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ORIGINAL ARTICLE

Mycobiome of sunflower rhizosphere in organic farming

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Physiologically active substances released from the roots of cultivated plants can significantly affect the structure and functioning of the fungal coenosis in the rhizosphere soil. Rhizosphere soil is one of the essential participants in the allelopathic interaction of plants and soil biota, especially in the agrocenosis of sunflower plants, which is characterized by significant allelopathic potential. Studies to determine the number of micromycetes in the rhizosphere soil of sunflower plants hybrids Dushko and Oliver were conducted during 2018-2020, using generally accepted methods prescribed in DSTU 4287:2004 (National Standard of Ukraine). The impact assessment of sunflower hybrid plants and their cultivation technologies on the number and species composition of micromycetes in the rhizosphere soil is presented. It is established that in the conditions of both organic and traditional technologies of sunflower cultivation, the number of micromycetes differs depending on the hybrid, which indicates a significant effect of root secretions on the micromycetes population in the rhizosphere soil. In the conditions of traditional technology, the number of micromycetes in rhizosphere soil ranged from 5.1 to 6.3 thousand CFU/g of dry soil, and in the conditions of organic technology - from 5.4 to 6.7 thousand CFU/g of dry soil. It is noted that the sunflower hybrid root exometabolites and technologies of their cultivation put significant pressure on the number and species of micromycetes biodiversity in the rhizosphere soil. The species composition of micromycetes in the rhizosphere soil of sunflower plants was determined, and it was found that fungi of the genera *Aspergillus* spp., *Alternaria* spp., *Penicillium* spp., and *Fusarium* spp., are dominant, which are characterized by the different frequency of occurrence, which ranged from 25 to 80%. These micromycetes cause significant damage to sunflower crops and increase the level of biological contamination of agrocenoses during plants' growing season. We suggested that under organic and traditional technologies of sunflower cultivation, the number and species composition of micromycetes depended on the hybrids and technology of their cultivation.
Keywords: micromycetes, phytopathogens, CFU, rhizosphere, sunflower, biological pollution of ecosystems, agro biocenosis.

Introduction

The rhizosphere soil of plants is a dynamic multi-component ecosystem in which beneficial, neutral, and harmful microorganisms actively function (Ellanska et al., 2017). Cultivated plants are in constant interaction with symbiotic and pathogenic microorganisms in soil biota (Broeckling et al., 2008). Microbial cenosis plays a vital role in the plant communities' functioning, influencing their growth, development, and physiological parameters. The vast majority of soil microbiota increases soil fertility and crop productivity. Pathogenic microorganisms colonizing the rhizosphere soil lead to diseases of cultivated plants (Mendes et al., 2013). Crop yield losses only from root rot caused by phytopathogenic fungi are 5-15%. However, they can reach a much higher level (40-70%) (Antonyak et al., 2013). Thus, the rhizosphere soil of cultivated plants is the most critical factor that regulates the phytosanitary condition of agrocenoses and determines the infectious potential of the soil-plant-pathogen-microbiota system (Kopylov, 2012).
Continuous oilseeds cultivation for a long time leads to the rhizosphere soil microbiome impoverishment in agro phytocoenoses due to reduced species microbial biodiversity. With the help of the literature analysis, it was established that due to the increase in phytopathogenic micromycetes number, the infectious load on other crops in crop rotation increases (Petrenkova, 2014). This significantly affects the processes that occur in the rhizosphere and the tissues of host plants and inhibits their growth and development, reducing yields (Kirichenko et al., 2010; Petrenkova et al., 2012). Necrotrophic phytopathogens, including fungi of the genera Aspergillus spp., Penicillium spp., Fusarium spp., Alternaria spp, the leading producers of mycotoxins that parasitize sunflower plants during the growing season (Kirichenko et al., 2010). Some stages of their development occur in the soil during interaction with host plants (Antonyak et al., 2013).
Physiologically active plant substances of different varieties and crop hybrids significantly affect the structure and functioning of microbial populations in the rhizosphere soil (Antonyak et al., 2013; Kostyuchenko, 2014; Sugiyama, 2019), where metabolites are exchanged between cultivated plants and microorganisms (Ellanska et al., 2017).
Sunflower is well known for its allelopathic potential. More than 200 natural allelopathic sunflower compounds, including heliannuols, terpenoids, flavonoids, chlorogenic acid, isochlorogenic acid, and scopolin, are characterized by inhibitory or stimulating effects against pathogenic microorganisms and segetal vegetation (Jabran, 2017).
Currently, considerable attention is paid to the study of crop interaction with the rhizosphere soil microbiome. It is known that the cultivated plant vegetative organs are a source of microbial biodiversity in the rhizosphere soil (Bruinsma et al., 2003). However, the influence mechanism of variety/hybrid on the number and microorganisms species composition has not been studied enough.
Therefore, our research was aimed at determining the influence of sunflower hybrids on the micromycetes number and species composition in rhizosphere soils in organic production.

Materials and methods

The research was conducted during 2018-2020 based on the laboratory of agroecosystems and organic production biocontrol at the Institute of Agroecology and Nature Management of NAAS, Ukraine. Samples of rhizosphere soil were taken in the fields of the Skvyra research station of organic production of Institute of Agroecology and Environmental Management NAAS of Ukraine by the envelope method on the diagonal of the field by DSTU 4287: 2004. The number of rhizosphere microorganisms of Dushko and Oliver sunflower hybrids was determined by budding, flowering, and physiological maturity. Soil type - low humus coarse-grained medium loam black soil.

The territory of Skvyra Research Station is characterized by a moderately warm, moderately humid climate, which is conducive to the growth and development of sunflower plants. It is well known that the ontogenesis of sunflower and the spread and development of diseases are significantly affected by temperature and rainfall. The integrated indicator of these factors is the hydrothermal coefficient (HHC, Selyaninov coefficient).

Sunflower grows well for an HHC of 1.0-1.5 (sufficient hydration). However, at HHC ≥1.0, the probability of culture damage by various diseases, mainly white and grey rot, increases sharply. Only specific disease development is possible for smaller hydration, except for theAlternaria, dry head rot, verticillium wilt, *Orobanche cumana* (sunflower broomrape) (Dermenko, 2017).

Table 1. The value of the hydrothermal coefficient (HHC) during the growing seasons.

Year	Month						The
	April	May	June	July	August	September	average
2018	0.3	0.9	2.6	2.9	0.1	1.3	1.35
2019	0.6	2.3	1.4	0.6	0.3	0.4	0.9
2020	1.7	1.8	0.9	0.8	0.5	0.4	1.0

Note: HHC ≥1 - sufficient hydration; HHC 0.8-1.0 - moderate hydration; HHC 0.6-0.7 - insufficient hydration.

According to the results of the calculation of HHC, presented in Table 1, we can conclude that the growing season in 2018 was characterized as sufficiently wet (HHC 1.35). At the same time, the vegetation period of 2019 was arid, the drought was not intense (HHC 0.9), and 2020 was sufficiently moist (HHC 1.0).

However, meteorological conditions during the research period, namely: high air temperature and high rainfall during the growing season, significantly impacted the formation of micromycetes in the sunflower agrocenosis.

Protection of sunflower crops from diseases was carried out according to the scheme presented in Table 2.

Table 2. Scheme of sunflower crops protection from diseases (2018-2020)

Growing technology	The remedy name	The phase of cultural development	The consumption rate
Organic technology	Avatar 2 Protection micro- and ultramicroelements (Mg, Cu, Zn, Fe, Mn, Co, Mo, La, Ni, V, Ti, Se, Ag, Si, I, B) and potassium citrate - chelated by natural di- and tricarboxylic organic acids (lemon, amber, wine, and apple)	4 – 5 pairs of original leaves; 8 – 10 pairs of original leaves;	0.1-0.2 l / ha
Traditional technology	Acanto Plus (a.s. cyproconazole 80 g/l + picoxystrobin 200 g/l)	8 – 10 pairs of original leaves; Budding — the beginning of flowering	0.5 - 1.0 l / ha

The number of micromycete populations in the rhizosphere was studied using dilution and surface sowing of soil suspension on nutrient media (DSTU 7847:2015, 2016). Chapek's medium was used for the study. The number of micromycetes was expressed in colony-forming units (CFU) in 1 g of dry soil. The number of microorganisms in 1 g of dry soil was determined by the formula (1):

$$N = \frac{a * 10^n}{V} \quad (1)$$ (DSTU 7847: 2015, 2016)

where N is the number of cells or colony-forming units (CFU) in 1 g of the studied soil;
a is the average number of colonies grown in the cup of this dilution;
n - breeding number from which the crop was made;
V- is the volume of soil suspension taken for sowing, 0.1 cm3.

The number of microorganisms in 1 g of dry soil is calculated by the formula (2):

$$x = N * K \quad (2)$$

where x is the number of cells or colony-forming units (CFU) in 1 g of dry soil;
N is the number of cells or colony-forming units (CFU) in 1 g of the studied soil;
K-conversion factor to dry soil.

For accurate calculations of the number of microorganisms in the soil, its humidity was determined in advance following DSTU ISO 11465. The count of the number of colonies of micromycetes in Petri dishes was performed using an automatic counter SCAN4000 (Interscience, France). Identification of isolates of microscopic fungi to genus and species was carried out on a biological microscope DN-200D by determinants (Litvinov, 1967; Pidoplichko, 1972), using an online database "MycoBank".

The rate of occurrence (RO) of phytopathogenic micromycetes in the rhizosphere soil of sunflower was determined by the formula (3) (Mirchyk, 1988):

$$A = \frac{B \times 100\%}{C} \quad (3)$$

Where A is the frequency of occurrence of species;
B-the number of samples in which these species were selected;
C-the total number of selected species.

Statistical processing of experimental data was performed using statistical and correlation methods of mathematical statistics using Microsoft Excel software.

Results

We revealed that the number of micromycetes in the rhizosphere is subject to significant fluctuations depending on the sunflower hybrids and the technology of their cultivation. The number of micromycetes in the rhizosphere of sunflower hybrids in the conditions of traditional technology with the use of chemical fungicides, depending on the hybrid, ranged from 5.1 to 6.3 thousand CFU/g of dry soil (Fig. 1-A). The rhizosphere soil of Dushko hybrid plants in the budding phase amounted to 5.9 thousand CFU/g of soil. In the flowering phase, there was a slight decrease (up to 5.6 thousand CFU/g of soil) in the number of micromycetes. During the physiological maturity phase of the rhizosphere plant of the Dushko hybrid, this indicator increased rapidly and reached an average of 6.3 thousand CFU/g of dry soil.

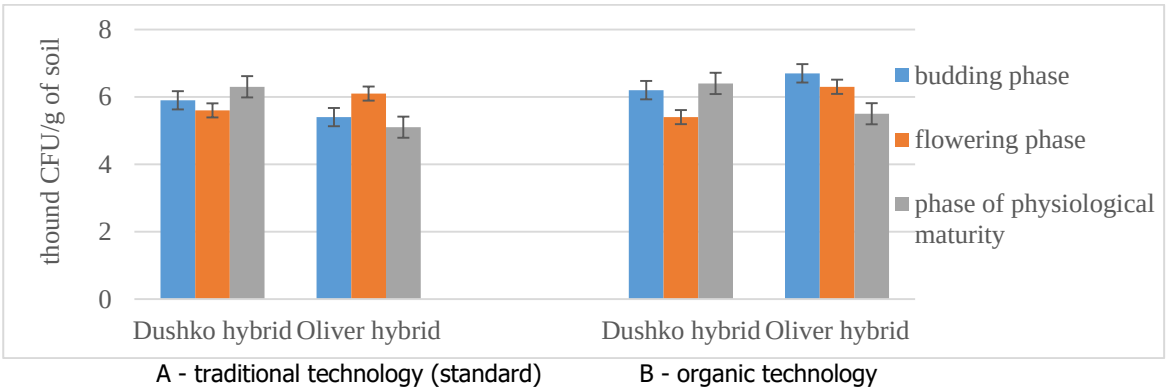


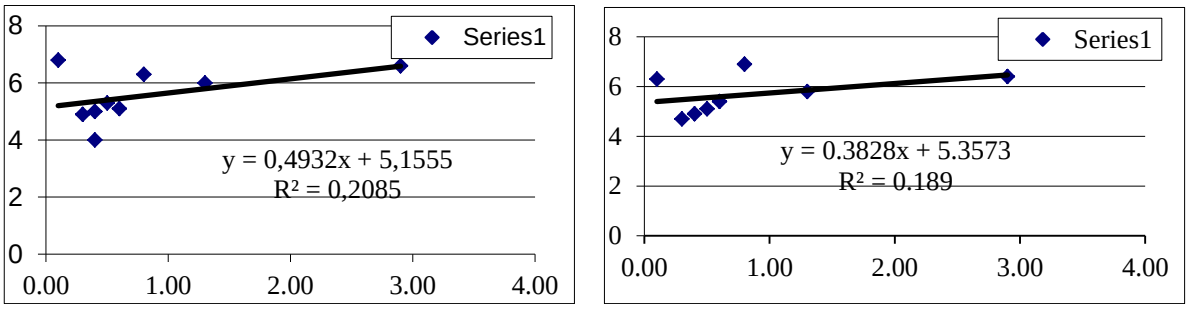
Fig. 1. The number of micromycetes in the rhizosphere soil of sunflower plants against the background of different technologies for growing crops.

The number of micromycetes in the rhizosphere soil of Oliver sunflower in the budding phase was slightly lower compared to the Dushko hybrid and amounted to 5.4 thousand CFU/g of soil. However, in the Oliver hybrid rhizosphere, in the flowering phase, the number of micromycetes increased significantly and averaged 6.1 thousand CFU/g, and in the phase of physiological maturity, it decreased rapidly and amounted to 5.1 thousand CFU/g of dry soil (Fig.1-A).
Thus, it was found that against the background of traditional technology during the plants' ontogenesis of the studied sunflower hybrids, the number of micromycetes in the rhizosphere soil varies depending on the hybrid and the phase of plant development.
During organic sunflower production against the background of biological remedies, the number of micromycetes in the rhizosphere soil of sunflower plants of the studied hybrids ranged from 5.4 to 6.7 thousand CFU/g of dry soil (Fig. 1-B). Thus, the number of CFU micromycetes in the Dushko sunflower hybrid rhizosphere soil in the budding phase was 6.2 thousand CFU/g of dry soil. Simultaneously, in the flowering phase, there was a significant decrease in the density of micromycetes (up to 5.4 thousand CFU/g). In the physiological maturity phase, there was a significant increase in the number of micromycetes in the rhizosphere of the Dushko sunflower hybrid (up to 6.4 thousand CFU/g).
A significant decrease in the number of CFU micromycetes during plant ontogenesis under organic production conditions was observed in the rhizosphere soil of Oliver sunflower hybrid. Thus, if the number of micromycetes in the rhizosphere of plants of the studied hybrid in the budding phase was 6.7 thousand CFU/g of dry soil, then in the flowering phase, the number was 0.4 thousand CFU lower. In the phase of physiological maturity, the number of micromycetes in the rhizosphere of the Oliver hybrid continued to decrease to 5.5 thousand CFU/g of dry soil (Fig. 1-B). The obtained results may indicate a significant pressure of the root exometabolites of Oliver sunflower plants on the population of micromycetes in the rhizosphere.
Simultaneously, the number of micromycetes in the rhizosphere soil of the studied hybrids was correlated with the value of HTC during the plant vegetation period for 2018-2020. (Table 3).

Table 3. Correlation indices between the number of micromycetes in the rhizosphere of the studied hybrids and the value of HTC.

	Correlation, (r)	The regression equation, (y)
	Hybrid Dushko	
Traditional technology	0.456	y = 0.4932X + 5.1555 (Fig. 2-A)
Organic technology	0.434	y = 0.3828X + 5.3573 (Fig. 2-Б)
	Hybrid Oliver	
Traditional technology	0.396	y = 0.4293X + 5.4407 (Fig. 3-A)
Organic technology	0.447	y = 0.1559X + 6.6718 (Fig. 3-Б)

Based on the statistical processing result, we found that the micromycetes number in the rhizosphere soil of the studied sunflower hybrids was directly correlated with the value of the HTC of the growing season 2018-2020.
Thus, we determined that the number of phytopathogenic micromycetes differs, indicating a significant effect of root exometabolites of different sunflower hybrids and their cultivation technologies on the micromycetes population in the soil rhizosphere.
The species composition and micromycetes occurrence rate selected from the rhizosphere soil of the studied sunflower hybrids during the growing season were determined. According to the research results, the species composition of micromycetes isolates of rhizosphere Dushko and Oliver sunflower hybrids is represented by fungi of the genera: *Aspergillus, Alternaria, Penicillium, Fusarium,* and *Trichoderma* (Table 4).



А) Б)

Fig. 2. Relationship between the number of micromycetes in the sunflower rhizosphere soil and the value of HTC (Dushko hybrid in the conditions of traditional (Fig. 1-A) and organic (Fig. 1-B) cultivation technology).

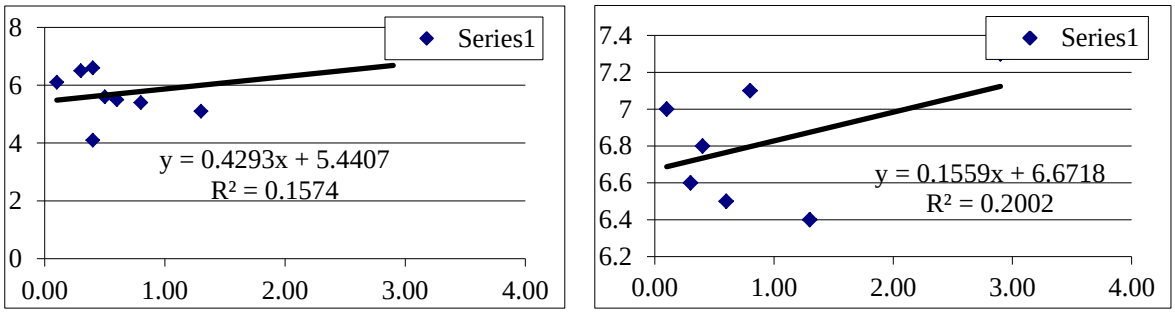


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Fig. 3. Relationship between the number of micromycetes in the rhizosphere soil of sunflower plants and the value of HTC (Oliver hybrid in the conditions of traditional (Fig. 2-A) and organic (Fig. 2-B) cultivation technology).

Table 4. The frequency of micromycetes selected from the rhizosphere soil of sunflower hybrids Dushko and Oliver during the growing season.

Species of micromycetes isolated from the sunflower rhizosphere soil	The frequency of micromycetes,% The phase of cultivated plant development		
	Budding	Inflorescence	Physiological maturity
	Hybrid Dushko		
Aspergillus flavus Link, 1809	50	60	70
Aspergillus niger Tieghem, 1867	55	-	-
Penicillium Link; Fr 1809	60	-	80
Alternaria alternata (Fr.) Keissl., 1912	-	50	60
Trichoderma viride Pers., 1794	30	-	-
Fusarium oxysporum Schltdl., 1824	-	25	-
	Hybrid Oliver		
Aspergillus flavus Link, 1809	60	60	40
Alternaria alternata (Fr.) Keissl., 1912	-	60	50
Fusarium oxysporum Schltdl., 1824	20	35	10
Trichoderma viride Pers., 1794	15	5	-

Note: if the frequency of micromycetes is ≥50%, these species are dominant; 30-50% - those that happen often; occurrence at the level of 10% and more minor - rare species (Zhdanova, 2002).

Dushko hybrid rhizosphere soil was characterized by the largest species of micromycetes diversity, from which 6 species were isolated (Fig. 4). Thus, in the rhizosphere soil of this hybrid in the phase, the species composition was represented by genus

Aspergillus fungi, which included species *A. niger* and *A. flavus* with a frequency of 55% and 50%. Typical representatives of Dushko hybrid rhizosphere soil were fungi of the genus *Penicillium*, the frequency of which in the budding phase was 60%, and in the phase of physiological maturity - 80%. Simultaneously, the phytopathogenic complex of the sunflower root zone of the studied hybrid formed the fungus *A. alternata* with a frequency of 50% in the flowering phase and 60% in the physiological maturity phase (Table 4).

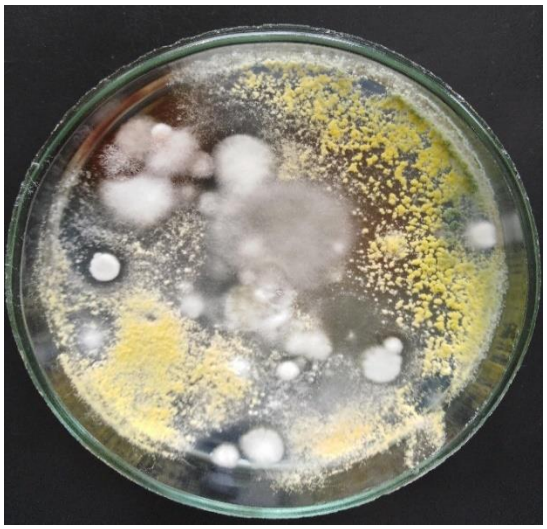

Fig. 4. The spectrum of micromycetes in the rhizosphere soil of sunflower plants of the Dushko hybrid.

A typical representative of the Oliver hybrid rhizosphere soil was the phytopathogenic fungus *F. oxysporum*, characterized by the different frequency of occurrence during plant ontogenesis (Table 4). Thus, in the budding phase, the frequency of this species occurrence was 20%, in the flowering phase, it increased to 35%, and in the physiological maturity phase, it significantly decreased (up to 10%). It can be assumed that Oliver sunflower hybrid root exometabolites can exert significant pressure on its population in agrocenosis.

In the phase of physiological maturity, not only the species composition of the mycological group changed, but the species diversity of potential phytopathogens expanded due to micromycetes: *A. alternata, F. oxysporum, and A. flavus*.

As can be seen from studies, in the rhizosphere of sunflower plants, hybrids Dushko and Oliver during the growing season formed a specific micromycetes group, among which the dominant toxin-forming species were fungi of the genera *Aspergillus* and *Penicillium* and species with phytopathogenic properties (known as *A. alternata*, *F. oxysporum*). Sunflower crops and leads to biological contamination of agrocenoses during the growing season of plants.

The scientific literature analysis shows that biotic environmental factors significantly influence the number and dynamics of the phytopathogenic micromycetes population in agrocenoses. Among them, the leading factor is the selective pressure of varieties and hybrids of cultivated plants. They can influence the qualitative and quantitative indicators of phytopathogenic background in agrocenoses, which significantly impairs the agroecosystem's biosafety (Parfeniuk et al., 2020).

Sunflower Hybrids, characterized by the appropriate structure of morphological and physiological, and biochemical characteristics, are factors in the mycobiome formation of the rhizosphere soil and vegetative organs of plants, its quantitative and qualitative composition. Our results showed that in the organic and traditional technologies of sunflower cultivation, the number of micromycetes depended on the hybrids and technology of their cultivation.

In the rhizosphere of sunflower hybrid plants, a specific group of micromycetes is formed during the growing season, among which toxin-forming fungi species belonging to the genera *Aspergillus* and *Penicillium* and the fungus *A. alternata* dominate.

Conclusion

The micromycetes number in the rhizosphere soil varies significantly depending on the sunflower hybrid and the technology of its cultivation. In the conditions of traditional technology, the number of micromycetes varies from 5.1 to 6.3 thousand CFU/g of dry soil, and in the conditions of organic technology - from 5.4 to 6.7 thousand CFU/g of dry soil. This can be explained by the significant influence of sunflower plants of different hybrids root exometabolites and technologies of their cultivation on the population of micromycetes in the rhizosphere soil.

Fungi of the genera represent the species composition of micromycete isolates in the Dushko and Oliver sunflower hybrids rhizosphere: *Aspergillus, Alternaria, Penicillium, Fusarium,* and *Trichoderma*. The budding phase is dominated by fungi of the genus *Aspergillus*, which are represented by *A. niger* and *A. flavus* species with a frequency of 50 - 55%.

In the physiological maturity phase, in the Dushko hybrid rhizosphere, soil fungi of the genus *Penicillium* parasitize with a frequency of 60-80%. The phytopathogenic complex of the root zone of sunflower plants is formed by phytopathogenic micromycetes of *A. alternata* and *F. oxysporum*. Their frequency is 50%. These micromycetes cause significant damage to sunflower crops and lead to biological contamination of agrocenoses during plants' growing season.

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