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Peculiarities of Taxonomic Structure of Micromycete Complex in Root Zone of Sunflower in Conditions of Southern Steppe of Ukraine

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Abstract: The taxonomic structure of microscopic fungi complex in root zone of sunflower under its growing in conditions of southern Steppe of Ukraine comparing with natural biocenosis and black vapor has been studied. Soil of background (natural biocenosis, rhizosphere), black vapor (arable layer 0–20 cm) and root zone (rhizosphere) of a sunflower hybrid Zaporozhsky-32 was investigated. Soil samples of sunflower rhizosphere were selected at the stages of 2–4 true leaves (May), flowering (June), head formation (August) and full maturity (October) during 2009–2011. Samples of background soil and black vapor soil were taken in the same terms as the crop. Almost throughout the entire vegetation period, the number of micromycetes in the root zone of sunflower did not differ from the natural biocenosis and black vapor, and only by the end of the vegetation it nearly doubled. Despite almost the same total number of identified genera, there are significant differences in genus composition of micromycetes isolated from background soil and root zone of sunflower. Micromycetes of the genera *Botrytis, Cladosporium, Metarrhizium* and *Rhizopus* were typical only for sunflower rhizosphere while micromycetes of the genera *Doratomyces* and *Acremonium* were exclusively found in natural biocenosis. In addition, in soil under sunflower the range of the species in *Penicillium* genus expanded, while the species variety in *Aspergillus* genus significantly reduced compared to background soil and black vapor. During the growing season, in natural biocenosis genus diversity practically did not change, whereas in root zone of sunflower some fluctuations in number of genera were observed. Based on the Sorensen index, it was found that the micromycete complexes of the background soil and the black vapor were the most similar, where 23 species of fungi were common, while in rhizosphere of sunflower very specific mycocenosis was formed.

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Introduction

Sunflower is one of the most common crops in Steppe zone of Ukraine, which takes one of the first places in the economy of this country. Oil sunflower valued for its high taste and benefits over other vegetable fats (Varenyk, 2009). The formation of sunflower yield is strongly influenced by agrometeorological factors and level of crop reaction to environmental conditions. Therefore, modern sunflower breeding along with increased productivity and oiliness, demands creating ecologically plastic, drought-resistant, adapted to adverse conditions varieties and hybrids (Tolmachev and Kyrpychova, 2008).

However, unjustified increase in sowing area and uncontrolled increase in the proportion of sunflower in modern intensive crop rotations leads to accumulation of infectious diseases. Especially dangerous for sunflower crop are White mold (*Sclerotinia sclerotiorum* pathogen), Gray mold (*Botrytis cinerea*), Fomopsis (*Phomopsis helianthi*), and in recent years Fusariosis (*Fusarium oxysporum* var. *orthoceras* and *Fusarium sporotrichiella* Bilai var. *poae* pathogens) and some others (Castano *et al*., 2005; Karov *et al*., 2011; Madhavi *et al*., 2005).

Solving the problem of protecting sunflower from diseases is associated with the development of comprehensive measures, including the creation of resistant hybrids and varieties, development of agricultural technologies cultivation, chemical and biological means of protection, which reduce the harmfulness of pathogens (Maslienko, 2005; Petrenkova, 2005). However, while there are significant changes in the structure of native soil microflora, particularly in saprotrophic complex of micromycetes that play a significant role in the formation and functioning agrophytocenosis (Babayants, 2011; Belyuchenko, 2016).

With this in mind, the main aim of research was to study the taxonomic structure of microscopic fungi complex in root zone of sunflower at different phases of plant development under bog growing in compared with natural biocenosis and black vapor.

Materials and methods

The research was conducted in the fields of rotation of the Institute of Oilseed Crops (IOC) of NAAS during the growing seasons of 2009–2011. Soil is leached low-humic chernozem.

Soil of background (natural biocenosis, rhizosphere), black vapor (arable layer 0–20 cm) and root zone (rhizosphere) of a sunflower hybrid Zaporozhsky-32 of IOC NAAS breeding was investigated. Predecessor of sunflower is winter barley. Agricultural technology of sunflower growing is common for the south of Ukraine. Samples of soil (20–25 combined samples, on an area of 25 m × 25 m) were selected according to the following phases of sunflower development: 2–4 true leaves (May), flowering (June), head formation (August) and full maturity (October). Samples of background soil and black vapor soil were taken in the same terms as the crop.

A complexes of soil micromycetes of agrocenosis (black vapor and sunflower) and natural biocenosis were studied. Isolation and cultivation of micromycetes was carried out on the Czapek's medium in accordance with the methods adopted in soil microbiology (Mirchink, 1988; Zvyagintsev, 1991).

Identification of fungi carried out by the structure and features of formation of reproductive organs, morphological and culture characteristics using handbooks and original works (Ellis, 1971; Bilaj and Koval, 1988; Bilaj and Kurbatskaya, 1990; Domsh *et al.*, 1993; Gerlach and Nirenberg, 1982; Gerlach and Nirenberg, 1982; Litvinov, 1967; Pidoplichko, 1972; Samson and Frisvad, 2004; Satton *et al.*, 2001; Watanabe, 2002).

The structure of the micromycete complex was characterized by the abundance (%) and frequency of occurrence. The number of micromycetes was determined by Zvyagintsev (1991). The differences in micromycete number were defined by the t-test at the 0.01 level of probability. To characterize the similarity of the species composition of the micromycetes of the investigated soils, the Sorensen similarity coefficient (Cs) was used (Megarran, 1992).

Results and discussion

Dynamics of micromycete number

Table 1 shows the data of seasonal dynamics of micromycete number in the root zone of sunflower compared to background soil and black vapor. Apparently, the number of microscopic fungi in the rhizosphere of sunflower varied from 49.6 to 119.6 thousands of CFU/g of soil and significantly differed from that of background soil and black vapor just in autumn. From May to October, the number of micromycetes in the background soil and black vapor did not change significantly, whereas in the soil under sunflower, by the end of its vegetation, it almost doubled.

Table 1: Number of micromycetes in background soil, black vapor soil and root zone of sunflower, average for 2009–2011 years.

Soil	Number of micromycetes, thousand CFU (g soil) $^{-1}$		
	May	**July**	**October**
Background	75.6 ± 12.42	61.1 ± 7.85	57.9 ± 6.13
Black vapor	58.6 ± 6.39	78.9 ± 12.11	63.9 ± 8.26
Root zone of sunflower	49.6 ± 8.53	68.8 ± 6.27	119.6 ± 8.29*,#

Notes: *The differences from background soil and black vapor are significant at the 0.01 level of probability; #The differences from May and July are significant at the 0.01 level of probability.

Genus and species spectrum of micromycetes

As a result of the study of species diversity of micromycetes derived from background soil and soils of agrocenosis, 55 species belonging to *Zygomycota* (2) and *Deuteromycota* (53) were revealed. During the growing season in the natural biocenosis we found 34 species, in black vapor – 30 and in sunflower root zone – 32 (Table 2).

An analysis of the taxonomic structure of micromycete complexes revealed differences in the genus variety of mycocenosis of the background soil and the soils of agrocenosis. It was found that in background soil a number of genera of micromycetes during season was fairly stable, while in the black vapor and root zone of sunflower we observed fluctuations in this index. The complex of typical microfungi species derived from the studied soils was formed by micromycetes belonging to the genera *Acremonium, Alternaria, Aspergillus, Botrytis, Cephalosporium, Cladosporium, Curvularia, Doratomyces, Fusarium, Gliocladium, Metarrhizium, Paecilomyces, Penicillium, Trichoderma, Verticillium, Mucor, Rhizopus* and *Stemphylium*.

The genera *Penicillium* (23 species), *Aspergillus* (9 species) and *Fusarium* (8 species), which are typical for soils of southern regions of Ukraine (Marfenina, 1988), were the most widely represented, both in background soil and in soils of agrocenosis.

It should be noted that despite almost the same total number of identified genera and species, there are qualitative differences in genus composition of micromycetes derived from background soil and soils of agrocenosis. Thus, in soil under sunflower the range of the species in *Penicillium* genus expanded, while the species variety in *Aspergillus* genus significantly reduced compared to background soil and black vapor. The representatives of the

Table 2: The systematic structure of micromycete complexes at the level of leading genera in the background soil, the black vapor soil and the root zone of sunflower.

Genus	Number of species, pcs. (%)		
	Background	Black vapor	Sunflower
Acremonium	2 (5.9)	1 (3.3)	–
Alternaria	1 (2.9)	1 (3.3)	1 (3.1)
Aspergillus	6 (17.7)	5 (16.7)	3 (9.4)
Botrytis	–	–	1 (3.1)
Cladosporium	3 (8.8)	2 (6.7)	2 (6.3)
Cephalosporium	1 (2.9)	1 (3.3)	1 (3.1)
Curvularia	1 (2.9)	1 (3.3)	–
Doratomyces	1 (2.9)	–	–
Fusarium	2 (5.9)	4 (13.3)	3 (9.4)
Gliocladium	–	–	1 (3.1)
Metarrhizium	–	–	1 (3.1)
Mucor	1 (2.9)	1 (3.3)	–
Paecilomyces	2 (5.9)	–	–
Penicillium	9 (26.5)	9(30.0)	13 (40.6)
Rhizopus	–	–	1 (3.1)
Stemphylium	1 (2.9)	–	1 (3.1)
Trichoderma	2 (5.9)	3(10.0)	2 (6.3)
Verticillium	2 (5.9)	2 (6.7)	2 (6.3)
Total species	34	30	32
Total genera	14	11	13

genera *Doratomyces* (*D. stemonitis*) and *Acremonium* (*Acremonium roseum, A. alternatum*) were typical only for background soil, while micromycetes of the genera *Metarrhizium* and *Botrytis* were typical for sunflower rhizosphere. Microfungi of the genus *Mucor* were mainly derived from background soil and black vapor, while micromycetes of genus *Rhizopus* were typical for soil under sunflower (Table 2).

Genus and species spectrum of melanin-containing micromycetes, presented by *Alternaria, Cladosporium, Curvularia* and *Stemphylium* genera which are pathogenic fungi, was less diverse in soils of agrocenosis compared to background soil. However, the genus *Stemphylium* (*Stemphylium botriosum*) was found only in the rhizosphere of sunflower. It is interesting to note that the greatest number of melanin-containing fungi among four oil crops such as rape, linseed, castor and sunflower was found just in the sunflower (Kostyuchenko, 2010).

Dynamics of genus composition

Dynamics study of composition of the isolated genera during plant vegetation revealed the differences between the complexes of the micromycetes of the soil of background soil and the soils of agrocenosis. The most stable by the number of genera was mycocenosis of natural biocenosis, where genus diversity practically did not change during the growing season, whereas in the rhizosphere of sunflower a fluctuations in this indicator during spring and autumn were observed (Figures 1–3).

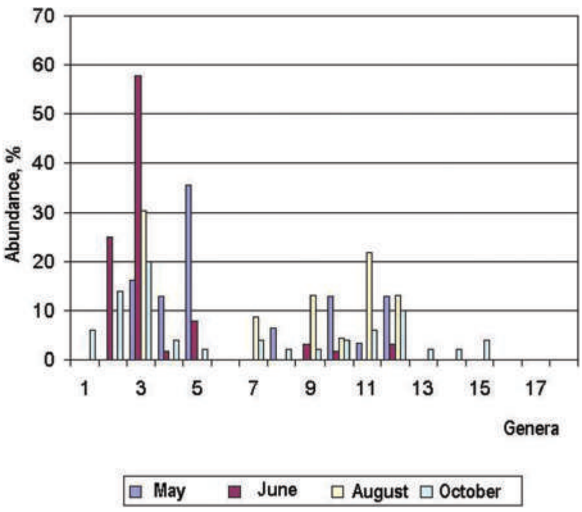

Figure 1: The dynamics of genus abundance of micromycetes isolated from background soil during the growing season: 1 – *Mucorales*; 2 – *Aspergillus*; 3 – *Penicillium*; 4 – *Paecilomyces*; 5 – *Trichoderma*; 6 – *Metarrhizium*; 7 – *Acremonium*; 8 – *Cephalosporium*; 9 – *Verticillium*; 10 – *Fusarium*; 11 – *Alternaria*; 12 – *Cladosporium*; 13 – *Stemphylium*; 14 – *Curvularia*; 15 – *Doratomyces*; 16 – *Gonytrichum*; 17 – *Botrytis*; 18 – other genera.

The most diverse genus composition of micromycetes isolated from the soil of agrocenosis in spring was in the soil of a black vapor (8 genera), less diverse – sunflower (6 genera). Representatives of the genus *Penicillium* dominated, but the species spectrum of the fungi of this genus was narrower than in other periods and was represented by only 4 species. Representatives of the genus *Aspergillus* were found only in the soil of the black vapor.

Micromycetes of the genera *Aspergillus, Cladosporium, Penicillium, Trichoderma, Vertitsillium* were permanent representatives as in a black vapor soil and rhizosphere of sunflower in summer.

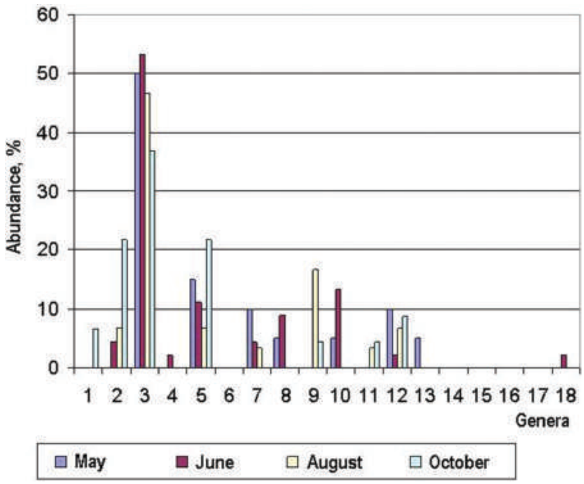

Figure 2: The dynamics of genus abundance of micromycetes isolated from black vapor soil during the growing season: 1 – *Mucorales*; 2 – *Aspergillus*; 3 – *Penicillium*; 4 – *Paecilomyces*; 5 – *Trichoderma*; 6 – *Metarrhizium*; 7 – *Acremonium*; 8 – *Cephalosporium*; 9 – *Verticillium*; 10 – *Fusarium*; 11 – *Alternaria*; 12 – *Cladosporium*; 13 – *Stemphylium*; 14 – *Curvularia*; 15 – *Doratomyces*; 16 – *Gonytrichum*; 17 – *Botrytis*; 18 – other genera.

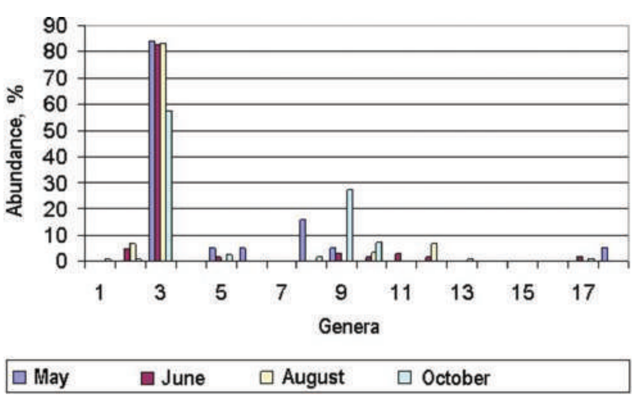

Figure 3: The dynamics of genus abundance of micromycetes isolated from root zone of sunflower during the growing season: 1 – *Mucorales*; 2 – *Aspergillus*; 3 – *Penicillium*; 4 – *Paecilomyces*; 5 – *Trichoderma*; 6 – *Metarrhizium*; 7 – *Acremonium*; 8 – *Cephalosporium*; 9 – *Verticillium*; 10 – *Fusarium*; 11 – *Alternaria*; 12 – *Cladosporium*; 13 – *Stemphylium*; 14 – *Curvularia*; 15 – *Doratomyces*; 16 – *Gonytrichum*; 17 – *Botrytis*; 18 – other genera.

At the beginning of the growing season in sunflower rhizosphere the species of the genera *Acremonium, Fusarium, Verticillium*, as well as saprotrophic saccharolytic micromycetes of the genera *Mucor, Rhizopus* and some fungi of the genus *Penicillium* were isolated. In summer, micromycetes with amylolytic and celluloseolytic activity of the genera *Aspergillus, Penicillium, Trichoderma, Fusarium, Cladosporium* were observed.

It should be noted that in summer in the soil of agrocenosis the number of species of genus *Fusarium* decreased. Representatives of this genus were derived only from sunflower rhizosphere. Micromycetes of the genus *Trichoderma* (*T. koningi, T. hamatum*) we revealed consistently throughout the growing season. It is known that this species of micromycetes is characterized by rapid growth and pectinolytic and chitin-destroying activities. In addition, volatile compounds, secreted by these fungi, are able to inhibit the growth of some types of fungi of the genus *Fusarium* (*F. oxysporum, F. solani, F. gibbosum*), and the species of the genus *Verticillium* and *Alternaria alternata* (Lugauskas *et al*., 2006).

Species richness of micromycetes and their dynamics

Both in the soil of the natural biocenosis and in the soil of the agrocenosis, the species of genera *Aspergillus* and *Penicillium* predominated in quantity, the proportion of which was 40–56 % of the total number of fungi isolated.

Background soil

Species richness of micromycetes isolated from soil of natural biocenosis, included 34 species belonging to 14 genera. Especially in summer, the micromycetes of *Penicillium* genus, abundance of which was 30.4–57.8 % were the most numerous. *Penicillium camembertii* (abundance 26.6 %), *P. thomii* (21.7 %) and *P. citrinum* (6.3–9.7 %) dominated in the cenosis. Often in this soil, *P. canescens* and *P. notatum* were identified. Much less often and, mainly in spring and autumn, we revealed *P. nigricans, P. purpurogenum*, who are permanent residents and the dominant species in the rhizosphere of sunflower. In addition to these species, *Trichoderma koningi* dominated in spring, and *Paecilomyces lilacinus, Penicillium citrinum, Fusarium graminearum* were typical common species (Figure 4).

Micromycetes of the genus *Aspergillus* were isolated quite often and in summer were represented by *Aspergillus melleus* species, *A. ochraceus*, and in

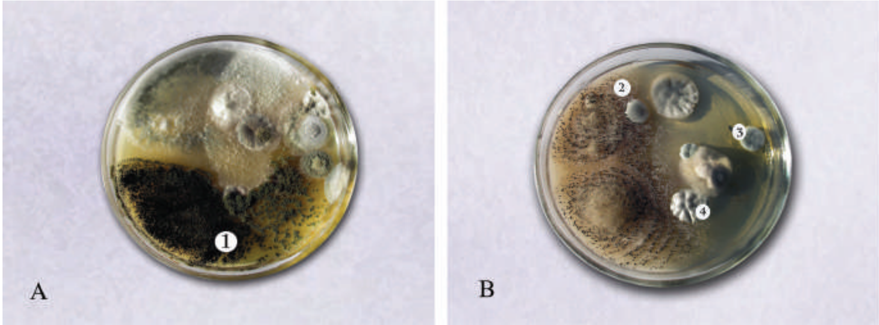

Figure 4: Typical micromycetes isolated from the background soil: A – 1 – *Trichoderma viride*. B – 2 – *Aspergillus alliaceus*; 3 – *Penicillium thomii*; 4 – *Paecilomyces* sp.

autumn – by *A. alliaceus*. Frequency of occurrence of other members of this genus (*A. ungius, A. candidus* and *A. niger*) in this soil was low.

The species composition of the genus *Fusarium* was less varied and was presented by *Fusarium oxysporum, F. lateritium, F. expansum, F. graminearum, F. semitectum*. Among the selected micromycetes of the genus *Trichoderma, Trichoderma koningi* and *T. viride* were more often distinguished than other species. Permanent representatives of micromycete complex of natural biocenosis were representatives of the family *Dematiaceae*: micromycetes of the genera *Cladosporium* (*C. cladosporioides, C. herbarum*), *Alternaria* (*Alternaria alternata*), *Curvularia* (*Curvularia* sp.) and *Stemphylium* (*Stemphylium botriosum*).

Black vapor soil

In the soil of black vapor the species diversity of micromycetes was slightly lower than in soil of natural biocenosis (11 genera, 30 species). The genus *Penicillium* (9 species) was the most diverse. Its abundance in different periods was 37.0–50.0 %. The complex of dominant species in summer formed *Penicillium thomii, P. nigricans, P. canescens, Trichoderma koningi, Verticillium* sp. (Figure 5). In autumn, species range expansion was observed due to genera *Paecilomyces, Aspergillus, Verticillium* and *Metarrhizium* but the frequency of occurrence of these and other species of fungi was not more than 20–40 %. In summer, *P. thomii* and *Penicillium crustosum* were the most abundant species and in autumn – *P. canescens* and *P. purpurescens*.

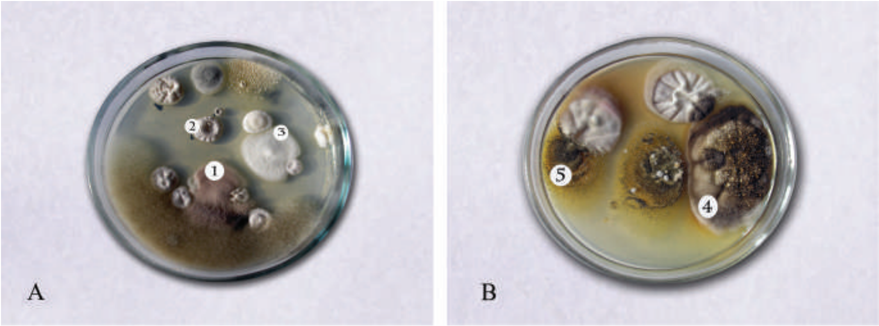

Figure 5: Typical micromycetes isolated from the black vapor soil: A – 1 – *Paecilomyces lilacinus*; 2 – *Paecilomyces* sp; 3 – *Verticillium* sp. B – 4 – *Aspergillus melleus*; 5 – *Metarrhizium* sp.

Sunflower rhizosphere

In the rhizosphere of sunflower 32 species from 13 genera were revealed. In all the terms of the study the genus *Penicillium* was the most diverse one (13 species). The species *Penicillium purpurogenum* dominated in cenosis with sufficiently high abundance (16.7–26.3 %). During flowering and head maturation *Penicillium notatum* and *P. thomii* were the most often isolated species. *P. citrinum, P. canescens, P. purpurescens, P. variabile* and *P. simplicissimum*, which are typical of this crop, were less abundant species (Figure 6).

The genus *Aspergillus* was represented by *A. melleus, A. ungius*, and *A. niveus* species, which were isolated from only sunflower rhizosphere.

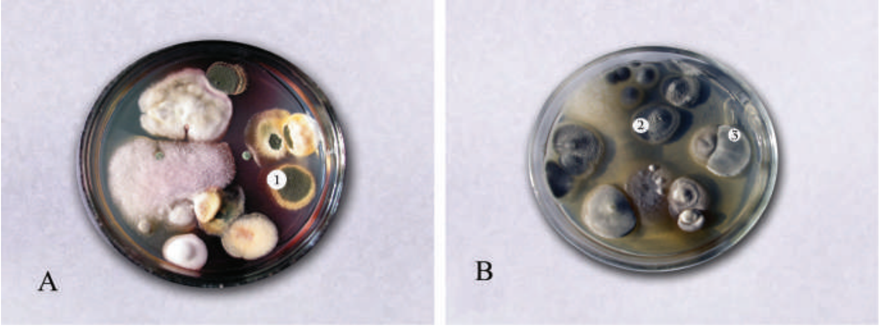

Figure 6: Typical micromycetes isolated from the root zone of sunflower: A – 1 – *Penicillium purpurogenum*. B – 2 – *Penicillium nigricans*; 3 – *Penicillium thomii*.

Species of the genus *Trichoderma* included *T. koningi, T. hamatum* and *T. viride* – typical frequent species in the rhizosphere of other oilseeds.

In autumn, species range expansion of *Penicillium* genus as well as other genera was observed. The genus *Verticillium* was abundant (27.4 %), mainly due to *Verticillium album*.

At root zone of sunflower mycocenosis, in which the species *Botrytis cinerea, Paecilomyces variotii, Rhizopus nigricans, Trichoderma viride* predominated, the species *Gliocladium roseum, Fusarium moniliforme var. subglutinans, F. oxysporum* var. *orthoceras, Penicillium nigricans* were constantly observed. At the end of the growing season, the proportion of *Penicillium nigricans*, which is typical of sunflower root zone, is considerably increased.

The phytopathogenic complex of the root zone in the phases of flowering and head formation formed species of the genera *Acremonium* and *Fusarium* (*F. moniliforme* var. *lactis, F. monoliforme* var. *subglutinans, F. oxysporum* var. *orthoceras, F. sambucinum* var. *sublunatum*). In the maturation phase, not only the species composition of this complex but also the dominant species changed due to the micromycetes *Alternaria alternata, Botrytis cinerea, Gliocladium roseum, Rhizopus nigricans*, which were not revealed at the early stages of plant development, and *F. sambucinum* var. *sublunatum*, whose specific density exceeded the summer period.

The similarity of micromycete complexes

As a result of comparing the lists of microscopic fungi species based on the calculated Sorensen similarity coefficients it was established that the most similar were the micromycete complexes of the background soil and the black vapor, where 23 species of fungi were common (Sorensen index is 0.72). In the rhizosphere of sunflower a very specific mycocenosis was formed that differ in species composition from a background soil and a black vapor (Sorensen index = 0.61–0.65) (Table 3). 10 species of micromycetes were common species that were revealed both in the soils of the natural biocenosis and the black vapor, and under the sunflower: *Alternaria alternata, Aspergillus candidus, Cladosporium herbarum, Penicillium canescens, P. citrinum, P. purpurogenum, P. thomii, P. nigricans, Verticillium album, V. terrestre*.

Thus, as a result of the conducted studies, it was established that the mycocenosis formed in the rhizosphere of sunflower differs from the mycocenosis of the natural biocenosis. The general pattern is the predominance of representatives of the genus *Penicillium* in terms of the number of species. At the same time, there was a different participation of representatives of other

Table 3: Sorensen similarity coefficients in micromycete complexes derived from background soil, black vapor soil and root zone of sunflower.

Soil	Number of species	Sorensen index		
		Background	Black vapor	Root zone of sunflower
Background	34	x		
Black vapor	30	0.72	x	
Root zone of sunflower	32	0.61	0.65	x

genera in the soils of agrocenosis and background soil. In general, it should be noted that the micromycete complexes of natural biocenosis and black vapor remained the most stable throughout the time of the research. The genus variety of mycocenosis in the root zone of sunflower changed significantly in different phases of plant development. It is characteristic that the change in the structure of the fungal complex in the rhizosphere of sunflower occurred due to an increase in the proportion of phytopathogenic species belonging to the genera *Alternaria, Cladosporium, Fusarium* and *Verticillium*.

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