
	A. pullulans 2.1		
	Penicillium sp. 1.4		
	Other species of fungi 25.1		
Falcon, EC	<i>Mucor</i> sp. 34.9	43.52	
(two	N. oryzae 11.6		
sprayings)	<i>Tr. roseum</i> 11.6		
	Trichoderma sp. 8.1		
	Curvularia sp. 5.8		
	Penicillium sp. 3.4		
	Cladosporium sp. 2.3		
	Aspergillus niger 2.3		
	Other species of fungi 20.0		
Imunotsytofit,	Alternaria sp. 21.6	46.22	
tb (two	<i>Mucor</i> sp. 61.8		
sprayings)+	Penicillium sp. 5.9		
Falcon, EC	Trichoderma sp. 4.9		
	Cladosporium sp. 2.9		
	A. pullulans 2.9		
Trykhofit, s.	Alternaria sp. 64.1	48.44	
(two	Mucor sp. 9.9		

sprayings)	Penicillium sp. 2.1	
	Chaetomium sp.1.4	
	N. oryzae 1.4	
	A. pullulans 0.7	
	Cladosporium sp. 1.0	
	Other species of fungi 19.7	
LSD ₀₅	Alternaria sp. 2.2	1.9
	<i>Mucor</i> sp. 5.1	
	Cladosporium sp. 1.4	
	Penicillium sp. 2.0	
	A. pullulans 0.9	
	Other species of fungi 1.8	

In 2019, spraying with chemical and biological preparations led to an increase in weight of 1,000 seeds (Table 2). An external examination of the seeds showed that the largest number of small seeds was in the variant using Falcon, and the largest amount of wrinkled ones was in the variant sprayed with a chemical fungicide and Imunotsytofit.

Table 2

Effect of spraying on mycobiota of winter wheat seeds and weight of 1000 grains (SNAU, 2019)

1000 grains (SNAU, 2019)				
Variant	Isolation of colonies, %	Weight of 1000		
		grains, g		
Control	A. pullulans 32.1	37.94		
	Alternaria sp. 29.6			
	<i>N. oryzae</i> 19.4			
	Penicillium sp. 3.2			
	Cladosporium sp.3.2			
	<i>F. poae 1.6</i>			
	Other species of fungi 11.4			
Falcon, EC	<i>N. oryzae</i> 67.8	38.52		
	A. pullulans 11			
	Acremonium sp. 3.4			
	Cladosporium sp. 1.7			
	Alternaria sp. 1.7			
	Penicillium sp. 0.8			
	Other species of fungi 13.6			
Trykhofit, s.	<i>N. oryzae</i> 36.8	44.72		
(two sprayings)	Alternaria sp. 23.2			
	A. pullulans 13.7			

	Cladosporium sp. 8.4	
	<i>F. poae</i> 2.1	
	Other species of fungi 15.8	
Imunoteutofit th	N. oryzae 67.3	39.86
Imunotsytofit, tb	•	39.80
+ Falcon, EC	A. pullulans 7.1	
	Cladosporium sp. 7.1	
	Penicillium sp. 3.5	
	Chrysonilia sitophila 2.7	
	Fusarium sp. 2.7	
	Trichoderma sp. 2.7	
	Alternaria sp. 1.8	
	Acremonium sp. 0.9	
	Other species of fungi 4.2	
LSD ₀₅	Alternaria sp. 1.9	2.2
	A. pullulans 2.5	
	N. oryzae 3.3	
	Penicillium sp. 1.6	
	Cladosporium sp. 1.7	
	Other species of fungi 2.5	

A. pullulans and Alternaria sp. dominated in control. A significant percentage of *N. oryzae* was also noted, which negatively affected the growth parameters of wheat seedlings, reducing the length of seedlings and roots. The use of fungicides led to a decrease in the number of dominant species and an increase in the isolation of the dangerous *N. oryzae*, which significantly affected the length of seedlings (Fig. 1). The highest number of this species was noted in the variants with use of Falcon. This preparation removed the minor presence of *F. poae*. After spraying with Imunotsytofit another species of *Fusarium* appeared.

In 2020, spraying with fungicide and biologicals also improved seed set. The best indicators of mass of 1000 grains were noted in variants using resistance inducers (Imunotsytofit and Chitosan) (Table 3).

All preparations reduced the number of dominant *Alternaria* sp. and caused a significant appearance of *A. pullulans*, which was absent in the control. Attention was also drawn to the greatest change in the composition of the mycobiota over three years of studying the effectiveness of fungicides. Only *Alternaria* sp., *Tr. roseum*, and unspecified fungal colonies remained in variants with preparations. After spraying with Falcon, additional colonies of three, Imunotsytofit – four, Trykhofit – five, Chitosan – six, Haupsyn – seven species were isolated, compared to the control.

Table 3

Effect of spraying on mycobiota of winter wheat seeds and weight of
1000 grains (SNAU, 2019)

Variant	Location of colonics 0/		
Variant	Isolation of colonies, %	Weight of 1000 grains,	
		g	
Control	Alternaria sp. 62.7	38.16	
	<i>Tr. roseum</i> 11.7		
	Acremoniella atra 7.4		
	<i>Mucor</i> sp. 3.7		
	<i>F. poae</i> 1.6		
	Rh. stolonifer 1.2		
	Other species of fungi 11.7		
Falcon, EC	Alternaria sp. 25.9	39.54	
	Tr. roseum 13.4		
	A. pullulans 10.7		
	Cladosporium sp. 1.8		
	Penicillium sp. 0.9		
	Other species of fungi 47.3		
Haupsyn, s.	Alternaria sp. 49.1	38.20	
(two sprayings)	A. pullulans 22.8		
	Trichoderma sp. 7		
	Mucor sp. 6.1		
	Tr. roseum 5.3		
	Penicillium sp. 1.8		
	Microdochium nivale		
	0.9		
	Cladosporium sp. 0.9		
	Bacteria 0.9		
	Other species of fungi 5.2		
Imunotsytofit,	Alternaria sp. 48.1	40.30	
tb	A. pullulans 27.8		
(two sprayings)	Tr. roseum 17.7		
(·····································	Trichoderma sp. 2.5		
	Mucor sp. 1.3		
	Arthrinium arundinis 1.3		
	Other species of fungi 1.3		
Trykhofit, s.	Alternaria sp. 37.6	38.88	
(two sprayings)	A. pullulans 32.3		
(the sprayings)	N. oryzae 3.2		
	Cladosporium 2.2		
l			

	Penicillium sp. 2.2	
	Tr. roseum 1.1	
	<i>Phoma</i> sp. 1.1	
	Other species of fungi 20.6	
Chitosan, tb	Alternaria sp. 39.7	43.00
(three	A. pullulans 35.1	
sprayings)	Tr. roseum 12.6	
	<i>Mucor</i> sp. 1.8	
	<i>Phoma</i> sp. 1.8	
	Trichoderma sp. 0.9	
	Cladosporium sp. 0.9	
	Penicillium sp. 0.9	
	Other species of fungi 6.3	
LSD 05	Alternaria sp. 1.8	3
	Tr. roseum 2.1	
	A. pullulans 2.2	
	Other species of fungi 1.5	

In 2020, the use of Chitosan resulted in the largest mass of 1000 seeds, possibly due to the fact that epiphytotia of *Septoria tritici* blotch (STB) was observed on wheat this year, and this elicitor, according to other studies, is capable of restraining its development. The high efficiency of chitosan complexes against foliar diseases of spring wheat was noted: "Chitosan II" and "Chitosan II" showed their effectiveness in restraining the development of STB, "Chitosan II" well restrained the entire complex of leaf diseases, especially brown rust (Kolesnikov et al., 2017).

Chemicals, reducing the number of some species, lead to an increase in others. For example, with direct treatment of wheat seeds with fungicides, the dominant species in the seed mycoflora was reduced (*Drechslera australiensis*), while the rare species *Aspergillus terreus* increased with the use of Acrobat MZ, Aliette and the combined use of Metalaxyl and Mancozeb. The species that were not isolated in the control (*Alternaria alternata* and *Fusarium oxysporum*) appeared after soaking in solutions of some fungicides (Javaid et al., 2016). In our research, the biggest changes in seed mycoflora were caused by two-time spraying with Falcon in 2018, which provoked the appearance of an atypical genus for our zone, and the use of Haupsyn in 2020.

If we consider the impact of spraying not on the mycobiota as a whole, but on its dominant genus *Alternaria*, then it is possible to claim a significant regulation of these fungi over three years (Table 4).

Table 4

of whiter wheat seeds mycobiota					
Variant	Isolation among fungal		Average	Effectiveness,	
		colonies, %		data for	%
	2018	2019	2020	three years	
Control	76.8	29.6	62.7	56.4	-
Falcon, EC					
(one	31.5	1.7	25.9	19.7	65.1
spraying)					
Trykhofit,					
s. (two	64.1	23.2	37.6	41.6	26.2
sprayings)					
LSD ₀₅	4	2.1	4.6	1.8	8.5

Effectiveness of spraying against the dominant genus (*Alternaria* sp.) of winter wheat seeds mycobiota

Together with the identification of fungi on the agar medium, the length of winter wheat seedlings was measured (Fig. 1). In 2019, spraying with fungicides led to a decrease in plant length. This can be explained by the fact that the drugs reduced the number of dominant *Alternaria* sp. and provoked the appearance of *N. oryzae*. According to our long-term studies, this species inhibits plant growth, significantly reducing the length of germinal roots and coleoptiles. The largest number of *N. oryzae* was isolated in variants with Falcon spraying (about 67%), therefore, the shortest length of seedlings was noted here. The worst variant for plant development was with the simultaneous use of Imunotsytofit and Falcon because in addition to *N. oryzae*, *Fusarium* sp. was also isolated, the phytotoxins of which also negatively affect plant germination.

The effectiveness of fungicides on the formation of mycobiota of wheat seeds is mostly devoted to *Fusarium* sp., because their significant toxicity has long been known. When studying their effect on one genus, as in our studies on *Alternaria* sp., there is a decrease in the number of fungi with an increase in productivity. The study of the effectiveness of Falcon (0.61/ha) against *Fusarium* sp. under the conditions of artificial inoculation showed an increase in the weight of 1000 seeds by 1.7 g, compared to the control. Analysis of seeds in laboratory conditions on the 7th day showed a decrease in Fusarium infection by 82% (Kremneva et al., 2018). The study of fungicides for artificial infection of wheat ears with *F. graminearum* together with the low effectiveness of fungicides Sportak (prochloraz) and Alto (cyproconazole) demonstrated an increase in yield against the

background of their fungicidal action against the significant development of rust diseases and root rots -0.63 and 0.67 t/ ha, respectively. At the same time, the effectiveness of these fungicides against the number grains with *Fusarium* sp. was 50.6 and 19.2% (Grushko et al., 2004). In our studies, fungicide application also resulted in an increase in 1000-grain weight, except in one year when spraying caused a significant field infestation by *Mucorales*, which presumably had such a negative effect.

In 2020, the length of seedlings after spraying was greater than in the control, with the exception of the variant with the use of Falcon. Plants germinated best in the variant with the use of Trykhofit. This variant differed from the others in the same dominant presence of *Alternaria* sp. and *A. pullulans*, as well as the lowest percentage of *Tr. roseum*, which is also able to suppress the development of wheat seedlings.

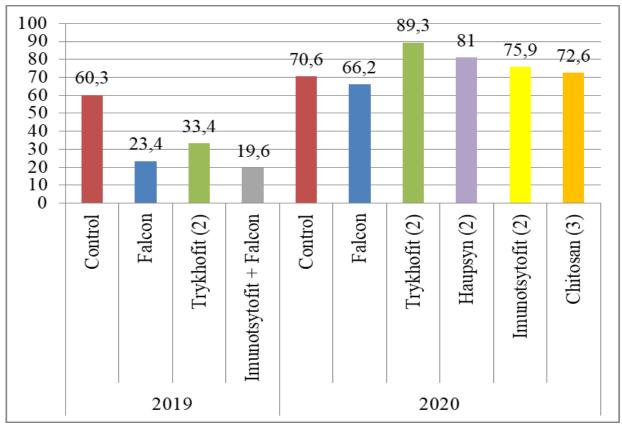


Figure. 1. The effect of spraying plants on the length of winter wheat seedlings (LSD₀₅ (2019)=1.4, LSD₀₅ (2020)=2.3)

The use of Falcon caused a decrease in the length of wheat seedlings, compared to the control. In 2019, the reason for this was an increase in the number of *N. oryzae*, and in 2020, the length of seedlings exceeded the control in other variants. A reduction in wheat germination and growth rates was noted for the study of different fungicides: Acrobat reduced seed

germination even at the recommended dose, compared to the control; Metalaxyl and Mancozeb, when used together with a reduced rate, negatively affected the length of seedlings; Acrobat, Dithane, Aliette, Metalaxyl and Mancozeb reduced plant biomass the most; the effect on root biomass was more positive, except for the negative effect of the combined use of preparations (Arshad et al., 2006).

Biofungicides based on microorganisms (Trykhofit and Haupsyn) reduced the number of the dominant *Alternaria sp.* less, compared to Falcon, but caused changes in the composition of the mycocomplex, thereby causing the best germination of plants. Similar studies with similar results were obtained for the study of biological preparations on rye. During the two-time spraying of winter rye plants with biofungicides Trykhodermin BT (2 1 / ha), and Haupsyn (5 1 / ha), a decrease in the release of *Alternarium* sp. from seeds was noted in the conditions of a humid chamber. The elements of the crop structure were also improved. In particular, an increase in the weight of 1000 grains by 8.8 and 8 g was noted, respectively. The use of drugs affected the germination of plants in laboratory conditions: an increase in the mass and length of seedlings, and the length of their roots was noted. The best growth parameters of rye were observed with the use of Trykhodermin BT (Polishchuk et al., 2018).

The simultaneous use of fungicides and resistance inducers allows reducing the risks of the emergence of resistant forms of microorganisms, to reduce the dependence of the latter on environmental conditions (Ons et al., 2020). The study of the simultaneous use of Falcon with Imunotsytofityt for two years had its positive results: in 2018, they better reduced the number of *Alternaria* sp.; in 2019 they increased the mass of 1000 seeds, compared to the use of only chemicals. But the study of one Imunotsytofityt had better results in terms of weight of 1000 grains and seedling length, compared to Falcon. Similar results were obtained by Soroka *et al.* (2017), when the treatment of winter wheat during the growing season with Imunotsytofityt (0.5 g /ha) obtained the best result during the germination of the obtained seeds in terms of germination energy and seedling length, than when using Rostok and Rybav-Extra. The simultaneous use of Rostok and Imunotsytofityt did not always have better results compared to spraying with one elicitor.

So, in the conditions of the North-East of Ukraine, after spraying with fungicides, the effect of biological and chemical preparations on the mycobiota of winter wheat seeds was established. This measure not only changed the number of selected species/genera, but also the overall

composition of fungi. The biggest changes in the latter were noted in 2020 for the use of Haupsyn. According to the analysis of three-year studies, Falcon had a greater effect on the mycocomplex, reducing the amount of dominant *Alternaria* sp., compared to biological preparations. Two-time spraying with a chemical preparation led to the appearance of the genus *Curvularia* sp., which is atypical for our area.

The use of fungicides also significantly affected the weight of 1000 seeds. For the most part, their use increased this indicator, with the exception of 2018, when they provoked the appearance of *Mucorales* in the mycobiota of seeds. The most complete seeds were formed in variants with biological preparations. The study of the effect of spraying plants on plant length during seed germination showed the best results for the application of biofungicides.

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