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Micromycete Composition in Pure and Combined Crops in Cultivated Land

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Micromycetes are actively involved in the transformation of biogenic elements in soil, providing life activity of other parts of the cultivated land and its functioning as an integral system. Change in crops in farming rotation and introduction of new ones are reasonable in order to reduce the negative development of the monoculture. When cultivating specific crops, certain microbiocenoses are formed in soil, including both useful and pathogenic forms.

Key words: Phytohormones, growth inhibitors, heterotrophic organisms, Pathogenic forms, rhizosphere, rhizoplane, micromycetes.

Microorganisms have a multifaceted impact on the plant vegetation: they synthesize phytohormones (development stimulants) and toxins (growth inhibitors), affect the nutrition status by increasing the solubility or immobilization of mineral compounds in the root zone, seize atmospheric nitrogen or determine the emission of gaseous compounds etc.[3, 7, 8]. Study of the microbiocenoses compound and features of life activity of heterotrophic organisms in the root zone of plants is necessary to solve successfully many practical tasks of agriculture.

Cultivation of certain crops determines the accumulation of specific species of microorganisms in soil, particularly fungi, including both useful and pathogenic forms. The study of the microbial population of plants is necessary to organize correct crop sequence in the farming rotation and to develop control measures for soil exhaustion by the monoculture, as well as to successfully implement microbial strains in order to increase plant yield and inhibit pathogenic organisms. To solve these problems, it is necessary to study the species composition and ratio (the domination level) of microorganisms in soil, rhizosphere, and rhizoplane. Despite the importance of these issues, there are a few studies on the determination of composition of pathogenic and saprotrophic microorganisms, particularly fungi, in soil, rhizosphere, and rhizoplane of the agricultural plants[4]. There are almost no works performing a comparative analysis of the abundance of micromycetes in soil and the root zone of plants on the basis of quantitative indicators. That is why there are no doubts concerning the relevancy of studies of the composition and abundance of various species of microscopic fungi in soil and root zone of the plants[8].

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MATERIALS AND METHODS

The microbiological studies were held at the Department of Biology and Ecology of Kuban State Agrarian University based on trials carried out on the vegetation plot with leached low-humic chernozemic soil having the humus content within 2.5% and moist available for plants about 40-45%

of maximum water capacity. Soil samples were collected in the middle of April and October during the monitoring, the sowing of blue panic grass, alfalfa, and the mixture of blue panic grass and alfalfa.

10 samples of soil and 10 samples of both rhizoplane and rhizosphere were collected prior to sowing. The composition of microscopic fungi was studied prior to sowing in pure crops of blue panic grass, alfalfa, and the herbage mixture (blue panic grass + alfalfa). Water suspensions of particular samples of soils and outwashes of peeled roots acidulated with the lactic acid were sown to the Czapek's and Rolen's media. Plates with inoculation were incubated in thermostats at the temperature 25°C within 7 to 10 days. Detachment of fungi into a pure strain was conducted in one or a group of similar colonies. To assess the abundance and typicality of fungi of a particular habitat, we used the indicators of abundance, density, and occurrence. An analysis of the species composition of fungi was carried out by means of inoculation of fine soil aleurite (dust) in an amount of 1 mg of soil particles in the Petri dish on Czapek's medium. Species identification of detached cultures of fungi was carried out in accordance with the

Table 1. Species composition, occurrence and abundance of species (*%)* of microscopic fungi in soil and rhizosphere of forage crops and cotton plant, field test, 1988)

Species	Soil		Rhizosphere					
			Blue panic grass		Alfalfa		Blue panic grass + alfalfa	
	April		October		October		October	
	occurrence	abundance of species	occur-rence	abundance of species	occu-rrence	abundance of species	occurrence	abundance of species
Aspergillus flavus	10	2.4	40	2.0	–	-	20	1.2
Aspergillus niger	5	3.0	20	0.4	40	1.2	40	1.2
Aspergillus ustus	40	3.6	20	2.4	40	1.8	20	0.3
Aspergillus fumigatus	–	–	20	2.0	–	–	40	0.6
Aspergillus ochraceus	–	–	–	–	60	1.8	100	9.3
Aspergillus sidovii	10	1.2	100	61.5	20	0.6	40	0.6
Aspergillus sclerotiorum	–	–	10	0.8	–	–	–	–
Aspergillus rugulosus	70	12.0	–	–	20	0.6	40	1.5
Aspergillus terreus	–	–	–	–	20	0.6	–	–
Acremonium sp.	20	1.2	–	–	20	0.6	20	0.6
Alternaria sp.	20	2.4	20	0.8	20	1.2	–	–
Arthrinium phaeospermum	–	–	–	–	–	–	–	–
Cladosporium sp.	70	17.2	60	4.4	80	13.2	40	2.4
Dematiaceae	40	6.0	–	–	60	6.7	–	–
Fusarium nivale	20	2.4	60	14.2	60	56.2	20	0.3
Fusarium sp.	30	1.8	–	–	40	1.8	–	–
Cladosporium sp.	40	7.2	40	1.2	20	3.6	60	74.5
Drechslera australiensis	20	2.4	40	1.2	20	2.9	40	0.6
Humicola grisea	–	–	20	0.8	–	–	–	–
Penicillium cyclopium	50	26.9	40	6.7	80	2.9	20	1.8
Penicillium funiculosum	10	1.2	–	–	–	–	40	4.2
Penicillium purpurogenum	10	1.2	–	–	–	–	–	–
Rhysopus orósàå	20	3.6	–	–	–	–	–	–
Scopulariopsis sp.	–	–	40	1.2	–	–	–	–
Stachybotrys atra	20	1.2	20	0.4	–	–	20	0.6
Ulocladium botrytis	10	0.6	–	–	–	–	–	–
Mucor sp.	30	2.4	–	–	80	4.3	20	0.3

determinants corresponding to each group of micromycetes on the basis of culture-morphological traits.

RESULTS AND DISCUSSION

The species composition of microscopic fungi in the chernozemic soil leached in the root zone of the introduced crops was carried out on the basis of the studies performed. The Analysis is represented by the territorial occurrence of microscopic fungi when extracting them to Czapek's and Rolen's solid nutritional media with sucrose allowed determining the typical species in the soil, rhizosphere, and rhizoplane of plants. We classified the species with territorial occurrence of more than 50% as typical dominating organisms, those with occurrence between 30 and 50% — as typical, and between 10 and 30% — to non-typical. The greatest variety of microscopic fungi was registered in soil prior to sowing during the first year of plant vegetation (Table 1).

Species differences in the composition of micromycetes were registered in soil, compared to the rhizosphere. For instance, *Aspergillus. Rugulosus,* micromycetes dominate in soil.as well as *Cladosporium, Aspergillus niger, Penicillium cyclopium.* Occurrence of *Aspergillus sgtewi* and *Fusarium nivale* increased in the rhizosphere of pure sowing of blue panic grass[2].

In the rhizosphere of the pure crop of alfalfa, *Aspergillus ochraceus, Pinicillium cyclopium, Cladosporium, Fusarium nivale* and *Mucor, Dematiaceae* are the dominating fungi, and in the double herbage mixture rhizosphere *Aspergillus ochraceus* dominate. In the pure crops of rhizosphere of the blue panic grass, the greatest abundance of *Aspergillus sgotevi* was observed (61.5%). In the rhizosphere of alfalfa, this indicator is greatest among *Fusarium nivale* (56.2 %). In the rhizosphere of the double herbage mixture and in the initial sample of soil, the dominating abundance of fungi was not registered[1].

The composition and abundance of microscopic fungi during the first year of plant development in the rhizoplane is slightly different from the complex of micromycetes of the rhizosphere in soil (Table 2).

The quantity of fungi on the roots of blue panic grass and alfalfa was found to be similar to

Table 2. Species composition, occurrence, and abundance of species (%) of microscopic fungi in the rhizoplane of forage crops (field test, October)

Species	Blue panic grass		Alfalfa	
	occurrence	abundance of species	occurrence	abundance of species
1	4	5	6	7
Aspergillus flavus	23.3	11.0	30.0	4.4
Aspergillus niger	23.3	36.2	53.3	39.8
Aspergillus ochraceus	23.3	2.2	30.0	3.5
Aspergillus sidovii	23.3	1.1	–	–
Aspergillus terreus	23.3	1.1	–	–
Aspergillus ustus	33.3	1.1	30.0	1.6
Aspergillus sp.	–	–	53.3	0.9
Alternaria sp.	–	–	53.3	2.7
Cladosporium sp.	33.3	2.2	53.3	0.9
Fusarium sp.	53.3	24.2	–	–
Cladosporium sp.	23.3	1.1	23.3	0.9
Dematiacae	33.3	4.4	–	–
Monocil	33.3	4.4	53.3	5.2
Mucorales	–	–	30.0	11.5
Penicillium Assum	23.3	4.4	–	–
Penicillium Divavicofa	23.3	1.1	30.0	0.9
Penicillium monover	23.3	5.5	53.3	14.2
Penicillium sp.	–	–	23.3	0.9
Scopulariopsis sp.	–	–	23.3	0.9

the soil and rhizosphere. The prevailing species of micromycetes in the dominating crops' rhizoplane (blue panic grass and alfalfa) was discovered: for blue panic grass—*Fusarium* (53 %); for alfalfa—micromycetes *Aspergillus niger* (53,3%) and *Aspergillus sp.* (53.3 %). Fungi *Aspergillus (A. niger, A. flavus, A. achraceus, A. ustus), Mucorales, Penicillium sp.* are typical of all studied plants in their rhizoplane[5].

Aspergillus niger is abundant in the rhizoplane of blue panic grass and alfalfa. In the rhizoplane of blue panic grass, there was an increase in the abundance of species of *Fusarium* genus (24.2%), as well as *Aspergillus niger* (36.2%), compared to the antecedent soil and rhizosphere (respectively, for fungi of *Fusarium* genus—4.2; 14.2%), for *Aspergillus niger*—respectively 3.0% and 0.4%. A similar trend was observed in alfalfa for the *Aspergillus niger* species: 1.2% in the rhizosphere and 39.8% in the rhizoplane. The diversity in the micromycetes complex increased in the rhizosphere of blue panic grass during the second year of development (Table 3).

Prevalence of *Aspergillus sidovii, Cladosporium sp., Fusarium nivale* micromycetes has remained. The territorial occurrence of *Cladosporium, Fusarium nivale* micromycetes has increased mildly, and the occurrence of *Aspergillus sidovii* species has decreased. A recession of the abundance level of *Aspergillus (A. flavus, A. niger,*

Table 3. Species composition and occurrence *(%)* of microscopic fungi in rhizosphere of forage crops (field test, October)

Species	Blue panic grass	Alfalfa	Blue panic grass + alfalfa
Aspergillus flavus	33.3	–	13.3
Aspergillus niger	13.3	26.7	26.7
Aspergillus ustus	13.3	33.3	13.3
Aspergillus fumigatus	13.3	6.7	33.3
Aspergillus ochraceus	13.3	66.7	93.3
Aspergillus sidovii	93.3	13.3	33.3
Aspergillus sclerotiorum	13.3	6.7	–
Aspergillus repens	6.7	–	–
Aspergillus rugulosus	6.7	26.7	46.7
Aspergillus terreus	20.0	30.0	20.0
Acremonium sp.	6.7	20.0	26.7
Alternaria sp.	33.3	26.7	33.3
Arthrinium phaeospermum	–	6.7	6.7
Cladosporium sp.	66.7	80.0	46.7
Dematiaceae	6.7	20.0	26.7
Drechslera australiensis	46.7	20.0	46.7
Fusarium nivale	86.6	46.7	33.3
Fusarium sp.	6.7	46.7	13.3
Humicola grisea	20.0	6.7	13.3
Mucor sp.	6.7	86.7	26.7
Penicillium canesceus	6.7	13.3	13.3
Penicillium cyclopium	46.7	86.7	26.7
Penicillium funiculosum	13.3	13.3	46.7
Penicillium lilacium	6.7	–	6.7
Penicillium meleagrium	–	6.7	6.7
Penicillium purgurogenum	13.3	6.7	–
Rhyzopus grysea	–	–	6.7
Scopulariopsis sp.	40.0	6.7	–
Stachybotrys atra	20.0	–	26.7
Trichoderma koningii	–	–	6.7
Ulocladium botrytis	6.7	13.3	6.7
Paecylomyces sp.	6.7	–	6.7

A. ustus, A. fumigatus) micromycetes was registered in the rhizosphere of blue panic grass during the second year. The trend of an increase in the micromycete species composition was observed in the rhizosphere of alfalfa, as well as the herbage mixture of blue panic grass + alfalfa. The prevalence of *Aspergillus ochraceus, Cladosporium, Mucor, Penicillium cyclopus* remained in the rhizosphere of alfalfa during the second year. *Aspergillus ochraceus* dominates in the rhizosphere of the double herbage mixture during the second year as well as during the first one. Typical representatives of micromycetes in this herbage mixture within the two-year period are: *Aspergillus* (*A. fumigates, A. sidovii, A. rugulosum*), *Penicillium funiculosum, Drechslera australienses, Cladosporium*[6].

Table 4. Species composition, occurrence and abundance (%) of microscopic fungi in the rhizoplane of forage crops (field test, October)

Species	Blue panic grass	Alfalfa
Aspergillus flavus	16.7	23.3
Aspergillus niger	16.7	60.0
Aspergillus ochraceus	30.0	26.6
Aspergillus sidovii	23.3	3.3
Aspergillus sp.	13.3	63.3
Aspergillus terreus	30.0	6.3
Aspergillus ustus	16.7	13.3
Aspergillus wentii	6.7	–
Cladosporium sp.	23.3	46.7
Ciocladium rosium	6.7	6.7
Dematiacae	16.7	20.0
Fusarium sp.	66.0	13.3
Monocil	26.6	3.3
Mucorales	40.0	50.0
Penicillium Assum	–	23.3
Penicillium Divavicofa	16.7	–
Penicillium Monover	20.0	26.7
Penicillium sp.	20.0	40.0
Scopulariopsis sp.	–	20.0
Trichoderma sp.	6.7	13.3
Alternaria sp.	13.3	60.0

CONCLUSION

Thus, species diversity of fungi increases for the rhizosphere of the studied crops. Practically constant presence of fungi *Aspergillus (A. niger, A. ustus, A. sidovii), Penicillium cyclopium, Drechslera auctraliensis, Cladosporium, Fusarium nivale* in the rhizosphere of all forage crops was registered. During the second year, more species of fungi were detached from the root surface of the plants than during the fist year (Table 4). In the rhizoplane of blue panic grass, *Fusarium* species occur more often, as well as micromycetes *Aspergillus terreus, Mucorales. Aspergillus (A. flavus, A. ustus, A. ochraceus)* is less common, as well as the families of*Dematiaceae, Penicillium, Gladosporium, Divavicofa, Monocil.* A decrease in the abundance of prevailing fungi *Fusarium* was registered in the rhizoplane of blue panic grass compared to the rhizosphere. During the second year, in opposition to the first year, alfalfa demonstrated an increase in the abundance of prevailing micromycetes: *Alternaria* and *Aspergillus*. *Fusarium, Asperillus (À. niger, A. flavus, A. ochraceus, A. ustus, A. niger, A. sp*.), *Mucorales, Penicillium grysogenum, Alternaria* are typical of the rhizoplane of all studied plants[9, 10].

The occurrence of *Fusarium* fungi in the rhizoplane of all introduced crops increased during the second year comparing to the first one. In long-term cultivation of forage crops in one place, the level of dominance of these pathogenic fungi in the root zone increases, which could affect the growth and development of plants. A substantial difference between the compositions of fungi of the rhizoplane of forage crops was determined[11].

Among the fungi found in the soil, the vast majority are typical saprotrophic organisms, such as *Aspergillus (A. terreus, A. ochraseus, A. niger, Trichoderma*, etc.), engaged in the processes of mineralization of organic matters. Some of these micromycetes are known as potential producers of phytotoxic substances reducing yields of agricultural crops: *Aspergillus* (*A. flavus, A. ustus*), *Penicillium* (*P. citrlauiii, P. funiculosim, P. nigricans)*.

The composition of microscopic fungi of the rhizoplane of plants is different from the complex micromycetes of the rhizosphere by lower number species. A similar trend in species richness of micromycetes complexes in the rhizosphere and rhizoplane of forage crops was detected[6, 7].

Cultivation of forage crops reduces the occurrence of such toxigenic fungi as *Aspergillus (A. flavus, A. ustus)*, and this fact can be considered

as a positive development in the "recovery" of the soil. Fungi of the *Trichoderma* genus, the overwhelming majority of which are antagonist to pathogenic and potentially pathogenic fungi, were registered in the root zone of these crops[6].

Biennial cultivation of forage crops has increased the biological activity of soil, escalated the species diversity of the named micromycetes. The increasing diversity of microscopic fungi in the rhizosphere and rhizoplane is a positive phenomenon contributing to the improvement of the soil microbial systems' resistance to various anthropogenic influences, in particular, to mineral fertilizers. The specific feature of root exudates determines the different composition of fungi in the root zone of plants: the rhizosphere of the cereal is dominated by fungi of the *Fusarium* and *Alternaria* genera, and the rhizosphere of legumes contains abundance of *Penicillium*.

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