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Diversity of Fungi in Rhizoplane, Rhizosphere and Edaphosphere of Sunflower at Different Stages of its Development

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Abstract: The genus and species composition of the micromycete complex in the root zone (rhizoplane, rhizosphere and edaphosphere) of sunflower at the beginning of flowering and full maturity stages during its cultivation on leached chernozem was studied. It was established that representatives of the genera *Aspergillus, Botrytis, Gliocladium, Fusarium, Paecilomyces, Penicillium, Rhizopus* and *Trichoderma* formed the complex of typical fungi of the rhizoplane and rhizosphere. At the stage of flowering in the mycocenosis, the species *Paecilomyces lilacinus, Paecilomyces variotii* and *Trichoderma viride* dominated, and in the maturity stage *Rhizopus nigricans, Penicillium nigricans, Botrytis cinerea* and *Fusarium moniliforme* var. *subglutinans* prevailed. The phytopathogenic complex at the flowering stage formed the species of the genera *Fusarium* and *Rhizopus*, and by the end of the vegetation the variety of potential pathogens was expanded by representatives of the genera *Alternaria, Botrytis* and *Gliocladium*. A comparative analysis of the micromycete complex revealed a similarity of the dominant species of the rhizoplane and rhizosphere in different stages of sunflower development. However, by the end of the vegetation in the rhizosphere, compared to rhizoplane, the abundance of fungi of the species *Fusarium moniliforme* var. *subglutinans* and *Penicillium nigricans* increased significantly. As for edaphosphere, *Rhizopus nigricans, Trichoderma viride* and *Penicillium nigricans* dominated during sunflower flowering, and by the end of the crop vegetation the number of micromycetes of the genus *Fusarium* was reduced while the proportion of micromycetes of the genus *Rhizopus* increased significantly.

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Résumé: Le genre et espèces composition du complexe de micromycètes dans la zone racinaire (rhizoplane, rhizosphère et édaphosphère) du tournesol au début de la floraison et à pleine maturité au cours de sa culture sur le chernozem lessivé ont été étudiés. Il a été établi que les représentants des genres *Aspergillus, Botrytis, Gliocladium, Fusarium, Paecilomyces, Pénicillium, Rhizopus* et *Trichoderma* formaient le complexe de hongos typiques du rhizoplane et de la rhizosphère. Au stade de la floraison dans la mycocénose, les espèces *Paecilomyces lilacinus, Paecilomyces variotii* et *Trichoderma viride* ont dominé, et au stade de maturité *Rhizopus nigricans, Penicillium nigricans, Botrytis cinerea* et *Fusarium moniliforme* var. *subglutinans* ont prévalu. Le complexe phytopathogène au stade de la floraison a formé les espèces des genres *Fusarium* et *Rhizopus*, et à la fin de la végétation, la variété des pathogènes potentiels a été élargie par des représentants des genres *Alternaria, Botrytis* et *Gliocladium*. Une analyse comparative du complexe de micromycètes a révélé une similitude entre les espèces dominantes du rhizoplane et de la rhizosphère à différents stades du développement du tournesol. Cependant, à la fin de la végétation dans la rhizosphère, par rapport aux rhizoplans, l'abondance des hongos de l'espèce *Fusarium moniliforme* var. *subglutinans* et *Penicillium nigricans* ont augmenté de façon significative. En ce qui concerne l'édaphosphère, *Rhizopus nigricans, Trichoderma viride* et *Penicillium nigricans* dominaient pendant la floraison du tournesol, et à la fin de la végétation, le nombre de micromycètes du genre *Fusarium* était réduit tandis que la proportion de micromycètes du genre *Rhizopus* augmentait significativement.

Resumen: Se estudió la composición de género y especies del complejo de micromicetos en la zona de la raíz (rizoplano, rizosfera y edafósfera) de girasol en el inicio de la floración y las etapas de madurez completa durante su cultivo en chernozem lixiviado. Se estableció que representantes de los géneros *Aspergillus, Botrytis, Gliocladium, Fusarium, Paecilomyces, Penicillium, Rhizopus* y *Trichoderma* formaron el complejo de hongos típicos del rizoplano y la rizosfera. En la etapa de floración en la micocenosis, predominaron las especies *Paecilomyces lilacinus, Paecilomyces variotii* y *Trichoderma viride*, y en la etapa de madurez *Rhizopus nigricans, Penicillium nigricans, Botrytis cinerea* y *Fusarium moniliforme* var. *subglutinans* prevalecieron. El complejo fitopatógeno en la etapa de floración formó las especies de los géneros *Fusarium* y *Rhizopus*, y al final de la vegetación la variedad de patógenos potenciales se expandió por representantes de los géneros *Alternaria, Botrytis* y *Gliocladium*. Un análisis comparativo del complejo de micromicetos reveló una similitud de las especies dominantes del rizoplano y la rizosfera en diferentes etapas del desarrollo del girasol. Sin embargo, al final de la vegetación en la rizosfera, en comparación con el rizoplano, la abundancia de hongos de la especie *Fusarium moniliforme* var. *subglutinans* y *Penicillium nigricans* aumentaron significativamente. En cuanto a la edafosfera, *Rhizopus nigricans, Trichoderma viride* y *Penicillium nigricans* dominaron durante la floración del girasol, y al final de la vegetación del cultivo se redujo el número de micromicetos del género *Fusarium* mientras que la proporción de micromicetos del género *Rhizopus* aumentó significativamente.

Keywords: *Helianthus annuus* L., micromycete complex, genus and species abundance, beginning of flowering, full maturity

Introduction

Sunflower is an important crop in the agrocenoses of the south of Ukraine. The areas under the sunflower are constantly increasing, leading to a crop rotations' distortion. The distortion of agricultural techniques of cultivation leads to a significant decrease in yield and deterioration in the quality of products, primarily due to the diseases. It is well known that a large number of species of pathogens of various origins are parasitic on sunflower. The most numerous are fungal diseases, the number of which is constantly growing (Gulya *et al.*, 1997; Leite, 2014).

It is also known that metabolites of soil micromycetes can have a negative effect on the soil, which leads to a decrease in the productivity of agricultural crops. Toxins and antibiotics produced by fungi can cause soil fatigue, which, first of all, affects the yield of plants. Fungal toxins and antibiotics, together with toxic substances released by plants, high doses of mineral fertilizers, can also reduce the biological activity of the soil (Svistova *et al.*, 2004).

Considering the foregoing, it is important to elucidate the direction of microbiological processes in the soil, and, in particular, the peculiarities of the formation of the micromycete complex, when sunflower is grown in agrocenoses (Belyuchenko, 2016; Maslienko, 2005).

A number of literature sources provide reviews of the results of studying the microbial complex of the root zone of different plant species. In most of them, a quantitative analysis of microscopic organisms in the rhizosphere and rhizoplane in a plant is given (Orazova *et al.*, 1999; Polyanskaya *et al.*, 1994). However, there are not enough data in them, except for legumes and cereals, on the species composition of the microbial complex, in particular micromycetes, which inhabit the rhizosphere and especially the rhizoplane in crops, including sunflower. But it is in the rhizoplane of the plant that the closest trophic and topical interrelations are formed.

The purpose of this work was to study the genus and species structure of the micromycete complex of the rhizoplane and rhizosphere in sunflower at the beginning of flowering and physiological maturity stages when it was grown on ordinary leached chernozem in comparison with the edaphosphere's mycocenosis.

Materials and Methods

The research was performed in 9-field 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 edaphosphere, rhizosphere and rhizoplane of a sunflower hybrid Zaporozhsky-32 created at IOC NAAS was investigated. Predecessor of sunflower was winter barley. Samples of soil (20–25 combined samples, on an area of 25 m × 25 m) were selected at the beginning of flowering (June) and full maturity (October) stages of sunflower development (Schneiter and Miller, 1981). Isolation and cultivation of micromycetes was carried out on the Czapek's medium according to standard methods (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 (Bilaj and Kurbatskaya, 1990; Domsh *et al.*, 1993; Samson and Frisvad, 2004; Satton *et al.*, 2001; Watanabe, 2002). The assistance in species identification of some microscopic soil fungi was also provided by specialists of the phytopathology department of the Plant Breeding and Genetics Institute – National Center of Seed and Cultivar Investigation (Odesa, Ukraine).

The structure of the micromycete complex was characterized by the genus and species abundance (%) (Mirchink, 1988).

Results and Discussion

During the period of research from the root zone of sunflower (rhizoplane, rhizosphere and edaphospere), we identified 32 species of microscopic fungi (12 genera) that belong to *Zygomycota* (2) and *Deuteromycota* (30). Of these, 28 species of micromycetes were isolated in a pure culture and identified. Analysis of the genus composition of microscopic fungi isolates showed that the vast majority of them belong to the genera *Aspergillus, Botrytis, Gliocladium, Fusarium, Paecilomyces, Penicillium, Rhizopus* and *Trichoderma*. The number of micromycetes of the genera *Acremonium, Alternaria, Metarrhizium* and *Mucor* was significantly less (Table 1).

It was established that in the rhizoplane and rhizosphere of sunflower a micromycete complex was formed, in which the fungi of the species *Botrytis cinerea, Paecilomyces variotii, Rhizopus nigricans* and *Trichoderma viride* dominated. *Gliocladium roseum, Fusarium moniliforme* var. *subglutinans, F. oxysporum* and *Penicillium nigricans* were constantly found. The proportion of *Penicillium nigricans*, a typical species for the root zone of sunflower, increased significantly at the end of vegetation (Table 1).

Certain differences were found in the composition and abundance of micromycete species on the surface of the roots (rhizoplane) and in the rhizosphere of plants. It was established that at the flowering stage on the roots and in the rhizosphere *Paecilomyces lilacinus* (abundance 46.5 and 22.9 %, respectively) and *Trichoderma viride* (abundance 10.7 and 18.1 %) dominated. The species *Acremonium roseum* and *Aspergillus* sp. were characteristic for sunflower rhizoplane during this period, whereas for the rhizosphere – *Paecilomyces variotii, Fusarium moniliforme* var. *lactis, F. oxysporum* and *Rhizopus nigricans*. At the maturity stage in the rhizoplane, compared to the rhizosphere, the abundance of *Alternaria alternata, Botrytis cinerea, Rhizopus nigricans, Gliocladium roseum* and *Penicillium steckii* was higher, while the abundance of *Fusarium moniliforme* var. *subglutinans* and *Penicillium nigricans* was more in the rhizosphere soil at 1.6 and 2.7 times, respectively (Table 1, Figure 1, 2).

At the flowering stage, the phytopathogenic complex of the root zone formed mainly species of the genus *Fusarium*. These species were found both in the rhizoplane and in the rhizosphere. In the rhizosphere during this period, micromycetes of the genus *Rhizopus* were also detected. At the maturity stage, the variety of potential phytopathogens was expanded by micromycetes of the genera *Alternaria, Botrytis* and *Gliocladium*. Both in the rhizoplane and in the rhizosphere, the abundance of species of the genus *Fusarium* increased significantly. This is especially true of the *species F. moniliforme* var. *subglutinans*, the abundance of which in the rhizosphere exceeded the summer period by 7.5 times. It should also be noted that by the end of the sunflower growing season, the abundance of potential phytopathogens in the rhizoplane was significantly higher than in the rhizosphere. Thus, this excess for species of *Botrytis cinerea, Rhizopus nigricans* and *Gliocladium roseum* was 2.8, 1.3 and 7.5 times. In total, the proportion of phytopathogens in the rhizoplane was more than 70 %, which

Table 1: The genus and species structure of the micromycete complex in rhizoplane, rhizosphere and edaphosphere of sunflower at different stages of its development.

№	Species	Species abundance, %					
		beginning of flowering			full maturity		
		rhizoplane	rhizosphere	edaphosphere	rhizoplane	rhizosphere	edaphosphere
Zygomycota, Zygomycetes, Mucorales							
Mucoraceae							
1	*Mucor racemosus*	-	4.8	-	-	-	-
2	*Rhizopus nigricans*	-	12.8	14.9	21.1	13.7	38.3
Deuteromycota, Hyphomycetes, Hyphomycetales							
Moniliaceae							
3	*Acremonium roseum*	3.6	-	-	-	-	-
4	*Aspergillus alliaceus*	-	-	10.5	-	2.4	-
5	*A. candidus*	-	-	-	-	0.6	4.9
6	*A. melleus*	-	-	9.7	-	4.2	1.2
7	*A. niger*	-	-	-	-	0.6	-
8	*A. ochraceus*	-	-	-	-	-	4.9
9	*Aspergillus sp*	1.8	-	1.5	-	-	2.5
10	*Botrytis cinerea*	-	-	2.3	22.8	8.3	-
11	*Gliocladium roseum*	-	-	1.5	8.8	1.2	1.2
12	*Metarrhizium anisoplie*	-	-	-	-	-	6.2
13	*Paecilomyces lilacinus*	46.5	22.9	-	3.5	-	1.2
14	*P. variotii*	-	13.3	3.0	-	-	-
15	*Penicillium canescens*	-	-	-	-	-	2.5
16	*P. citrinum*	-	2.4	5.2	-	-	6.2
17	*P. melinii*	-	2.4	-	-	-	-

(*continued*)

Table 1: (*continued*)

№	Species	Species abundance, %					
		beginning of flowering			full maturity		
		rhizoplane	**rhizosphere**	**edaphosphere**	**rhizoplane**	**rhizosphere**	**edaphosphere**
18	*P. nigricans*	6.2	-	20.2	10.5	29.2	7.4
19	*P. purpurogenum*	-	-	-	-	-	1.2
20	*P. steckii*	-	-	-	12.3	4.8	-
21	*Trichoderma koningii*	7.1	8.4	-	-	-	2.5
22	*T. viride*	10.7	18.1	20.2	-	-	11.1
Dematiaceae							
23	*Alternaria alternata*	-	-	-	1.8	0.6	-
Tuberculariales							
Tuberculariaceae							
24	*Fusarium lateritium*	-	-	-	-	3.6	-
25	*F. moniliforme var. lactis*	-	2.4	-	-	-	-
26	*F. moniliforme var. subglutinans*	8.9	2.4	1.5	10.5	17.9	-
27	*F. oxysporum*	-	9.6	9.0	8.8	6.0	2.5
28	*F. sambucinum var. sublunatum*	-	2.4	-	-	-	-
	Other species	5.4	8.4	-	-	7.1	6.2
	Total species	*9*	*14*	*12*	*9*	*14*	*18*
	Total genera	*6*	*7*	*8*	*6*	*8*	*9*

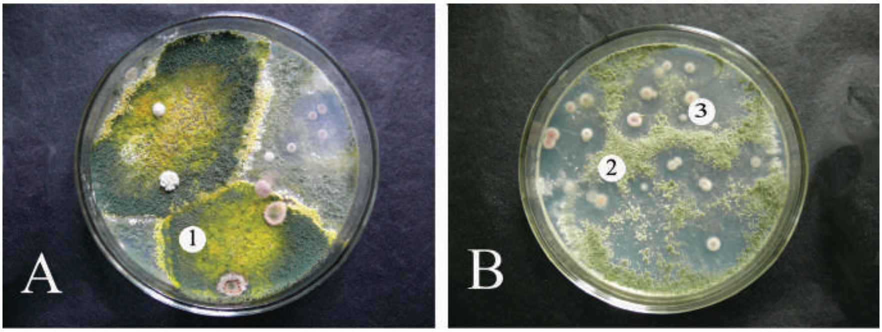

Figure 1: Typical micromycetes isolated from sunflower rhizoplane: A – 1 - *Trichoderma viride*. B – 2 - *Trichoderma koningii*; 3 - *Paecilomyces lilacinus*.

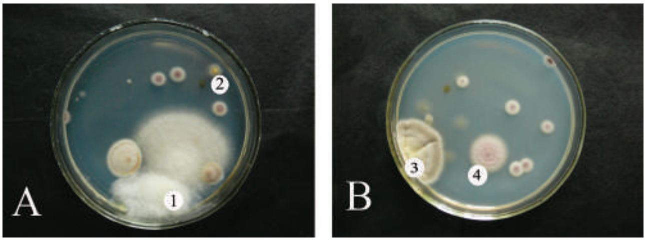

Figure 2: Typical micromycetes isolated from sunflower rhizosphere: A – 1 - *Fusarium oxysporum*; 2 - *Aspergillus alliaceus*. B – 3 - *Aspergillus melleus*; 4 - *Paecilomyces lilacinus*.

is significantly higher than in the rhizosphere and especially in the edaphosphere.

The edaphosphere was characterized by a much larger genus and species diversity of micromycetes than the rhizoplane and sunflower rhizosphere, especially at the end of its vegetation. For example, only in autumn in the edaphosphere the fungi of the genus *Metarrhizium* were found. Their appearance at the end of the season, as well as the expansion of the entire species spectrum of micromycetes, was noted by us in the black vapor soil (Kostyuchenko and Lyakh, 2017). At the same time, in the edaphosphere, in comparison with the rhizoplane and the rhizosphere of sunflower, the number of fungi of the genus *Fusarium* was reduced and the proportion of micromycetes of the genus *Trichoderma* increased.

Figure 3 represents the ratio of the genus abundance of micromycetes isolated from the rhizoplane, rhizosphere and the edaphosphere of sunflower at the end of its vegetation (maturity stage). In addition to the fact that the whole variety of the micromycete complex of the rhizoplane was represented by only 6

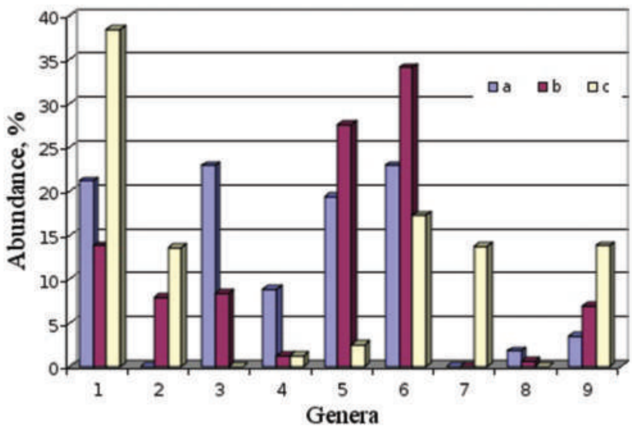

Figure 3: The genus abundance of micromycetes isolated from rhizoplane (a), rhizosphere (b) and edaphosphere (c) of sunflower at the end of its vegetation: 1 - *Mucorales*, 2 - *Aspergillus*, 3 - *Botrytis*, 4 - *Gliocladium*; 5 - *Fusarium;* 6 - *Penicillium*, 7 - *Trichoderma*, 8 - *Dematiaceae;* 9 - other genera.

genera, in contrast to the rhizosphere (8 genera) and the edaphosphere (9 genera), four of them – the fungi of the genera *Penicillium, Mucor, Botrytis* and *Fusarium* – were characterized by a high and almost equal level of abundance (about 20 %). The micromycetes of the last two genera are phytopathogenic species and in total constitute about 50.1 % of fungi isolated in the rhizoplane. In the rhizosphere, fungi of the genus *Penicillium* (abundance 34 %) prevailed, as well as phytopathogenic fungi of the genus *Fusarium* (abundance 27.5 %). In the edaphosphere, there was the maximum abundance of fungi of the genus *Mucor* (mainly due to the species *Rhizopus nigricans*), while the proportion of phytopathogenic fungi of the genus *Fusarium*, in comparison with the rhizoplane and rhizosphere, was respectively 7.7 and 11.0 times less. In contrast to the rhizoplane and rhizosphere, where no fungi of the genus *Trichoderma* were found, in the edaphosphere the fungi of this genus were abundant enough.

It is believed that fungi of the genus *Fusarium* play a leading role in the damage of the root system of cultivated plants. Their species composition is to a certain extent determined not only by the type of vegetation of phyto- and agrocenoses. It also depends on temperature, humidity, soil type (De Cal *et al.*, 2000; Magan *et al.*, 2002; Whipps, 2001). Thus, *Fusarium oxysporum* predominates in podzolized chernozem. This species, along with *F. moniliforme* var. *subglutinans*, in our studies was the predominant species of the genus *Fusarium* both in the rhizoplane and in the rhizosphere of sunflower at the end of its vegetation.

As is known, micromycetes of the genus *Trichoderma* can suppress the development of fungi of phytopathogenic genera, including such as *Fusarium* and *Alternaria* (Lugauskas *et al*., 2006). The ratio of suppressor fungi and phytopathogenic fungi indicates the nature of the interaction of micromycetes in the soil and, in general, the state of microbiota development (Frunze, 2018). In this connection, it is of interest to analyze the ratios of the fungi of the genera *Fusarium* and *Trichoderma* in the rhizoplane, rhizosphere and edaphosphere at the beginning and the end of the growing season of sunflower. It can be seen, that at the beginning of flowering in the rhizoplane and the rhizosphere of sunflower the spesies abundance of micromycetes of the genus *Trichoderma* significantly exceeded the abundance of fungi of the genus *Fusarium*. By the end of vegetation, the suppressor fungi were not detected at all in the micromycete complex, whereas the proportion of the phytopathogenic genus increased compared to the beginning of flowering. In addition, at the end of vegetation, representatives of another phytopathogenic genus, *Alternaria*, appeared in the rhizoplane and rhizosphere. As for edaphosphere, both in June and October, there was a significant excess in the abundance of fungi of the genus *Trichoderma* over the phytopathogenic genus *Fusarium*. A possible cause of this may be the composition of root exudates of plants from different root zones, which is known to determine the ratio of phytopathogens and their antagonists (Costa *et al*., 2006).

Summarizing the obtained data, it should be noted that the sunflower rhizoplane was characterized by a much smaller species and generic variety of microscopic fungi in comparison with the rhizosphere and edaphosphere. By the end of vegetation in the edaphosphere of crop, there was a tendency to increase the diversity of micromycetes. As for the fungi phytopathogenic complex, in the rhizoplane and rhizosphere of sunflower at the stage of full maturation in comparison with the beginning of flowering, it was represented by a large generic and species diversity of micromycetes. By the end of the vegetation in this complex, representatives of the genera *Botrytis, Fusarium* and *Rhizopus* dominated. In the edaphosphere in this respect only fungi of the genus *Rhizopus* were isolated.

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