ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

The Micromycetae Composition Of The Soil Under The Crops Of A Summer Grain Cultures And A Specificity Of *Bipolaris Sorokiniana* And *Fusarium Spp* Strains.

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ABSTRACT

For the first time was isolated and identified a generic (9 genera) and a species composition (25 species) determining a micocenosis under the sown spring crops on a leached humus in the forest-steppe belt of the European part of Russia. According to the results of a research all the detected micromycetes were divided into 3 groups in a frequency of occurrence: the most common types, rare but typical types and the random species. In the rhizoplane of a spring grain crops were met the germs of various micromycetes. Thus, under the spring wheat crops were dominated the species of *Aspergillus wentii, Mucor pusillus, Penicillium purpurogenum.* On barley and oats the appearance of a community was determined by the species of the genera *Penicillium, Acremonium, Aspergillus, Mucor.* It was revealed that the species composition of causative agents of a root rot in the Republic of Mordovia, that is located in the forest-steppe belt of the European part of Russia, is represented by the micromycete *Bipolaris sorokiniana* and *Fusarium* species. These pathogens produce hydrolytic enzymes and toxins. Affecting plants, they are able to disrupt the metabolism and physiological processes in plant tissues. On the example of a spring wheat (the released varieties 'Prokhorovka') was studied a nature of the manifestation of pathogenic and toxic properties of causative agents of a root rot. Were established the differences in an action of strains of *Bipolaris sorokiniana* and *Fusarium* species on the development of diseases of the organs of seedling. It is proved that strains of the pathogen *Bipolaris sorokiniana* had a greater pathogenicity and toxicity than species of the genus *Fusarium.* The results showed a high toxicity of the strains of *F. heterosporum, F. sporotrichioides, F. oxysporum, F verticillioides, F. tricinctum.* Strains of *F. redolens and F. heterosporum* under the code 3l/2 were moderately toxic, and the types *F. sporotrichioide F. verticallioides, F. tricinctum* were toxic but not pathogenic due to low representation in the mycobiota of roots.
Keywords: micromycete, root rot, barley, spring wheat, oats, pathogenity, phytotoxicity, *Fusarium, Bipolaris sorokiniana.*

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INTRODUCTION

The main reasons for the increasing of the incidence of a plant root rot are the saturation of crop rotations by the grain crops and the increasing of a share of a minimum soil processing [1]. The role of fungi living in the soil is very significant. Fungi and their metabolites play an important role in microbiological processes, significantly affect the structure of the soil structure and growing plants on it. Diverse species composition of micromycetes, submitted mainly by the soil facultative parasites, which includes the causative agents of a root rot, hampering the effective protection of grain crops against them. The environmental factors such as a soil dwelling pests on the formation and a spread of a root rot pathogenesis, especially when their number exceeds the threshold of harmfulness in 2-3 times, weeds of the bluegrass that are the reservoirs of the diseases and a low soil microbiological activity due to the lack of organic matter [2]. Root rot cause the largest losses in dry years. However, conditions of high humidity early in the season also increase the incidence of infection, and a waterlogging at the end of the season results in the shrunken grain and a white plague [3].

The lack of information on the impact of crops on soil fungi, and about the specifics of strains in a particular region does not allow purposefully and reliably protect crops. To solve this problem, in 2007-2012 and 2015 we conducted a comprehensive study of features of distribution of various genera and species of micromices under the spring crops, and especially of fungi – agents of root rot in southern non-humus zone. The causative agents of root rots in this region are mainly microscopic fungi *Bipolaris sorokiniana* and species of genera *Fusarium* which belong to the class of imperfect fungi *Ascomycetes*, ordines *Pleosporales and Hypocerales*, families *Nectriaceae and Pleosporaceae*. The aim of our research was to identify the composition of micromycetes rhizoplane of a spring crops, a pathogenic complex of root rot pathogens and their pathogenic and phytotoxic properties.

MATERIALS AND RESEARCH METHODS

The experimental studies on this question was conducted in crops of spring grain crops on a leached hums of a heavy loam granulometric composition, where the humus content was 8 %, total nitrogen – 0,36 %, mobile forms of phosphorus and potash - 45 and 80 mg/kg of soil, the degree of a base saturaton is 91 %.

The allocation of the pathogen from plants was carried out according to methodical instructions[4]. Identification of genera and species was carried out on determinants [5-8]. The spatial frequency of occurrence of fungi was determined by the method proposed by T. G. Mirchink [9].

To study the properties of strains of causative agents of root rot were used plants of a spring wheat, barley and oats, collected in the different soil areas of the Republic of Mordovia in June 2007 to 2012 and 2015. All the samples of plants had signs of damage of leaves spotting and a root rot. Pathogenicity and toxicity of the isolates were investigated by the method of bioassay on seeds [10]. The testing objects were the organs of seedling spring wheat of a plant variety 'Prokhorovka'.

Indicators of pathogenicity were determined by the degree of development and prevalence of the disease on the organs of seedling. Toxicity was determined by the results of inhibition of germination, the length of the coleoptile or roots of the testing cultures. The samples germinated in water served as a control, their length was conceded as 100 %. The statistical processing of the obtained results was performed by the analysis of a dispersive variance.

RESULTS AND DISCUSSION

The mycological analysis of basal part of a harvested spring wheat, barley and oats with the following identification of fungi isolates allowed us to determine in accordance with the systematics and nomenclature of fungi their generic and species diversity. A composition of fungi allocated by the crop included 9 genera and 25 species (table 1).

Table 1: A species composition of micromycetes rhizoplane of the spring crops and their frequency of occurrence (2007-2011)

№	Species of micromicete	The frequency of occurrence in crops, %					
		Spring wheat		Barley		Oats	
		S *	T *	S	T	S	T
1	*Acremonium charticola* (*Lindau)* W. Gams	3	33	6	36	6	33
2	*Acremonium strictum* W. Gams	4	66	6	38	6	33
3	*Alternaria alternate* (Fr.) Keissl.	8	33	7	3	–	–
4	*Aspergillus ustus* (Bain.) Thom et Church	55	33	61	66	41	33
5	*Aspergillus wentii* Wehmer, Centralbl.	64	100	64	33	37	33
6	*Bipolaris sorokiniana* (Sacc.) Schoemaker	3	33	8	66	–	–
7	*Cladosporium brevi-compactum* (Pidopl. et Deniak.)	33	33	39	33	47	66
8	*Drechslera teres(*Sacc) Shoemaker	–	–	9	33	–	–
9	*Drechslera graminea* Rabenh.	3	33	11	66	–	–
10	*Fusarium verticillioides* (Sacc.) Nirenberg	4	3	5	8	3	5
11	*Fusarium heterosporum* Nees et T. Nees	12	63	3	32	–	–
12	*Fusarium tricinctum* (Corda) Sacc.	3	8	6	7	5	8
13	*Fusarium oxysporum* Schltdl.	8	33	6	66	–	–
14	*Fusarium sporotrichioides* Sherb.	7	33	8	33	6	33
15	*Fusarium redolens* Wollenw.	6	5	5	7	6	7
16	*Gliocladium penicilloides* Gorda	52	34	48	68	26	34
17	*Haetomium globosum* Kunze ex Fr.	10	100	4	34	63	35
18	*Mucor hiemalis* Wehmer	43	67	62	34	66	67
19	*Mucor pusillus* Lindt, Schipper	64	100	61	67	68	67
20	*Penicillium funiculosum* Thom	32	34	64	34	62	68
21	*Penicillium lapidosum* Raper et Fennell	45	34	64	34	67	98
22	*Penicillium purpurogenum* Stoll	62	99	69	68	65	34
23	*Penicillium raciborskii* Zaleski	44	32	61	67	63	69
24	*Penicillium tardum* Thom	42	35	63	99	66	68
25	*Trichoderma hamatum* (Bonorden) Bainier	39	34	31	69	38	65

*S –a spatial frequency; T –a timing frequency.

The distribution of species based on their frequency of occurrence characterizes the structure of the complex of microorganisms. According to this basis all the micromycetes were divided into 3 groups. The most common fungi (1-5 species) were included into the first group, and the frequency of its occurrence varied from 31 to 100 %.

These are the species of the genera *Penicillium, Aspergillus, Mucor*, *Cladosporium*, *Gliocladium*, *Trichoderma*. A special attention was deserved by fungi of the genera *Penicillium, Aspergillus* и *Mucor,* which are in the conditions of normal or high humidity increased the growth of plants, and with the insufficient hydration – oppressed them. The majority of the genus *Penicillium* was characterized by the types relative to the category of typical species, while among the representatives of the genus *Mucor* were identified the atypical types, which allowed us to refer them to the micromycetes of a typical basal area of spring cereals.

The quantitative composition of micromycetes changed depending on the soil moisture. So, abundantly plants bearing spores of the genus *Penicillium* gave a high percentage of isolates in the arid years (2009-2010), while the number of members of the genus *Mucor* dominated in the wet year - 2008.

Into the second group were included rare, but typical micromycetes genera *Acremonium*, *Helminthosporium*, *Alternaria*, *Fusarium* with a spatial frequency of less than 10 % and a timing frequency – more than 30 %. The peculiarity of this group is in the fact that the majority of species of these fungi are the causative agents of root rot.

In the third group were included the rare accidental species of the genus *Fusarium* that have a frequency incidence of less than 10 %.

In the root zone of different crops, but within the same soil type, a significant variation in the number of fungi of various species was observed. Thus, under the spring wheat crops the dominant species were: *Aspergillus wentii, Mucor pusillus, Penicillium purpurogenum*. The spatial frequency varied from 62 to 64 %, the timing frequency – 99-100%. Were identified the following common types: *Aspergillus ustus, Gliocladium penicilloides, Trichoderma hamatum*, species of the genus *Penicillium – P. raciborskii, P. lapidosum, P. tardum, P. funiculosum*. Their spatial frequency of occurrence was 32-55 %, and the timing – 32-35 %. On the young roots of spring wheat were mainly developing the fungi of the genera *Mucor, Fusarium, Penicillium*, and on the old roots – *Cladosporium brevi-compactum*, which did not cause diseases.

The community of micromycetes on barley and oats were represented by species of the genera *Penicillium, Acremonium, Aspergillus, Mucor*. The following species were mostly met under the crops: under the barley - *Penicillium purpurogenum,* under the oats – *P. lapidosum*. It was also observed a beneficial effect of barley on the survival of fungi of the genus *Drechslera* and some species of the genus *Fusarium*. These groups of micromycetes on barley were attributed to rare, but typical species. The above-mentioned microorganisms were not met on the oats, and the micromycetes *Alternaria alternata* and *Bipolaris sorokiniana* were classified as a random species.

Root rot pathogens have a wide phylogenetic specialization. So, micromicete *B. sorokiniana* revealed 37 physiological races capable of hitting 83 wild species and 65 plant species of the grass families [11]. Duration of its survival in the soil is ranged from 3 to 5 years and depends on its physico-chemical properties and micromycetes composition. Having a weak saprotrophic properties fungus *B. sorokiniana* belongs to the temporary inhabitants of soils.

Fusariose fungi, on the contrary, are considered as natives of the soil. Their life cycle is associated with crop plant residues, but they can also be the saprotrophs on the live plants. Crop residues of the affected cultures are the main source of the infection of this pathogen. A large number of infections is also on the weeds that are growing in the fields, where the predecessors were the grain crops [12]. The population density of *Fusarium* fungi is heterogeneous, and the number of spores in 1 g of soil can vary from 2 to 10 thousand.

The *Fusarium* root rot is becoming one of the most serious diseases in many regions of Russia where the seeds are sowing and in the whole world [13]. Currently, the genus *Fusarium* includes at least 300 physiological varieties, however less than a half was described [14].

Recently conducted in 11 regions of Russia the review of species of fungi of the genus Fusarium, causing root rot development, helped to identify 12 species, including high incidence of observed micromycete F. oxysporum. Studies have shown that the zonation of the distribution of species was dependent on soil type and climatic characteristics of the region. Fungi of the genus *Fusarium* have high intraspecific variability. Depending on the plant organs the proportion of fungi pathogens of root rot in the pathogenic complex is different. So the micromycete *B. sorokiniana* is better adapted to above-ground organs of the plants and fungi of the genus *Fusarium* to the underground [15].

Root rot pathogens are able to produce hydrolytic enzymes and toxins that are affecting the plant and are responsible for the manifestation of their pathogenic and toxic properties. So, the micromycete *B. sorokiniana* produces helminthosporol, helminthosporal, victoxin, cytokinin, and the species of the genus *Fusarium* **–** fusaric acid, isomarticin, zearalenone, etc. Enzymes contribute to penetration of the pathogen in the host tissues, thus destroying the cell structure. The effect of toxins is seen in the metabolic and physiological processes. This in turn contributes to the weakening of plants in general, decreases their resistance to the disease and accelerates the onset of the infectious process. Differences in aggressiveness and virulence as measures of pathogenicity are manifested in the quantity of the infective onset, duration of infection and climate conditions, resulting in different pathogenic and toxic properties of a root rot pathogens.

To study the characteristics of the strains of the local populations of pathogens of root rot were tested the isolates that were isolated from the different organs of plants in the beginning and at the end of the growing season. Were studied pathogenic and phytotoxic properties of micromycete *B. sorokiniana*, as well as widespread species of *Fusarium spp. – F. heterosporum, F. oxysporum, F. sporotrichioides* and rare species of fungi – *F. redolens, F. verticillioides, F. tricinctum*. The results showed different manifestations of the properties of pathogenicity and toxicity of the studied strains.

So, the strains of the pathogen *B. sorokiniana* had a higher pathogenicity, indicating their significant aggressiveness and virulence in relation to the testing cultures. Within this population the isolates of the pathogen possessed a stronger pathogenicity, isolated from the plants of barley. From all the analyzed organs, contaminated with a spore suspension of the fungus *B. sorokiniana*, coleoptile was affected stronger.

High damage and a death of seedlings were observed while contamination with the strain marked with the cipher 4K/5 (table 2).

Table 2: Pathogenicity of strains of causative agents of root rot, isolated from a spring wheat and barley, in a % to the control (water) (2009, 2011-2012, 2015)

Cipher of the isolates	Culture	The prevalence, %	A disease developing, %		Pathogenity %
			Coleoptile	Roots	
B. sorokiniana					
4к/4	wheat	76,2±5,4	29,7±3,1	18,5±1,9	24,1 (LP *)
4к/5	wheat	87,8±6,3	70,8±5,9	53,8±2,3	62,3 (P)
F. heterosporum					
1к/3	wheat	73,1±7,1	4,2±0,4	2,2±0,1	3,2 (NP)
1к/11	wheat	57,2±4,3	48,3±2,7	28,1±2,4	38,2 (MP)
2к/2	barley	68,4±4,7	11,0±0,6	5,2±0,8	8,1 (NP)
2к/3	barley	77,1±5,9	31,6±1,3	28,8±2,3	30,2 (MP)
3к/2	barley	62,0±3,3	9,8±0,5	3,8±0,2	6,8 (NP)
3к/1	barley	52,6±4,3	33,4±2,7	27,0±1,9	30,2 (MP)
3L/1	barley	88,1±7,0	5,9±0,3	4,3±0,3	5,1 (NP)
3L/2	barley	73,2±6,2	42,3±3,3	26,1±1,3	34,2 (MP)
F. oxysporum					
2к/4	barley	59,6±4,4	20,8±0,9	16,4±0,7	18,6 (LP)
5к/1	barley	61,8±4,3	11,2±0,9	22,4±1,6	16,8 (LP)
7к/1	barley	71,5±4,7	34,6±2,3	25,6±1,3	30,1 (LP)
7L/1	barley	69,2±5,3	30,5±2,6	25,1±1,9	27,8 (LP)
F. sporotrichioides					
6L/5	wheat	65,6±6,0	5,0±0,3	2,8±0,1	3,8 (NP)
F. redolens					
4к/1	barley	72,0±4,9	3,6±0,1	1,4±0,1	2,5 (NP)
F. verticillioides					
1к/1	oats	55,6±4,9	7,2±0,4	4,4±0,2	5,8 (NP)
F. tricinctum					
1к/7	oats	70,2±5,9	8,3±0,7	3,9±0,3	6,1(NP)

*P – Pathogenic; MP – moderately pathogenic; LP – light pathogenic; NP – non-pathogenic

Strain with a cipher 4K/4 was low pathogenic, but without showing pathogenic properties, it was more toxic than the pathogenic species. During the contamination of seedlings and roots with the pathogenic species we could observe their browning and darkening. During the contamination by the moderate pathogenic strains, the roots of seedlings were normally developed; it was only a small browning in the form of dots or dashes on the separate parts of the root. In the embodiments with *Fusarium* species the manifestation of the pathogenic properties of strains of the common species was lower than in *B. sorokiniana*.

While studying the influence of cultural liquid of *B. sorokiniana* and *Fusarium spp.* on the organs of the seedling strains from these populations, were observed the differences according to the degree of toxinogenesis, and therefore the causative agents in a different way inhibited the growth and the development of seedling and a primary root system of a spring wheat. The development of seedlings oppressed the most strongly the contamination of seeds by the fungus *B. sorokiniana*. It was also revealed that the culture filtrate of *V. sorokiniana* with cipher 4K/4 inhibited stronger the growth of seedlings and roots of the testing object, which, presumably, is due to the large number of the excreted toxins (table 3).

Table 3: Phytotoxicity of strains of causative agents of root rot, isolated from the spring wheat and barley, in a % to the control (water) (2009, 2011-2012, 2015)

Cipher of the isolates	Culture	Germination, %	The length of Coleoptile		The length of roots		Toxity, %
			mm	in % to the control	mm	in % to the control	
Bipolaris sorokiniana							
4к/4	wheat	86,6±9,3	12,2±0,8	22,8±1,5	7,2±1,1	9,1±1,4	90,9 (T*)
4к/5	wheat	82,3±8,0	15,4±1,2	28,3±2,3	11,5±1,6	14,9±2,1	85,1 (T)
F. heterosporum							
1к/3	wheat	96,7±6,1	14,0±1,0	27,0±1,9	12,7±1,0	15,6±1,2	84,4 (T)
1к/11	wheat	42,9±4,2	17,2±1,3	29,6±2,2	14,2±1,4	18,2±1,8	81,8 (T)
2к/2	barley	90,0±9,9	13,6±0,5	22,0±0,8	16,2±1,6	20,1±2,0	79,9 (T)
2к/3	barley	95,0±4,6	7,4±1,0	11,0±1,5	5,1±0,9	6,5±1,1	93,5 (T)
3к/2	barley	93,3±7,3	18,8±1,8	33,6±3,2	15,3±1,3	19,5±1,7	80,5 (T)
3к/1	barley	90,2±8,1	17,4±0,7	27,6±1,1	22,8±1,6	28,6±2,0	71,4 (T)
3L/1	barley	88,7±3,9	7,3±1,2	13,0±2,1	10,2±1,5	13,0±1,9	87,0 (T)
3L/2	barley	90,3±6,6	24,6±2,1	44,4±3,8	37,7±2,5	48,1±3,2	51,9 (MT)
F. oxysporum							
2к/4	barley	91,2±7,4	12,8±1,1	22,2±1,9	16,7±1,2	20,8±1,5	79,2 (T)
5к/1	barley	88,3±7,3	15,2±1,3	26,0±2,2	15,0±1,4	19,5±1,8	80,5(T)
7к/1	barley	90,4±8,1	13,4±1,7	22,2±2,8	11,1±1,5	14,3±1,9	85,7 (T)
7L/1	barley	31,6±2,7	15,8±1,4	27,8±2,5	23,5±1,9	29,8±2,4	70,2 (T)
F. sporotrichioides							
6L/5	wheat	56,9±7,1	14,1±1,2	22,2±1,9	14,7±1,2	18,2±1,5	81,8 (T)
F. redolens							
4к/1	barley	98,1±7,4	37,2±1,8	66,6±3,2	36,8±2,1	46,7±2,7	53,3 (MT)
F. verticillioides							
1к/1	oats	99,0±10,2	8,4±1,1	14,8±1,9	12,4±1,3	15,6±1,6	84,4 (T)
F. tricinctum							
1к/7	oats	30,0±3,3	6,6±0,5	11,0±0,8	5,3±0,7	6,5±0,9	93,5 (T)

*T– toxic; MT–moderately toxic.

The adverse effects on a root length and a coleoptile of a testing culture provided the most toxic strains of the species *F. heterosporum, F. sporotrichioides, F. oxysporum, F verti-cillioides, F. tricinctum.*

A moderate toxic, do not significantly affecting the inhibition of the growth of the organs of the germ, were strains of *F. redolens* and *F. heterosporum* with cipher 3l/2.

Strains of the species *F. sporotrichioides* and the rare species *F.verticallioides, F. tricinctum* were toxic for the seedlings of the testing crops, but turned out to be non-pathogenic, which may explain their low representation in the microbiota of a root rot.

The manifestation of toxicity as a result of a defeat of organs of seedling by the pathogens of a root rot was stronger relative to the roots of the testing culture.

CONCLUSION

The conducted studies have shown that the strains of the local population of a root rot pathogens adherents of spring cereals contributed to the changes of products-activities of a test-culture that have arisen as a result of illness, which undoubtedly proves their involvement in the pathological process.

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