DE GRUYTER HELIA 2021; 44(75): 181–190

Natalia Kostyuchenko, Viktor Lyakh* and Anatoliy Soroka

The state of soil microbiotes during sunflower growing with an herbicide of imidazolinone group

https://doi.org/10.1515/helia-2021-0005
Received March 31, 2021; accepted April 21, 2021;
published online May 3, 2021

Abstract: The effects of various concentrations of herbicide Euro-Lightning Plus on the state of microbiota in the root zone of sunflower have been studied. Soil of plant rhizosphere and interrow soil after treatment with the herbicide at the doses of 1.2 and 2.5 l/ha were taken for the analysis at the end of sunflower growing season. Rhizosphere soil without herbicide application was used as a control. The herbicide was applied at the stage of 2–4 true leaves. The total number of bacteria in the rhizosphere of control plants was 12.82 million CFU/g of soil while in the rhizosphere and in the interrow soil after herbicide treatment with a dose of 2.5 l/ha it decreased by 1.4–1.5 times. A general trend of decline in number of the basic ecological and trophic groups of bacterial microorganisms with the increase in a dose of herbicide was established. Microbiological coefficients that reflect the functional activity of the microflora indicate changes in its biological activity under the influence of the herbicide Euro-Lightning Plus, which leads to deterioration in the agroecological state of the studied soils. It was also found that herbicide application resulted in a rearrangement of micromycete complexes in the root zone of sunflower which led to a two-fold reduction, compared to the control, of mycobiota species diversity and the formation of a specific species composition of mycocenoses. A greater genus and species diversity of fungi of the microflora in the rhizosphere of control plants, in comparison with the herbicide-treated soil, was revealed. A reduction in species diversity of the genus *Penicillium* from six species in the control to 1–2 species in the rhizosphere of experimental sunflower

***Corresponding author: Viktor Lyakh**, Zaporizhzhia National University, Zhukovsky Str., 66, Zaporizhzhia 69600, Ukraine; and Institute of Oilseed Crops of NAAS, Institutskaya Str., 1, Zaporizhzhia 70417, Ukraine, E-mail: lyakh@iname.com
Natalia Kostyuchenko, Zaporizhzhia National University, Zhukovsky Str., 66, Zaporizhzhia 69600, Ukraine, E-mail: kostuchenko.zp@gmail.com
Anatoliy Soroka, Institute of Oilseed Crops of NAAS, Institutskaya Str., 1, Zaporizhzhia 70417, Ukraine, E-mail: bvryffy@hotmail.com

plants as well as the absence of rare saprophytic fungi species from the genera *Acremonium*, *Verticillium*, *Trichoderma* and *Paecilomyces* were noted.

Keywords: herbicide; microbial complex; mycocenosis; root zone; sunflower.

Introduction

Sunflower occupies the largest area among all oilseeds grown in Ukraine. It is a crop whose products are easily exported and bring significant income to the economy of this country. Modern cultivation of sunflower involves the use of pesticides at different stages of plant development. Without them obtaining high and stable yields is impossible. The most important among the currently used means of plant protection are herbicides (Tkalich 2014).

Euro-Lightning Plus is the most widely used herbicide of imidazolinone group in sunflower. This herbicide usually accompanies sunflower cultivation technology to control monocotyledonous and dicotyledonous weeds, – ambrosia, broomrape, sow thistle. The herbicide acts through the leaves and the soil. The decomposition of the agent in the soil occurs microbiologically.

Soil microcoenoses play a significant role in the plant life, as they are able to process the organic matter necessary for plant growth. However, the interaction of microbes and plants is not always limited to trophic functions. In many cases, it is caused by metabolic connections mediated by physiologically active substances produced by microorganisms and affecting plant growth (Tkacz and Poole 2015).

The use of herbicides has a significant and ambiguous effect on the soil microflora, because it is known that the reaction of microbial groups depends on the soil and climatic conditions and the chemical composition of pesticides. Some pesticides are quickly converted into less harmful compounds, others – accumulate in the soil, which is especially observed when using the same chemicals. The accumulation of pesticides in the soil leads to a decrease in crop yields, as it has a direct effect on the fertile soil layer. The consequence of the accumulation of pesticides is an increase in the general toxicity of soils, which is especially noticeable in agrocenoses, where natural vegetation is removed and monoculture predominates (Brovko et al. 2017).

The use of herbicides leads to imbalance of soil microflora in agrocenoses due to the selective sensitivity of the microbiota to these pesticides. Usually treatment with chemicals leads to the destruction of certain genera of microorganisms which are sensitive. However, some of them are able to dispose of herbicides using them as a source of energy (Chabaniuk et al. 2016).

The purpose of our study was to elucidate the effects of various concentrations of Euro-Lightning Plus on the state of the soil microbiota of sunflower agrocenoses when grown on dry land.

Materials and methods

The research was conducted in 2017–2018. Soil samples for analysis were taken from the rhizosphere of plants and from between rows at the end of the sunflower growing season according to the following scheme: 1 – control (rhizosphere soil without herbicide application), 2 – rhizosphere soil after treatment with the herbicide (dose 1.2 l/ha); 3 – soil between rows (1.2 l/ha); 4 – rhizosphere soil (2.5 l/ha); 5 – soil between rows (2.5 l/ha). Application time – 2–4 pairs of true sunflower leaves.

Optimal nutrient media were used to record the number of major ecological and trophic groups of bacteria: meat-peptone agar for ammonifiers, starch-ammonia agar for bacteria utilizing nitrogen compounds, soil agar for oligotrophs, and 'hungry' agar for oligonitrophils. For microscopic fungi, Chapek-Dox medium with sucrose was used. The cultivation time for the bacteria was 3–5 days, for the fungi – 7–14 days at the temperature of 28 °C. The experiment was repeated five times. The number of grown microorganisms was expressed in colony-forming units (CFU) in one g of air-dry soil.

A deep method for inoculation of microorganisms from the prepared soil extract in the corresponding decimal dilutions was used for the research. The soil suspension was sown on Czapek-Dox medium with a dilution of 1:1000, on meat-peptone, soil and starvation agar with a dilution of 1:10,000; on starch-ammonia agar – with a dilution of 1: 100,000 (Dudka et al. 1982; Mirchink 1988; Zvyagintsev 1991).

To evaluate the activity of microbiological processes occurring in the studied soils, the coefficients of mineralization-immobilization and pedotrophy were used (Andriyuk et al. 2001).

Identification of microorganisms was performed using standard handbooks and original works (Bilaj and Koval 1988; Satton et al. 2001).

To characterize the similarity of the species composition of mycobiota, the Sorensen coefficient (Cs) was calculated. Reliable indicators of similarity when comparing the species composition of two different associations were considered those in which the Sorensen coefficient was more than 50% (Megarran 1992).

The differences in number of microorganisms were evaluated by the t-test at the 0.05 and 0.01 levels of probability.

Results and discussion

Comparative analysis of quantitative characteristics of microbial complexes of the root zone of sunflower grown in a stationary infectious nursery of the Institute of Oilseed Crops of NAAS of Ukraine showed significant differences from the control in the number of major ecological and trophic groups of bacterial microflora, depending on the dose of herbicide Euro-Lightning Plus (Table 1).

Table 1: Total number of microorganisms of the main ecological-trophic groups in the root zone of sunflower plants non-treated and treated with herbicide (CFU/g soil).

Root zone	Number of bacteria, million CFU/g of soil			
	Ammonifiers, million	Bacteria, utilizing mineral nitrogen, million	Oligotrophs, million	Oligonitrophils, million
Control				
Rhizosphere	1.67 ± 0.10	8.93 ± 1.22	0.73 ± 0.08	1.49 ± 0.1
Euro-Lightning Plus (1.2 l/ha)				
Rhizosphere	1.40 ± 0.17	6.93 ± 0.82	0.34 ± 0.07[a]	1.32 ± 0.11
Row spacing	1.32 ± 0.03	6.10 ± 1.11	0.59 ± 0.04	0.91 ± 0.22
Euro-Lightning Plus (2.5 l/ha)				
Rhizosphere	1.20 ± 0.06[a]	4.19 ± 0.43[a]	0.30 ± 0.04[a]	1.23 ± 0.03
Row spacing	1.08 ± 0.04[a]	2.93 ± 0.15[a]	0.35 ± 0.02[a]	1.16 ± 0.13

[a]The differences from the control are significant at the 0.05 level of probability.

Thus, the total number of bacteria in the rhizosphere of control plants (without herbicide treatment) was 12.82 million CFU/g of soil. After treatment with the herbicide at a dose of 1.2 l/ha, the total amount of bacterial microflora in the rhizosphere of sunflower and between rows was 9.99 and 8.92 million CFU/g of soil, respectively. The microbiocenoses formed in the rhizosphere and between rows during herbicide treatment with a dose of 2.5 l/ha were the least numerous – 6.92 and 5.52 million CFU/g of soil. In general, the total number of bacterial microflora of the root zone of sunflower after treatment with the herbicide at a dose of 1.2 l/ha decreased by 1.2–1.3 times compared with the control, and in the treatment with a dose of 2.5 l/ha – by 1.4–1.5 times.

The general tendency of decrease in the number of main ecological and trophic groups of bacterial microorganisms with increase in a dose of herbicide is established. For example, the number of ammonifiers in the rhizosphere of sunflower without herbicide treatment (control) was 1.67 million CFU/g of soil and that exceeded the experimental treatments by 1.2 times (dose 1.2 l/ha) and 1.4 times (dose 2.5 l/ha). The number of microorganisms that utilize mineral nitrogen in the rhizosphere and between rows decreased compared to the control, respectively, by 1.3 and 1.5 times (dose 1.2 l/ha) and by two and three times (dose 2.5 l/ha).

Quantitative indicators of bacterial microflora (oligotrophs) grown on soil agar were significantly lower in samples of soil treated with the herbicide. This pattern was characteristic of both the rhizosphere and the interrow soil. The number of oligonitrophils grown on a ‘hungry’ agar generally tended to decrease in the treatments, but did not differ significantly from the control.

Analysis of the activity of biological processes occurring in the studied soils, according to the calculated coefficients of mineralization-immobilization and pedotrophy showed the differences of microbiological parameters under herbicide treatment. According to the data, the microbiological activity of the soil decreased with increasing a dose of the herbicide. Thus, the maximum value of the mineralization-immobilization coefficient was 5.35 in the control, while after the herbicide application at a dose of 2.5 l/ha there was noted a decrease in the number of ammonifiers and nitrogen immobilizers, which affected microbiological parameters (rhizosphere = 3.49; row spacing = 2.71). The activity of autochthonous microflora which is capable of decomposing humus and consuming fulvic acids was evaluated by the coefficient of pedotrophy. It was found that with the use of the herbicide the coefficient of pedotrophy in the rhizosphere of sunflower dropped almost two times (0.24–0.25) compared with the control (0.44).

The total number of microscopic fungi in the rhizosphere of control plants was 2.3 times higher than in the soil between rows of sunflowers when treated with the herbicide at a dose of 1.2 l/ha. Treatment with this chemical agent at a dose of 2.5 l/ha has led to a greater reduction in the number of fungi. Thus, the quantitative indicators in the control were 2.0 and 2.9 times higher than the same indexes for the rhizosphere of plants and for the soil between rows, respectively (Figure 1).

From the selected soil samples 33 microscopic fungi from 13 genera were isolated and analyzed. The genera *Aspergillus* (eight species) and *Penicillium* (seven species) were represented by the largest number of species.

Analysis of the taxonomic structure of the studied mycocenoses revealed both quantitative and qualitative differences in the genus and species composition of micromycetes in the rhizosphere of sunflower and in the soil between rows under the action of different doses of the herbicide. It was established that the mycocenosis of the rhizosphere of control plants was the most diverse in terms of species

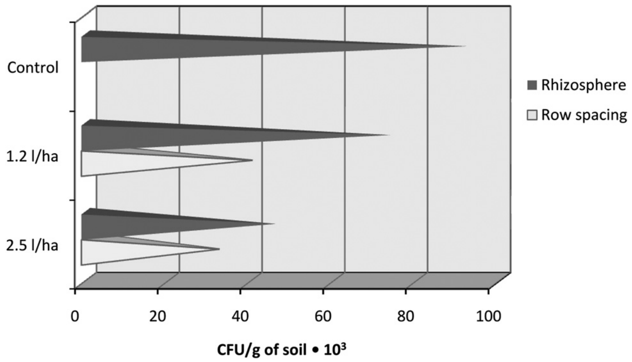

Figure 1: The total number of fungal colonies on the Czapek-Dox medium.

richness, where 29 species of microscopic fungi from 12 genera were found. The species diversity in the soil of the areas treated with the herbicide was meager and represented by 21 species from 10 genera (dose 1.2 l/ha) and by 20 species from 11 genera (dose 2.5 l/ha), respectively (Table 2).

In general, as shown in Table 2, the application of the herbicide led to a reduction in species diversity by almost half compared with the control in both the sunflower rhizosphere and in the soil between rows.

Typical species representatives that formed the mycocenosis of the rhizosphere of control sunflower plants were members of the genus *Aspergillus* (*Aspergillus alliaceus*, *Aspergillus candidus*, *Aspergillus melleus*, *Aspergillus niger*, *Aspergillus niveus*, *Aspergillus ustus*) and the genus *Penicillium* (*Penicillium canescens*, *Penicillium crustosum*, *Penicillium nigricans*, *Penicillium solitum*, *Penicillium thomii*, *Penicillium digitatum*), of which *A. ustus*, *P. canescens* and *P. digitatum* were not found in other treatments. In addition to these species of micromycetes, in control soil samples *Acremonium charticola* var. *subglutinans* and *Mucor hiemalis* were detected. These species were not isolated from the rhizosphere of sunflower and interrow specimen treated with herbicide (Table 2, Figure 2).

Our studies demonstrated a reduction in species diversity of the genus *Penicillium* from six species in the control to 1–2 species in the rhizosphere of experimental sunflower plants and the absence of representatives of this genus in interrow soil samples after treatment with the herbicide at a dose of 2.5 g/l. In addition, in the rhizosphere of sunflower and in the soil between rows treated with the herbicide, species diversity was dropped due to vanishing of rare saprophytic fungi from the genera *Acremonium*, *Verticillium*, *Trichoderma*, and *Paecilomyces*, which includes the entomopathogenic species *Paecilomyces lilacinus* – the causative agent of insect mycoses.

Comparative analysis of species composition of micromycete complexes and calculated Sorensen coefficients (Cs) indicate differences in species composition between mycocenoses in the rhizosphere of control and herbicide-treated sunflower plants. The similarity with the control for species representatives of mycocenoses in the rhizosphere of the experimental plants amounted to only 51–53% (Cs = 0.51–0.53). However, mycocenoses of sunflower rhizosphere which were treated with the herbicide in the doses of 1.2 and 2.5 l/ha were quite similar for species representatives (Cs = 0.83).

A large number of biological processes occur in the soil of agrocenoses, which can be estimated by calculating the coefficients that reflect the functional activity of the microflora (coefficients of oligotrophy, mineralization-immobilization, pedotrophy). These coefficients make it possible to determine what changes develop in the soil under the influence of herbicides – the restoration of fertility or its degradation (Sherstoboeva et al. 2017). Our analysis of the activity of biological

Table 2: The species structure of micromycete complexes in the root zone of non-treated and treated with herbicide sunflower plants.

Species	Control	Dose 1.2 l/ha		Dose 2.5 l/ha	
	R	R	I	R	I
Zygomycota, Zygomycetes, Mucorales					
Mucoraceae					
Mucor hiemalis	+	–	–	–	–
M. racemosus	+	–	–	–	+
Mucor. sp.	–	–	+	–	+
Rhizopus nigricans	+	+	+	+	+
Hyphomycetes, Hyphomycetales					
Moniliaceae					
Acremonium charticola var. *subglutinans*	+	–	–	–	–
Aspergillus alliaceus	+	+	+	+	+
A. candidus	+	–	+	–	–
A. melleus	+	+	+	+	+
A. niger	+	+	–	+	–
A. niveus	+	–	–	+	+
A. ochraceus	–	+	+	–	–
A. ustus	+	–	–	–	–
Aspergillus sp.	–	+	+	+	+
Cephalosporium gramineum	+	–	+	–	+
Paecilomyces lilacinus	+	–	+	–	–
Paecilomyces sp.	+	–	+	+	+
Eupenicillium ochrosalmoneum	+	+	+	+	+
P. canescens	+	–	–	–	–
P. crustosum	+	–	+	–	–
P. digitatum	+	–	–	–	–
P. nigricans	+	+	–	+	–
P. solitum	+	–	+	+	–
P. thomii	+	–	+	–	–
Trichoderma viride	+	–	–	–	+
Verticillium album	+	+	–	–	–
Verticillium sp.	+	–	–	–	+
Dematiaceae					
Alternaria helianthi	–	+	–	+	–
Cladosporium cladosporioides	+	–	–	–	–
Tuberculariales, ***Tuberculariaceae***					
Fusarium moniliforme var. *lactis*	+	+	–	+	–
F. moniliforme var. *subglutinans*	+	+	–	+	–
F. oxysporum var. *orthoceras.*	+	+	+	+	+
Fusarium sp.	+	–	–	–	–
Agonomycetales, ***Agonomycetaceae***					
Mycelia sterilia (white)	+	+	+	+	+
Total genera (species)	12 (29)	7 (14)	8 (16)	7 (15)	10 (14)

R – rhizosphere; I – row spacing; "–" – species were not found.

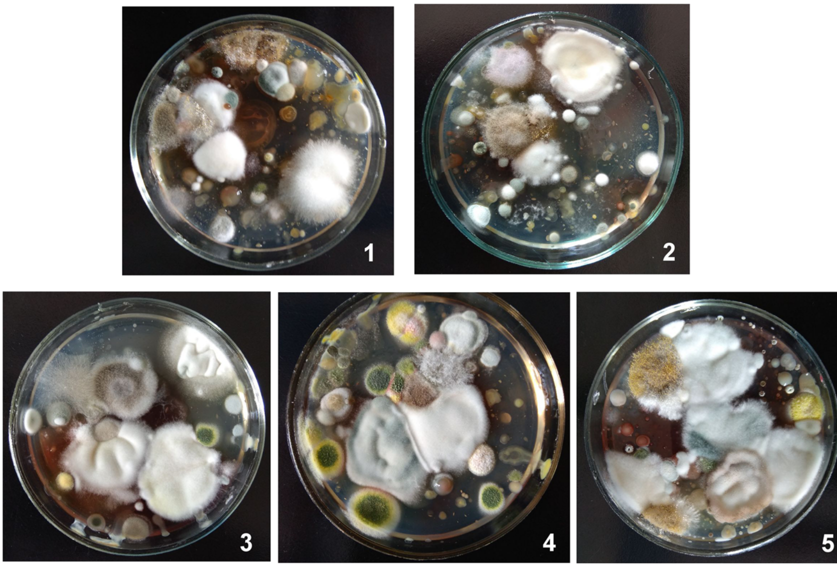

Figure 2: Typical micromycete complexes isolated from the root zone of non-treated (1) and treated with herbicide sunflower plants (2–5): 1 – rhizosphere without herbicide, 2 – rhizosphere, herbicide dose 1.2 l/ha; 3 – soil between rows, herbicide dose 1.2 l/ha; 4 – rhizosphere, herbicide dose 2.5 l/ha; 5 – soil between rows, herbicide dose 2.5 l/ha.

processes occurring in the studied soils, according to the calculated coefficients, showed significant differences in microbiological parameters for the rhizosphere of sunflower without treatment (Kostyuchenko and Lyakh 2017) and when treated with different doses of herbicide.

Our data are consistent with the results of studies to establish the effect of herbicides on the microbiota during the cultivation of soybeans (Chabaniuk et al. 2016). According to the authors, the use of herbicides did not reduce the number of microorganisms of the main ecological and trophic groups and their biological activity in the soybean rhizosphere, however a regrouping of the dominant forms of microorganisms and reduction in microbial biodiversity were noted. Such changes have led to an increase in the proportion of toxic and opportunistic micromycetes in the soil.

Conclusion

Thus, the general tendency of decrease in number of the basic ecological and trophic groups of bacterial microorganisms with increase in a dose of drug is established. Microbiological coefficients that reflect the functional activity of the microflora indicate changes in its biological activity under the influence of the herbicide Euro-Lightning Plus, which leads to deterioration of the agroecological condition of the studied soils. It was also found that under the action of the herbicide Euro-Lightning Plus there was a restructuring of micromycete complexes of the root zone of sunflower, which led to a two-fold reduction compared to the control of species diversity of mycobiota and the formation of a special species composition of mycocenoses. The microflora of the rhizosphere of control plants was characterized by a greater genus and species diversity of fungi than the soil of the areas treated with the herbicide. The findings indicate the need for a more balanced approach to the use of herbicides in sunflower cultivation, taking into account their possible negative impact on subsequent crops.

Author contributions: All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.
Research funding: None declared.
Conflict of interest statement: The authors declare no conflicts of interest regarding this article.

References

Andriyuk, K.I., Iutynska, G.O., and Antipchuk, A.F. (2001). *Functioning of microbial coenoses of soil in the conditions of anthropogenic loading*. Oberegy, Kiev, pp. 240.
Bilaj, V.I. and Koval, E.Z. (1988). *Aspergillus*. Nauk. Dumka, Kiev, pp. 204.
Brovko, I.S., Chabanyuk, Y.V., Mazur, S.V., and Koretskyi, A.P. (2017). Relationships between biological soil indicators due to herbicide action. Agroecol. J. 1: 86–93.
Chabaniuk, Y., Brovko, I., Koretskyi, A., and Mazur, S. (2016). Functioning of soil microbiota under the influence of herbicides. Agroecol. J. 4: 122–125.
Dudka, I.A., Vasser, S.P., Ellanskaya, I.A., and Koval', E.Z. (1982). Methods of experimental mycology, 1982. In: Bilaj, V.I. (Ed.), *Reference book*. Nauk. Dumka, Kiev, p. 550.
Kostyuchenko, N.I. and Lyakh, V.A. (2017). Peculiarities of taxonomic structure of micromycete complex in root zone of sunflower in conditions of southern Steppe of Ukraine. Helia 40: 147–159.
Megarran, E. (1992). *Ecological diversity and its measurement*. Mir, Moscow, p. 184.
Mirchink, T.G. (1988). *Soil mycology*. MGU, Moscow, p. 220.
Satton, D., Fothergill, A., and Rinaldi, M. (2001). *Guide to pathogenic and opportunistic fungi*. Mir, Moscow, p. 486.
Sherstoboeva, O.V., Demyanyuk, O.S., and Chabanyuk, Y.V. (2017). Biodiagnostics and biosafety of soils of agroecosystems. Agroecol. J. 2: 141–148.
Tkacz, A. and Poole, Ph. (2015). Role of root microbiota in plant productivity. J. Exp. Bot. 66: 2167–2175.

Tkalich, Y.I. (2014). Productivity and economic evaluation of sunflower cultivation when using different cultivations of soil and herbicides. Sci. Tech. Bulletin Inst. Oilseed Crop. NAAS 20: 198–203.
Zvyagintsev, D.G. (1991). *Methods of soil microbiology and biochemistry*. MGU, Moscow, p. 303.