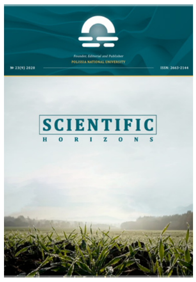
SCIENTIFIC HORIZONS

Journal homepage: https://sciencehorizon.com.ua
***Scientific Horizons*, 26(8), 52-61**



UDC 633.2:577.486:581.524.34
DOI: 10.48077/scihor8.2023.52

The influence of agrotechnical measures on the number of melanin-synthesizing microorganisms

Iryna Malynovska*
Corresponding Member of the National Academy of Sciences, Doctor of Agricultural Sciences, Senior Research Fellow
NSC "Institute of Agriculture of NAAS of Ukraine"
08162, 2-b Mashynobudivnykiv Str., Chabany vil., Ukraine
https://orcid.org/0000-0001-8002-7139

Viktor Kaminskyi
Academician of NAAS, Doctor of Agricultural Sciences, Professor
NSC "Institute of Agriculture of NAAS of Ukraine"
08162, 2-b Mashynobudivnykiv Str., Chabany vil., Ukraine
https://orcid.org/0000-0002-9668-6742

Yelyzaveta Zadubynna
PhD in Agricultural Sciences, Senior Research Fellow
Panfyly Research Station of the National Scientific Center
"Institute of Agriculture of the National Academy of Agrarian Sciences of Ukraine"
07750, 2 Tsentralna Str., Panfyly vil., Ukraine
https://orcid.org/0000-0002-9428-5603

Volodymyr Kurhak
Corresponding Member of the National Academy of Sciences, Doctor of Agricultural Sciences, Professor
NSC "Institute of Agriculture of NAAS of Ukraine"
08162, 2-b Mashynobudivnykiv Str., Chabany vil., Ukraine
https://orcid.org/0000-0003-2309-0128

Viktor Karpenko
Doctor of Agricultural Sciences, Professor
Uman National University of Horticulture
20301, 1 Instytutska Str., Uman, Ukraine
https://orcid.org/0000-0001-5607-7371

Article's History:
Received: 6.04.2023
Revised: 7.08.2023
Accepted: 23.08.2023

Abstract. In connection with the growing scale of soil dehumification, studies of the patterns of synthesis of the precursors of humus molecules – melanins of bacterial origin – are becoming increasingly relevant. The purpose of this study was to establish the influence of the main factors of agricultural production on the spread of melanin-synthesizing microorganisms: the method of basic soil cultivation, mineral fertilizers,

Suggested Citation:
Malynovska, I., Kaminskyi, V., Zadubynna, Ye., Kurhak, V., & Karpenko, V. (2023). The influence of agrotechnical measures on the number of melanin-synthesizing microorganisms. *Scientific Horizons*, 26(8), 52-61. doi: 10.48077/scihor8.2023.52.

**Corresponding author*

and the type of agricultural crop. Research methods: microbiological, laboratory-analytical, statistical, correlational. As a result, the study found for the first time that the main cultivation of the soil affects the number of melanin-synthesizing microorganisms: micromycetes, bacteria, *a*zotobacter. In the variant without the application of mineral fertilizers (control), the soil was characterized by the minimum amount of melanin-synthesizing bacteria using the No-till technology, 28.8% more – using disking, and 2.4 times more – using ploughing. The number of melanin-synthesizing micromycetes in the variant without fertilizers was also maximum upon ploughing, the average number was observed with No-till technologies, and the minimum number – using shallow disk tillage. Application of mineral fertilizers in a dose of $N_{30}P_{30}K_{65}$ leads to an increase in the number of melanin-synthesizing bacteria in the ploughing option by 3.58 times, disking by 3.53 times, No-till technology by 2.28 times. An increase in the dose of mineral fertilizers to $N_{150}P_{100}K_{120}$ contributes to an increase in the number of melanin-synthesizing bacteria by 5.04 times upon ploughing, 5.78 times upon disking, and 2.24 times upon no-till technologies. The medium-significant nature of the relationship between the content of humus and the number of melanin-synthesizing bacteria (r=0.528) and their share in the total number of microorganisms (r=0.470) suggested that in chernozem inherent in growing sunflowers, humus is formed mainly with the participation melanins of bacterial origin. Metabolites of polysaccharide-synthesizing bacteria take part in the formation of the resistance of humus molecules to microbial mineralization, the probability of the formation of colonies of which has a moderately significant relationship with the content of humus (r=0.532). The results of these studies can be used in the development of recommendations for regulating the content of humus in agricultural soils, preventing its excessive mineralization, which will allow preserving and increasing soil fertility

Keywords: melanins; bacteria; micromycetes; azotobacter; humus; proportion; correlation

INTRODUCTION

Soil organic matter, and humus in particular, is the final product of microbial transformation of molecules released from dead plant and animal cells (Lorenz *et al.*, 2021, Savarese *et al.*, 2021, Soil Humus Formation, 2018). Humus is of fundamental importance in ecosystems, regulating global carbon and nitrogen cycles, plant and microorganism growth, and soil functions. Pan *et al.* (2009) and Ping *et al.* (2009) noted that the accumulation of humus in agricultural ecosystems is necessary not only for preserving soil fertility and forming the productivity of agricultural crops, but for stabilizing soil organic carbon and reducing greenhouse gas emissions into the atmosphere. Nevertheless, the growth of food production increases the risk of depletion of arable land, which leads to dehumification, erosion, and desertification. In this regard, the study of the patterns of the synthesis of humic compounds and their precursors – melanins of bacterial origin – is relevant and timely.

Melanins are produced by heterotrophic bacteria, micromycetes, and are dark-coloured pigments. For many producers, these pigments are secondary metabolites that contribute to photoprotection (a photoprotector that effectively absorbs and dissipates solar radiation as heat), predation, predation protection, metal resistance (metal chelating sorbents) (Fisher *et al.*, 2019, Dornelas *et al.*, 2022). Tran Ly *et al.* (2020) believe that melanins bind organic compounds for detoxification, and act as an organic semiconductor.

The study (Reddy *et al.*, 2007) investigated the patterns of melanin synthesis by the actinobacterium *Modestobacter versicolor sp. nov.* isolated from biological crusts of the surface layer of the soil of the Colorado Plateau (USA), the cells of which produce melanin-like pigments under conditions of oligotrophy, i.e., when grown on an oligotrophic medium, but not when grown on a copyotrophic medium. Induction of melanogenesis by *Modestobacter versicolor sp. nov.* does not depend on the growth phase of the crop or lighting conditions, including UVB and UVA-irradiation, but UVB exposure can increase total pigment synthesis, while growth under low nitrogen conditions inhibits its synthesis.

Fungi, especially ascomycetes, synthesize melanin molecules in their cell walls to protect against fungivores and harsh abiotic conditions (Ekblad *et al.*, 2013, Baskaran *et al.*, 2019). The authors (Edwards *et al.*, 2021) believe that the synthesis of melanins also protects producers from the damaging effects of ultraviolet radiation.

Thus, to date, the regularities of melanin synthesis as a biotechnological product in producer monocultures and the possibilities of their application in practice have been investigated. However, the ecological aspects of the distribution of melanin-synthesizing microorganisms in nature, and especially in the soils of agrocenoses, are still understudied. The issue of influence on the spread of melanin-synthesizing microorganisms of the main factors of agricultural production: the method of basic soil cultivation and mineral fertilization are still unexplored. The present study aims to solve these problems, the results of which are presented below.

MATERIALS AND METHODS

The investigation was conducted in a stationary experiment on the study of short-rotational crop rotations at the Panfilsk research station of the National Research Centre "Institute of Agriculture of the National Academy of Agrarian Sciences of Ukraine", established in 2009.

The soil of the experimental plots is a typical shallow coarse-grained and light-loam chernozem, which lies on a carbonate light loamy forest. Before starting the experiment, the soil was characterized by the following agrochemical parameters: humus content – 3.17%; soil pH 6.15; hydrolytic acidity – 3.15 mg-eq/100 g of soil; the amount of absorbed bases – 14.1 mg-eq/100 g; exchange bases: calcium – 7.43; magnesium – 1.14 mg-eq/100 g of soil; degree of saturation with bases – 56%, easily hydrolysable nitrogen content – 72.8 mg/kg; mobile phosphates – 300.0 mg/kg, exchangeable potassium – 95.5 mg/kg of soil.

The study was conducted in crop rotation: sunflower – soybean – winter wheat – spring barley – sunflower. The object of the study was the variants of the stationary experiment: 1 – No-till technology, without fertilizers (control); 2 – No-till technology, $N_{30}P_{30}K_{65}$, 3 – No-till technology, $N_{150}P_{100}K_{120}$; 4 – Disking (shallow disk cultivation 10-12 cm deep), without fertilizers; 5 – Disking, $N_{30}P_{30}K_{65}$; 6 – Disking, $N_{150}P_{100}K_{120}$, 7 – Ploughing 25-27 cm deep, without fertilizers; 8 – Ploughing 25-27 cm deep, $N_{30}P_{30}K_{65}$, 9 – Ploughing 25-27 cm deep, $N_{150}P_{100}K_{120}$. In 2021, the variants under study were used to cultivate the sunflower hybrid "Tor", the predecessor of which was spring barley. The size of the sowing area was 25*6=150 m^2, the accounting area was 100 m^2. The experiment was repeated three times, the placement of variants and repetitions was systematic.

The number of microorganisms of the main ecological-trophic, functional, and systematic groups was estimated according to the method of sowing soil suspension on the corresponding general, elective, and special nutrient media in three repetitions (Dubey *et al.*, 2020, Lee *et al.*, 2021). The number of colonies was counted for 21 days depending on the growth rate and physiological characteristics of microorganisms of a certain ecological-trophic group. Indicators of the intensity of processes of mineralization of nitrogen compounds, soil organic matter, humus, probability of bacterial colony formation (PCF) were calculated as described earlier (Malynovska *et al.*, 2023). For a general assessment of the biological state of the soil, the indicator of total biological activity (TBA) was calculated using the method of relative values (Rusakova, 2013). The content of humus in the soil was determined according to (DSTU 4289:2004).

Statistical processing of experimental data was performed according to methods generally accepted in soil science and soil microbiology using Microsoft Excel and Statistica 10 computer programs. Determination of correlations between the indicators under study and their closeness was assessed based on the results of correlation analysis obtained using Microsoft Excel 2010.

RESULTS

The results of the study are presented in Table 1, Figure 1. The analysis of the results of these studies suggested that the main tillage affects the number of melanin-synthesizing microorganisms: micromycetes, bacteria, *a*zotobacter. In the variant without fertilizers, the soil was characterized by the maximum number of melanin-synthesizing bacteria when ploughing was used, by 28.8% less – when disking, and 2.4 times less – when using No-till technology. With the minimum dose of fertilizers ($N_{30}P_{30}K_{65}$), this regularity was preserved: the number of melanin-synthesizing bacteria in the ploughing variant exceeded the number of such bacteria by disking by 30.7%, upon No-till technology – 3.76 times. At the maximum dose of fertilizers ($N_{150}P_{100}K_{120}$), the corresponding indicators were 12.4% and 5.4 times.

Table 1. *The number of microorganisms in chernozem typical for growing sunflowers, million CFU*/g of dehydrated soil*

Variant	*Azotobacter*, % fouling of soil clods	Pedotrophs	Cellulose-destroying bacteria	Polysaccharide synthesizing	Actinomycetes	Micromycetes	Melanin-synthesizing micromycetes	Share of melanin-synthesizing micromycetes in the total amount, %	Melanin-synthesizing bacteria	Share of melanin-synthesizing bacteria in the total number, %	Autochthonous	Total number
No-till technology, without fertilizers	41.3	86.3	65.5	12.0	10.3	0.308	0.0220	7.14	2.48	0.26	20.1	950.3
No-till technology, $N_{30}P_{30}K_{65}$	18.7	116.8	54.8	14.2	16.5	0.423	0.0350	8.27	5.66	0.56	9.83	1,010.7
No-till technology, $N_{150}P_{100}K_{120}$	18.7	79.0	94.4	16.7	13.0	0.525	0.0560	10.7	5.56	0.52	10.5	1,079.0
Disking, without fertilizers	26.7	150.8	69.3	14.4	18.1	0.430	0.0142	3.30	4.62	0.37	15.2	1,250.7
Disking, $N_{30}P_{30}K_{65}$	36.0	152.5	81.6	25.7	20.8	0.824	0.0645	7.82	16.3	1.15	17.5	1,421.6
Disking, $N_{150}P_{100}K_{120}$	40.7	192.4	86.5	6.60	19.8	0.535	0.0452	8.45	26.7	2.03	18.0	1,315.5
Ploughing, without fertilizers	96.0	88.9	90.2	12.3	12.1	0.250	0.0317	12.7	5.95	0.62	15.8	959.1

Table 1, Continued

Variant	*Azotobacter*, % fouling of soil clods	Pedotrophs	Cellulose-destroying bacteria	Polysaccharide synthesizing	Actinomycetes	Micromycetes	Melanin-synthesizing micromycetes	Share of melanin-synthesizing micromycetes in the total amount, %	Melanin-synthesizing bacteria	Share of melanin-synthesizing bacteria in the total number, %	Autochthonous	Total number
Ploughing, $N_{30}P_{30}K_{65}$	22.0	98.0	51.2	10.4	13.1	0.356	0.0346	9.72	21.3	2.72	13.1	783.4
Ploughing, $N_{150}P_{100}K_{120}$	25.3	46.4	63.2	4.21	8.77	0. 239	0.0175	7.32	30.0	4.63	10.5	648.6
LSD_{05}	3.55	8.74	2.12	3.15	1.88	0.05	0.002		0.08		0.98	

Note: *CFU * – colony-forming unit*
Source: *developed by the authors of this study*

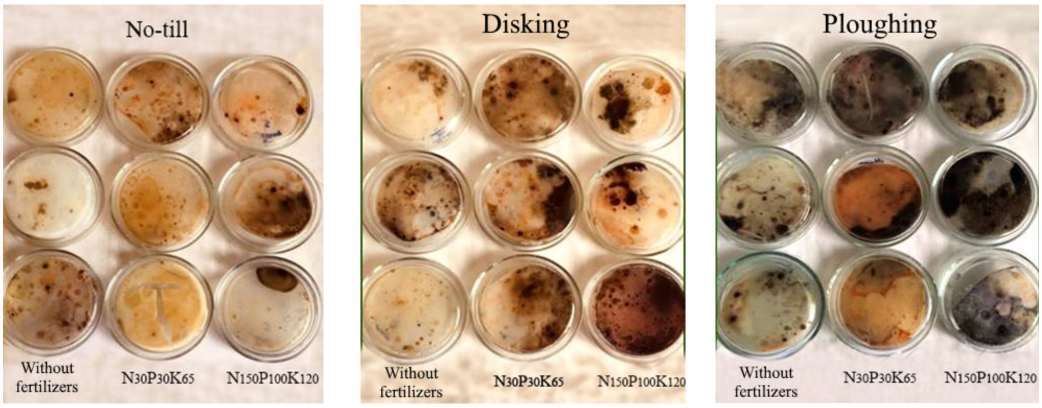


Figure 1. *Synthesis of melanins by microorganisms of chernozem typical for the use of no-till technology as the main tillage, disking 10-12 cm deep and ploughing 25-27 cm deep*

The share of melanin-synthesizing bacteria in the total number of microorganisms also depends on the method of the main soil cultivation: in the variant without fertilizers, the maximum value of this indicator is characterized by the soil with the use of ploughing, by 67.6% less – the soil with the use of disking, by 2.38 times smaller value – the soil upon No-till. As a result of the incertion of mineral fertilizers, the share of melanin-synthesizing bacteria in the total number of microorganisms increases under all methods of soil cultivation, especially significantly – under ploughing.

Somewhat different patterns are observed for melanin-synthesizing micromycetes, specifically, in the version without fertilizers, the maximum number of these microorganisms is observed when ploughing is used, but the average number is not observed when disking is used (as for melanin-synthesizing bacteria), but with No-till technologies. The insertion of mineral fertilizers in the minimum dose changes the pattern even more: the maximum number of melanin-synthesizing micromycetes is found when using disking, the same – when using ploughing and No-till technology. At the maximum dose of fertilizers, the highest number of melanin-synthesizing micromycetes is sown from the soil in the No-till technology variant, the average – from the soil of the disking variant, the minimum – after ploughing. The number of melanin-synthesizing micromycetes under No-till technology significantly increases compared to the option without fertilizers when applying the maximum dose of fertilizers, which correlates with an increase in phytotoxicity of the soil by 9.52% (Tables 1, 2). Perhaps this is explained by the fact that micromycetes are the main toxin producers in the soil.

Table 2. *Indicators of the intensity of mineralization processes and phytotoxic properties of typical chernozem under various agrotechnical and agrochemical measures*

No.	Variant	Pedotrophicity index	Oligotrophy coefficient	Nitrogen mineralization coefficient	Humus mineralization activity, %	Total biological activity	Weight of 100 plants of the test culture – winter wheat, g
1	No-till technology, without fertilizers	0.175	0.117	0.282	23.3	727.2	11.5
2	No-till technology, $N_{30}P_{30}K_{65}$	0.226	0.183	0.268	8.42	765.6	10.3

Table 2, Continued

No.	Variant	Pedotrophicity index	Oligotrophy coefficient	Nitrogen mineralization coefficient	Humus mineralization activity, %	Total biological activity	Weight of 100 plants of the test culture – winter wheat, g
3	No-till technology, $N_{150}P_{100}K_{120}$	0.146	0.196	0.297	13.3	904.9	10.5
4	Disking, without fertilizers	0.248	0.112	0.373	10.1	910.8	12.8
5	Disking, $N_{30}P_{30}K_{65}$	0.229	0.209	0.350	9.11	1221.4	10.3
6	Disking, $N_{150}P_{100}K_{120}$	0.303	0.113	0.285	9.36	1094.8	9.32
7	Ploughing, without fertilizers	0.228	0.238	0.339	17.8	784.5	10.9
8	Ploughing, $N_{30}P_{30}K_{65}$	0.304	0.190	0.440	13.4	839.8	11.0
9	Ploughing, $N_{150}P_{100}K_{120}$	0.155	0.180	0.308	22.6	586.9	10.2
	LSD_{05}						0.11

Source: *developed by the authors of this study*

Upon disking, the highest number of melanin-synthesizing micromycetes is observed at the lowest of the investigated doses of fertilizers, which exceeds the indicator of the option without fertilizers by 4.54 times, the indicator of the option with the maximum dose of fertilizers – by 1.43 times. Upon ploughing, the maximum number of melanin-synthesizing micromycetes is also observed in the variant with the lowest of the investigated doses of fertilizers and significantly exceeds the number of these microorganisms only in the variant with the dose of fertilizers $N_{150}P_{100}K_{120}$ – by 1.98 times. This is explained, perhaps, by the fact that the dose of $N_{150}P_{100}K_{120}$ fertilizers turned out to be inhibitory for such tillage as disking and ploughing.

An important indicator is not only the number of melanin-synthesizing micromycetes, but their share in the total number of fungi. In the variant without fertilizers, the share of melanin-synthesizing micromycetes is maximum when ploughing is carried out, average – when using No-till technology, minimum – upon disking. Application of fertilizers leads to an increase in the share of melanin-synthesizing micromycetes in their total number under No-till technologies and disking. Conversely, upon ploughing, the share of melanin-synthesizing micromycetes decreases by 30.7% when $N_{30}P_{30}K_{45}$ is applied, and by 73.5% when $N_{150}P_{100}K_{120}$ fertilizer doses are applied. When disking is used as the main tillage, the share of melanin-synthesizing micromycetes gradually increases: from 3.30% (without fertilizers) to 7.83% ($N_{30}P_{30}K_{45}$) and up to 8.45% ($N_{150}P_{100}K_{120}$), which corresponds to the classic ideas about the increase in the toxic load with an increase in the dose of mineral fertilizers containing heavy metal impurities.

The spread of *a*zotobacter as a melanin-synthesizing microorganism is influenced, on the one hand, by doses of nitrogen fertilizers, and on the other hand by soil pollution with pollutants. A manifestation of the inhibitory effect of nitrogen fertilizers is a decrease in the number of *a*zotobacter with an increase in their dose: when using No-till technology by 2.21 times, when ploughing – by 4.36 and 3.79 times, respectively, with an increase in the dose of fertilizers. When disking, the number of *a*zotobacter ranges from 26.7 to 40.7% of soil clod growth. In addition, the number of *a*zotobacter is affected by the method of main soil cultivation, in the variant without fertilizers, its maximum number is observed when ploughing is used, possibly because ploughing provides better soil aeration, and representatives of the genus *a*zotobacter are obligate aerobes and require the presence of oxygen for their existence. With the incertion of mineral fertilizers, the development of *a*zotobacter in ploughed soil is suppressed to a greater extent than with disk shallow tillage, but to a lesser extent than with No-till technology, which is due, perhaps, to the difficulty of nitrogen diffusion of fertilizers into the soil.

One of the important characteristics of the microbial cenosis is the specific content of autochthonous microorganisms. According to the obtained data, the highest level of humus mineralization is observed in the version without fertilizers when using No-till technology (23.3%), the average level – when ploughing (17.8%), the minimum – when disking (10.1%) (Table 2). When using No-till technology, the application of mineral fertilizers in the dose of $N_{30}P_{30}K_{45}$ leads to a decrease in the activity of mineralization of humus by 2.77 times, as a result of the application of fertilizers in the dose of $N_{150}P_{100}K_{120}$ – by 75.0%, which is associated with better vegetative growth of plants, greater allocation root exudates, which, in turn, slows down the destruction of humus as a less available substrate. When disking, the application of mineral fertilizers slows down the mineralization of humus by 10.9% and 7.91%, respectively. The tendency to activate the decomposition of humus compounds at the maximum of the investigated doses of fertilizers is observed both upon disking and upon ploughing. In the variant using ploughing, the activity of humus destruction exceeds the indicators of the control (without fertilizers) and the variant with the minimum dose of fertilizers, which indicates the suboptimal dose of fertilizers $N_{150}P_{100}K_{120}$. The regularities regarding the activation of humus decomposition under the influence of various agricultural practices coincide with those previously established.

The index of pedotrophicity, which reflects the level of consumption of organic matter, in the variant using No-till technology, with the application of the minimum dose of fertilizers, increases by 29.1%, and with the application of the maximum dose of fertilizers, it decreases by 19.9% compared to the control. In the variant using disking, the pedotrophicity index slightly decreases with the minimum dose of fertilizers, while with the maximum dose it increases by 22.2%. When ploughing, the patterns of changes in the activity of consumption of soil organic matter are comparable to those observed when using No-till technology. The pedotrophicity index in the version without fertilizers has the maximum value upon disking, the average value upon ploughing, the minimum value upon using no-till technology. As a result of applying fertilizers in the dose of $N_{30}P_{30}K_{45}$, ploughing begins to be characterized by the maximum level of consumption of soil organic matter, when applying fertilizers in the dose of $N_{150}P_{100}K_{120}$ – soil cultivation with disc tools.

To establish the relationship between the indicators under study, a correlation analysis was conducted, which showed that the content of humus in typical chernozem correlates with the number of autochthonous microorganisms, melanin-synthesizing bacteria, their share in the total number of microorganisms, the PCF of polysaccharide-synthesizing bacteria and the activity of humus mineralization (Table 3).

Table 3. *Correlation coefficients between indicators of the state of the microbial cenosis and the content of humus in chernozem typical for growing sunflowers*

Indicator	**Azotobacter, % fouling of soil clods**	**Polysaccharide synthesizing**	**Autochthonous**	**Micromycetes**	**Melanin-synthesizing micromycetes**	**Share of melanin-synthesizing micromycetes in the total amount, %**	**Melanin-synthesizing bacteria**	**Share of melanin-synthesizing bacteria in the total number, %**	**Total number of microorganisms**	**PCF of azotobacter**	**PCF of micromycetes**	**PCF of polysaccharide-synthesizing bacteria**	**Humus mineralization activity, %**	**Total biological activity**	**Humus content, %**
Azotobacter, %	1.000														
Polysaccharide synthesizing	-0.038	1.000													
Autochthonous	0.460	0.156	1.000												
Micromycetes	-0.268	0.757	0.213	1.000											
Melanin-synthesizing micromycetes	-0.060	0.627	0.038	0.815	1.000										
Share of melanin-synthesizing micromycetes in the total amount, %	0.505	-0.017	-0.164	-0.135	0.444	1.000									
Melanin-synthesizing bacteria	-0.186	-0.477	-0.101	0.050	0.078	0.004	1.000								
Share of melanin-synthesizing bacteria in the total number, %	-0.226	-0.597	-0.322	-0.257	-0.190	-0.005	0.909	1.000							
Total number of microorganisms	0.033	0.626	0.477	0.830	0.551	-0.256	-0.193	-0.543	1.000						
PCF of azotobacter	0.543	-0.088	0.050	-0.439	-0.450	0.079	-0.228	-0.089	-0.179	1.000					
PCF of micromycetes	-0.645	0.498	1.000	0.480	0.553	0.064	-0.075	-0.048	0.070	-0.330	1.000				
PCF of polysaccharide-synthesizing bacteria	-0.059	0.375	-0.399	0.079	0.415	0.515	-0.621	-0.589	-0.027	-0.259	0.499	1.000			

Table 3, Continued

Indicator	Azotobacter, % fouling of soil clods	Polysaccharide synthesizing	Autochthonous	Micromycetes	Melanin-synthesizing micromycetes	Share of melanin-synthesizing micromycetes in the total amount, %	Melanin-synthesizing bacteria	Share of melanin-synthesizing bacteria in the total number, %	Total number of microorganisms	PCF of azotobacter	PCF of micromycetes	PCF of polysaccharide-synthesizing bacteria	Humus mineralization activity, %	Total biological activity	Humus content, %
Humus mineralization activity, %	0.294	-0.373	-0.152	-0.608	-0.448	0.127	0.049	0.312	-0.654	0.141	-0.191	-0.059	1.000		
Total biological activity	-0.039	0.773	0.156	0.944	0.788	-0.046	0.101	-0.183	0.775	-0.245	0.391	-0.035	-0.415	1.000	
Humus content, %	-0.099	-0.014	-0.338	-0.114	-0.052	-0.053	0.528	0.470	0.029	0.382	0.134	0.532	-0.335	-0.231	1.000
Yield, t/ha	-0.160	-0.587	0.135	-0.105	0.045	0.148	0.913	0.899	-0.329	-0.244	-0.052	-0.499	0.138	-0.079	0.320

Source: *developed by the authors of this study*

The content of humus is inversely correlated with the physiological and biochemical activity of azotobacter cells. Since both azotobacter, which can synthesize melanins (Noar *et al.*, 2018), and polysaccharide-synthesizing bacteria are related to the synthesis of humus molecules and take part in the formation of its resistance to bacterial mineralization (Iutinskaya, 1989), the emergence of these connections is understandable. The number of melanin-synthesizing bacteria in typical chernozem has an inverse highly significant relationship with the PCF of polysaccharide-synthesizing bacteria (r=-0.625). The share of melanin-synthesizing bacteria in the total number of microorganisms is inversely correlated with the number of polysaccharide-synthesizing bacteria (r=-0.597), the total number of microorganisms (r=-0.543), and the PCF of polysaccharide-synthesizing bacteria (r=-0.589).

The effect of mineral fertilizers, especially nitrogen fertilizers, on the number of melanin-synthesizing bacteria and micromycetes indicates that the main macroelements are necessary to reproduce representatives of this valuable agronomic group of microorganisms and their synthesis of melanins in natural soil conditions. This is confirmed by observations of the growth of microorganisms in monocultures. Thus, Reddy *et al.* (2007) showed that the growth of *Modestobacter versicolor sp. nov.* in conditions of low content of nitrogen compounds is accompanied by inhibition of melanin synthesis. Therefore, the incertion of mineral fertilizers into the soils of agrocenoses is necessary not only to optimize the mineral nutrition of agricultural plants, but to preserve and reproduce such an important factor of potential soil fertility as the humus content.

Soil microorganisms take part in the synthesis of humus in three ways: 1) through the synthesis of melanins, 2) the synthesis of exopolysaccharides and other secondary metabolites, 3) the synthesis of enzymes (Soil Humus Formation, 2018). The highly significant nature of the relationship between the content of humus and the number of melanin-synthesizing bacteria (r=0.528) and their share in the total number of microorganisms (r=0.470) suggests that humus is formed mainly with the participation of melanins of bacterial and not fungal origin, which is also confirmed by the low inverse correlation coefficients between the humus content and the number of melanin-synthesizing micromycetes (r=-0.052) and their share in the total number of fungi (r=-0.053). Since a low inverse correlation coefficient was found between humus content and the number of azotobacter (r=-0.099), it can be assumed that melanins of bacteria of the genus *Azotobacter* take a minor part in the synthesis of humus molecules when growing sunflowers on typical chernozem, but this is contradicted by a sufficiently high correlation coefficient between humus content and physiological and biochemical activity of azotobacter cells (r=0.372). It is known that with a small number of bacteria of a certain group, they can quickly activate the synthesis of metabolites due to an increase in the physiological and biochemical activity of cells without increasing the number of cells, i.e., reproduction.

Earlier, Iutinskaya (1989) showed that decomposition products of microbial exopolysaccharides react with humus molecules, resulting in the formation of complexes more resistant to microbial degradation. The obtained data confirmed the existence of a direct, moderately significant relationship between the physiological and biochemical activity of cells of polysaccharide-synthesizing bacteria and the content of humus (r=0.532), which reflects the increase in the resistance of humus molecules to mineralization by autochthonous microorganisms. This phenomenon contributes to the accumulation of humus since the mineralization activity of such polysaccharide-humate complexes decreases.

Between the content of humus and the number of autochthonous (humus-degrading) microorganisms, a moderately significant inverse correlation was found (r=-0.338), which emphasizes the negative influence of autochthonous bacteria on the accumulation of humus. In addition, a moderately significant inverse correlation (r=-0.335) was found between the humus content and the activity of humus mineralization by soil microorganisms, which is quite natural and is confirmed by the results of previous studies (Malynovs`ka *et al.*, 2021)

The analysis of the obtained data shows that the number of polysaccharide-synthesizing microorganisms has a direct correlation with the number of micromycetes (r=0.757), melanin-synthesizing micromycetes (r=0.627), and an inverse correlation with the number of melanin-synthesizing bacteria (r=-0.477), their share in the total number of microorganisms (r=-0.597), insignificant connection – with the number of azotobacter. This suggests that when there are such conditions in the soil that melanins of micromycetes take a greater part in the synthesis of humus molecules, the role of polysaccharide-synthesizing bacteria becomes more significant.

The highly significant correlation coefficient between the number of melanin-synthesizing bacteria (r=0.913), their share in the total number of microorganisms (r=0.899) and the crop yield are noteworthy. Perhaps this is a reflection of the constantly operating mechanism of the influence of melanin synthesis on the content of humus in typical chernozem, since the connection between the synthesis of humus and the productivity of agricultural crops is not so direct, it can become direct only under conditions of long-term action.

It was previously shown that in grey forest soil, closer relationships are formed between the humus content and the number of melanin-synthesizing micromycetes (r=0.811) and their share in the total number of fungi (r=0.915), with the number of melanin-synthesizing bacteria forming medium-level relationships significance (r=0.417), as with their share in the total number of microorganisms (r=0.450). In typical chernozem, the synthesis of humus is more influenced by melanin-synthesizing bacteria, the physiological and biochemical activity of azotobacter cells and polysaccharide-synthesizing bacteria. Thus, the processes of humus synthesis with the participation of melanins take place in diverse types of soils according to various patterns, depending on the agricultural methods used.

CONCLUSIONS

It was found that the main tillage and doses of mineral fertilizers affect the number of melanin-synthesizing microorganisms in typical chernozem. The soil upon ploughing at 25-27 cm is characterized by the largest number of melanin-synthesizing bacteria, the average – upon shallow disc tillage at 10-12 cm, the smallest number – when using No-till technology.

Application of mineral fertilizers in a dose of $N_{30}P_{30}K_{65}$ increases the number of melanin-synthesizing bacteria by 3.6 times (ploughing), 3.5 times (disking), and by 2.3 times – when using No-till technology. The use of mineral fertilizers in a dose of $N_{150}P_{100}K_{120}$ contributes to the growth of the number of melanin-synthesizing bacteria by 5.0 times when ploughing, 5.8 times when disking, and 2.2 times when using no-till technologies.

The maximum number of melanin-synthesizing micromycetes is observed in the soil of the variant without fertilizers when ploughing is used, the average number is observed when using the No-till technology, and the minimum number is observed using disking. Application of mineral fertilizers in a dose of $N_{30}P_{30}K_{65}$ changes the pattern: the largest number of melanin-synthesizing micromycetes is found in the soil when using disking, while ploughing and no-till technology reveal the same number of melanin-synthesizing micromycetes.

The number of melanin-synthesizing bacteria (r=0.528) and their share in the total number of microorganisms (r=0.470) has the greatest influence on humus synthesis in chernozem typical of sunflower cultivation. Melanins produced by azotobacter cells take part in the synthesis of typical chernozem humus, which is confirmed by the direct average correlation coefficient between the humus content and the physiological and biochemical activity of azotobacter cells (r=0.372).

Polysaccharide-synthesizing bacteria take part in the formation of the resistance of humus molecules to microbial mineralization, the probability of the formation of colonies of which has a moderately significant relationship with the content of humus (r=0.532). In the synthesis of humus molecules of chernozem typical for growing sunflowers, melanins of fungal origin take a minor part, which is confirmed by the low inverse values of the correlation coefficients between the humus content and the number of melanin-synthesizing micromycetes (r=-0.052) and their share in the total number of fungi (r=-0.053).

The study of the impact of agrotechnical and agrochemical factors on the processes of synthesis of melanins, which are the precursors of humus molecules, has the prospect of continuing, involving other agricultural crops grown on typical chernozem, since the type of agricultural crop substantially affects the course of mineralization and synthesis processes in the microbial community of the rhizosphere soil.

ACKNOWLEDGEMENTS

None.

CONFLICT OF INTEREST

The authors of this study declare no conflict of interest.

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Вплив агротехнічних заходів на чисельність меланінсинтезувальних мікроорганізмів

Ірина Михайлівна Малиновська
Член-кореспондент НААН, доктор сільськогосподарських наук, старший науковий співробітник
ННЦ «Інститут землеробства НААН»
08162, вул. Машинобудівників, 2-б, смт. Чабани, Україна
https://orcid.org/0000-0001-8002-7139

Віктор Францевич Камінський
Академік НААН, доктор сільськогосподарських наук, професор
ННЦ «Інститут землеробства НААН»
08162, вул. Машинобудівників, 2-б, смт. Чабани, Україна
https://orcid.org/0000-0002-9668-6742

Єлизавета Валеріївна Задубинна
Кандидат сільськогосподарських наук, старший науковий співробітник
Панфільська дослідна станція ННЦ «Інститут землеробства НААН»
07750, вул. Центральна, 2, с. Панфіли, Україна
https://orcid.org/0000-0002-9428-5603

Володимир Григорович Кургак
Член-кореспондент НААН, доктор сільськогосподарських наук, професор
ННЦ «Інститут землеробства НААН»
08162, вул. Машинобудівників, 2-б, смт. Чабани, Україна
https://orcid.org/0000-0003-2309-0128

Віктор Петрович Карпенко
Доктор сільськогосподарських наук, професор
Уманський національний університет садівництва
20301, вул. Інститутська, 1, м. Умань, Україна
https://orcid.org/0000-0001-5607-7371

Анотація. В зв'язку із зростанням масштабів дегуміфікації ґрунтів все більш актуальними стають дослідження закономірностей синтезу попередників гумусових молекул – меланінів бактеріального походження. Мета досліджень полягає в установленні впливу на розповсюдження меланінсинтезувальних мікроорганізмів основних чинників агровиробництва: способу основного обробітку ґрунту, мінерального удобрення, виду сільськогосподарської культури. Методи дослідження: мікробіологічний, лабораторно-аналітичний, статистичний, кореляційний. В результаті проведених досліджень вперше встановлено, що основний обробіток ґрунту впливає на чисельність меланінсинтезувальних мікроорганізмів: мікроміцетів, бактерій, азотобактера. У варіанті без внесення мінеральних добрив (контроль) мінімальною кількістю меланінсинтезувальних бактерій характеризується ґрунт за застосування No-till-технології, на 28,8 % більшою – із застосуванням дискування і у 2,4 раза більшою – за проведення оранки. Кількість меланінсинтезувальних мікроміцетів у варіанті без добрив також максимальна за застосування оранки, середня кількість спостерігається за No-till-технології, мінімальна кількість – за використання мілкого дискового обробітку. Внесення мінеральних добрив у дозі $N_{30}P_{30}K_{65}$ призводить до збільшення чисельності меланінсинтезувальних бактерій у варіанті оранки в 3,58 рази, дискування – 3,53, No-till –технології – в 2,28 рази. Зростання дози мінеральних добрив до $N_{150}P_{100}K_{120}$ сприяє збільшенню чисельності меланінсинтезувальних бактерій за оранки в 5,04 раза, за дискування – 5,78, за No-till-технології – в 2,24 раза. Середньозначимий характер зв'язку між вмістом гумусу і чисельністю меланінсинтезувальних бактерій (r=0,528) та їхньою часткою у загальній кількості мікроорганізмів (r=0,470) дозволяє зробити висновок про те, що у чорноземі типовому за вирощування соняшнику гумус утворюється, в основному, за участі меланінів бактеріального походження. У формуванні стійкості молекул гумусу до мікробної мінералізації приймають участь метаболіти полісахаридсинтезувальних бактерій, вірогідність формування колоній яких має середньозначимий зв›язок із вмістом гумусу (r=0,532). Результати цих досліджень можуть бути використані при розробленні рекомендацій щодо регулювання вмісту гумусу в агроземах, запобігання його надмірної мінералізації, що дозволить зберегти та підвищити родючість ґрунтів

Ключові слова: меланіни; бактерії; мікроміцети; азотобактер; гумус; частка; кореляція