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The Effect of Chemicals of Plant Protection Products on Soil Microbiocenoses

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Abstract. With the emergence of agriculture, humankind faced numerous difficult tasks. For example, how to use the potential of the soil while saving its quality and functional properties, how to apply agricultural technologies effectively and environmentally friendly, how to make them safe for human health and biota, and many others. There are several informative and reliable recording criteria and indicator systems that fully and comprehensively describe changes in the ecological condition of the soil and agrocenoses. However, all these systems have one defect – the time from the impact of the factor to the "reaction" of the indicator. Early diagnosis of changes in the agrocenosis is possible due to the biological component of the soil, namely the microbiocenosis. Notably, that microorganisms have a large contact surface with the environment, high rates of reproduction in space and time, sensitivity to changing living conditions. The reaction of soil microbiocenosis and its activity (number of microorganisms of ecological-trophic and taxonomic groups, respiration intensity, microbial biomass content, and soil phytotoxicity) under the action of chemicals that are the basis of plant protection products (PPP) was studied in the laboratory. It was found that the number of microorganisms of different ecological-trophic and taxonomic groups under the action of a composition of cymoxanil with dimetomorph decreased by 1.5-4.5 times relative to control, chlorperifos-methyl in 1.1-2 times, and prometryn – not more than 1.5. The content of microbial biomass and the intensity of carbon dioxide emissions when using cymoxanil with dimethomorph compared to the control variant decreased by 44% and 51.4-64.8%, respectively; prometryn – by 10-13% and by 8-12%. The highest level of soil phytotoxicity was observed for variants using prometryn (20-24%), the lowest for a composition of cymoxanil with dimetomorph (7-12%). It was shown, that the high level of inhibition of test culture development with the use of prometryn associated with the class of PPP and the mechanism of its effect on the plant organism. Low indicators of soil phytotoxicity and microbiocenosis activity when using cymoxanil with dimetomorph are explained by the influence of the studied composition not only on phytopathogenic micromycetes, but also on all groups of soil micromycetes (cellulose-destroying, saprophytic) that dominate. Therefore, the influence of PPP chemicals on the microbiocenosis can be shown as follows: *PROMETRYN (the lowest level of influence) → CHLORPYRIFOS-METHYL → CYMOXANIL + DIMETOMORF (the highest level of influence)*

Keywords: microbiocenosis, microorganisms of the main ecological-trophic and taxonomic groups, emission of carbon dioxide, microbial biomass

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INTRODUCTION

The issues of soil quality, its functional properties, and the saving of a high yield were of the highest priority for humankind since the emergence of agriculture, which happened at least 10 to 23 thousand years ago [1; 2]. Soil, a natural body at the atmosphere-lithosphere interphase, is a dynamic entity teeming with life. Soil is an organic-C-mediated realm in which solid, liquid, and gaseous phases interact at a scale ranging from nanometers to kilometers and create dynamic environments conducive to the growth and development of plants and other biota [3]. One of the most important indicators for agricultural producers, both in Ukraine and in the world, is soil fertility. The territory of Ukraine concentrates in itself about 28% of the world's chernozem (black soil), which has the highest fertility rates [4].

The beginning of the XXI century combined a wide range of progressive, intensive, environmentally friendly, organic technologies for the most productive agricultural production. However, at the same time, scientists note that Ukraine's agricultural landscapes are in crisis. Sharp and ubiquitous, declining soil fertility and large-scale spread of degradation processes determine the need for significant changes in human economic activity and environmental management. Ecologically safe usage of natural resources is one of the necessary conditions for sustainable development not only of the agricultural sphere but also of society in general [5].

There are several informative and reliable recording criteria and systems of indicators that fully and comprehensively describe changes in the ecological condition of the soil and agrocenoses [6]. In practice, the system of normative agrochemical indicators – the base of physicochemical, agrophysical, and ecotoxicological indicators – is widely used. Appropriate to notice that all these bases are reliable and maximally characterise all the processes occurring in agricultural landscapes, but there is one defect – the time from the impact of the factor to the "reaction" of the indicator, which is recorded. However, the researchers from the Institute of Agroecology and Environmental Management of NAAS for several decades have been developing a system of indicators of the biological component of the soil, namely the microbiocenosis, which allows an early diagnosis of changes in the soil environment [7; 8].

Early diagnostics is possible because the microbiota is multifunctional and, participating in opposite reactions, performs a stabilising function of metabolic balance in nature. Due to the large surface of contact with the environment, it is very sensitive to changing living conditions, and the high rate of reproduction makes it possible in a short time to detect changes that occur under the influence of environmental factors [9]. Substances used in agro-industrial production, namely fertilisers, fungicides, herbicides, insecticides, acaricides, defoliants, adhesives, and many others directly affect both plants and animals and can accumulate in the soil. The stockpiling active substances of pesticides and agrochemicals are able to inhibit or stimulate the development of agronomically useful and saprophytic microflora. Direct or indirect action of pesticides may extend to the development of soil pathogens, growth, sporulation, germination of propagules, survival and competitive saprophytic activity of soil fungi [10-12].

Most of the works are dedicated to the effects of pesticides and agrochemicals on soil microflora, based on field studies that included the composition of the study preparation, soil moisture, pH, heavy metal content, weather conditions, and many other biotic and abiotic factors [13-15]. All this shows a whole system of influence, which complicates the interpretation of the results and generalisation of conclusions that relate directly to the impact of agrochemicals. Therefore, the evaluation and results of exposure to the active substance of any agrochemical or pesticide should be carried out in a laboratory with well-controlled conditions for all study variants.

The purpose of the study was to determine the response and the level of influence of active substances of the most common plant protection products (PPP) on the structure of the microbiocenosis.

MATERIALS AND METHODS

Laboratory studies were performed on soil samples taken at the Skvyra Research Station of the Institute of Agroecology and Environmental Management of NAAS (Table 1). The studies used chemicals that are the basis of the most widely used plant protection products in agriculture (Table 2).

Table 1. *Agrochemical characteristic of soil in stationary field experiments, 0-20 cm*

Type of soil	pH salt	Humus, %	Content, mg/kg of soil		
			Nitrogen which easily hydrolises	Active phosphorus	Exchangeable potassium
Typical chernozem	6.5	4.3	110	240	85

***Table 2**. Characteristics of chemicals used for research*

Name of active substance	Alternative names	Chemical formula (class)	Action	Class PPP
Prometryn	Prometryn, mercazine, gezagard, selectin, zirazin, A-1114, G-34161	$C_{10}H_{19}N_5S$ (triazines)	Inhibits the level of photosynthesis of plants by inhibiting the photosystem II	Herbycide
Cymoxanil	Cymoxanil, kurzat	$C_7H_{10}N_4O_3$ (other)	Inhibits sporulation of micromycetes by inhibiting RNA biosynthesis in their cells	Fungicide
Dimetomorf	Dimetomorf, acrobat, CME 151, forum, WL 127294	$C_{21}H_{22}ClNO_4$ (morpholines)	Breaks the development cycle of micromycete, causes antispore-forming effect due to modification of the cell wall of the micromycete	Fungicide
Chlorpyrifos-methyl	Chlorpyrifos-methyl; chlorpyrifos-methyl; cooper greencott; dowco 214; dowco 214; dowco-214; dursban methyl; dursbanmethyl	$C_7H_7Cl_3NO_3PS$ (organophosphorus compounds)	Neurotoxic effect, hydrolyses the enzyme acetylcholine esterase to acelcholine. Accumulation of acetylcholine in the synaptic cleft blocks the transfer of nerve impulses	Insectecide-acarcide

The influence of each chemical on the state of the microbiocenosis was studied in 3 concentrations (min, max, 10 * max), in accordance with the regulations of research established by the Ministry of Environmental Protection and Natural Resources of Ukraine [16; 17]. After sampling and before the beginning of the experiment, the soil was sieved through a 2 mm sieve and the remains of plants, roots, insects, worms, etc. were removed. The sifted soil was transferred to vegetative ceramic pots at the rate of 3 kg of soil (in terms of dry weight). The study included the following variants: 1. Control – whithout using chemical substances; 2. Prometryn; 3. Composition Cymoxanil + Dimetomorf; 4. Chlorpyrifos-methyl.

Control and experimental soil samples were moistened to 60% moisture content, 1-fold min concentration of active substance (1 min) was added to the experimental, 1-fold max concentration of active substance (1 max) and 10-fold max concentration of active substance (10 max), carefully mixed and composted at a temperature of + 22±2ºC for 30 days. Determination of the main indicators of the study was performed on 14 and 28 days of soil incubation [17-19].

Reactions of microbiocenosis and the level of influence on the structure were determined by the number of microorganisms of different ecological-trophic and taxonomic groups (ammonifiers, bacteria using mineral nitrogen, spore-forming bacteria, nitrogen-fixing and phosphate-mobilising microorganisms, *Azotobacter*, oligotrophs, pedotrophs, streptomycetes, micromycetes), carbon dioxide emission intensity, microbial biomass content, and phytotoxicity.

Microbiological analyses such as soil sampling, making 10-fold serial dilution of soil suspensions in physiological solution and their sowing on agar nutrient media were performed by conventional methods. The number of soil microorganisms was determined by sowing appropriate dilutions of microbial suspensions on agar media, which correspond to the trophic needs of microorganisms of the main ecological-trophic and taxonomic groups, the subsequent colonies counting. The amount of ammonifiers was determined on meat-peptone agar, spore-forming bacteria – on meat-peptone agar with the addition of glucose, micromycetes – on Czapek medium, bacteria that use mineral forms of nitrogen (on the 4th day of incubation of plates) and streptomycetes (on the 7th day of incubation of plates) – on starch-ammonia agar, oligonitrophils – on Ashbys medium diluted 100 times, pedotrophs – on medium containing soil extract [20; 21].

The content of total microbial biomass (C_{mic}) in the soil was determined by the rehydration method due to drying of soil samples at a temperature of 65-70ºC for 24 h followed by extraction with 0.5 M K_2SO_4 solution [22]. Determination of CO_2 released by the soil was performed in the laboratory from a constant temperature and humidity by the adsorption method – after alkaline adsorption and titration method determined the amount of CO_2 released from the soil [21; 23]. The phytotoxicity of the soil was measured by the Grodzinsky method in the Mochalov-Sherstoboev modification. *Raphanus sativum* seeds were used as a test culture. Inhibition of germination of radish seeds with a white tip was expressed as a percentage (%) [24].

Statistical processing of the research results, calculation of the standard deviation, and the smallest significant difference were carried on using the standard computer software package Microsoft Excel. The tests were performed in 5-fold repetition [25].

RESULTS AND DISCUSSION

Many years of soil research and determination of its impact on human life contributed to the fact that in the 1970-s 3 separate, but at the same time similar and maximally related, terms were formulated [26-28]. Namely, soil quality, soil health, and soil functionality. "Soil quality" usually describes the suitability of the soil for use in agricultural production [29]. The term "soil functionality" means the complex characteristics of particular land use with the productivity of plants and animals. "Soil health" – the ability of soil to function as a living system to maintain biological productivity and stabilisation of the health of plants, animals, and humans with the highest quality of the environment [27; 28]. Thus, soil health affects life and its characteristics, depending entirely on biodiversity and its dynamics. Moreover, numerous studies have shown that there is an integral connection between human health and soil condition [30-32].

Microbial groups of soil are represented by a set of interacting organisms that inhabit ecological niches with homogeneous conditions and carry out the transformation of energy and organo-mineral substances of this ecosystem. All connections in the grouping between members of associations of microorganisms are based on trophic interactions. Most soil microorganisms, with the exception of oligotrophs, remain inactive until the arrival of exogenous substrate [33]. The biological activity of soil is a function of its living component, which is manifested through the intensity of biological processes and is critical for ensuring the stability of ecosystems [34; 35]. Among the biodiagnostic parameters, the assessment of the number of microorganisms of the main ecological-trophic and taxonomic groups, the content of microbial biomass, the intensity of carbon dioxide emissions is especially important. The biological activity of soils is determined by the amount and composition of microbial metabolites, such as enzymes, amino acids, carbohydrates, nucleic acids, etc. These indicators not only correlate with microbial biomass but also reflect the nature of the transformation of organic matter and the specifics of environmental conditions [9; 36; 37]. Soil organic matter, comprising about 45-60% of its mass as soil organic C, is a principal source of energy for soil microorganisms [3].

The introduction of test substances presumably caused a reaction of a group of microorganisms to an exogenous factor. It was found that the number of microorganisms of the main ecological trophic and taxonomic groups directly depended on the type and composition of the test substance. The indirect effect, within statistically significant intervals, of the studied substances on the number of microorganisms of the main ecological-trophic and taxonomic groups was recorded depending on the study period (14 or 28 days) and the concentration of the introduced substance (min, max, 10 max). The number of ammonifiers for the introduction of prometryn in comparison with the control variant decreased, in the range of 10-12% (Table 3). The application of chlorperifos-methyl to the soil reduced the number of ammonifiers on the 14th day of the study by 1.3 times and on the 28th day by 1.5 times compared to the control. A significant decrease in ammonifiers was caused by the introduction of cymoxanil with dimethomorph. On the 14th day, the number of ammonifiers was noted at the level of 6.4 million CFU/g of soil, which is 1.6 times lower than in the control, on the 28th day the number of ammonifiers was 6.2 million CFU/g of soil, which is 1.7 times below control. Similar results were observed for spore-forming bacteria. On the 14th day of the study, the number of spore bacteria decreased by 28% and amounted to 1 million CFU/g of soil, and with the use of chlorperiphos-methyl – by 43% (0.8 million CFU/g of soil) compared to the control. The introduction of cymoxanil with dimetomorf caused a decrease in spore-forming bacteria 2.5 times on day 14 and 2.7 times on day 28. The introduced substances significantly affected the number of nitrogen-fixing microorganisms and bacteria that use mineral nitrogen. On the 14th day, the introduction of prometryn reduced the number of nitrogen fixers from 6.3 million CFU/g of soil (control) to 5 million CFU/g of soil on average at the applied concentrations. On day 28, reductions in nitrogen fixers fluctuated in statistically significant intervals on day 14 of the study. The introduction of prometryn into the soil reduced the number of bacteria using mineral nitrogen by no more than 10%. The introduction of chlorperiphos-methyl into the soil caused a decrease in bacteria using mineral nitrogen and nitrogen-fixing microorganisms, regardless of the applied concentration and the day of the study, by 1.1-1.3 times. Cymoxanil with dimetomorf reduced the number of nitrogen fixers compared to the control by 2-2.3 times, and the number of bacteria that use mineral nitrogen by 1.4 times.

***Table 3**. The number of microorganisms of the main-ecological trophic and taxonomic groups for the introduction of active substances PPP*

		Ammonifiers, *106 CFU/g of soil	Spore-forming bacteria, *106 CFU/g of soil	Mineral nitrogen immobilisers, *106 CFU/g of soil	Azotfixators, *106 CFU/g of soil	Phosfatmobilisers, *106 CFU/g of soil	Streptomicetes, *106 CFU/g of soil	Oligotrophs, *106 CFU/g of soil	Pedotrophs, *106 CFU/g of soil	Micromicetes, *103 CFU/g of soil	*Azotobacter*, % lumps of fouling
						14 day					
Control	–	*10.3±0.8*	*1.4±0.07*	*9.3±0.6*	*6.3*±0.4	*1.24±0.05*	*3.6±0.08*	*11.3±0.9*	*13.9±0.8*	*41.0±2.6*	*84*
Prometryn	min	9.9±0.6	1.1±0.06	8.0±0.6	5±0.3	0.9±0.06	2.2±0.1	10.7±0.5	11.9±0.6	30.4±2.1	67
	max	9.3±0.7	1±0.04	8.3±0.7	4.7±0.2	1.0±0.05	1.8±0.2	10.0±0.5	12.0±0.6	32.8±2.3	62
	10 max	9.6±0.8	0.98±0.03	8.6±0.7	5.1±0.3	0.8±0.06	2.5±0.1	10.2±0.5	11.6±0.5	31.9±2.2	68
Cymoxanil + Dimetomorf	min	6.4±0.7	0.47±0.01	6.7±0.5	3.0±0.1	0.4±0.03	0.9±0.1	8.5±0.9	9.2±1.1	17.0±2.7	45
	max	6.5±0.6	0.58±0.02	6.4±0.6	2.9±0.1	0.3±0.02	0.8±0.08	8.0±0.8	9.8±1.0	16.7±2.8	33
	10 max	6.4±0.7	0.51±0.02	6.8±0.6	2.6±0.1	0.4±0.02	1.0±0.06	8.8±0.8	9.9±1.0	17.5±2.7	48
Chlorpyrifos-methyl	min	8.1±0.6	0.81±0.06	7.1±0.8	4.1±0.2	1.0±0.06	1.6±0.13	9.2±0.7	10.8±0.7	21.8±1.4	58
	max	7.9±0.7	0.77±0.04	7.0±0.6	5.3±0.3	0.9±0.08	1.4±0.15	9.0±0.7	10.6±0.7	23±1.3	60
	10 max	8.2±0.6	0.82±0.03	7.7±0.6	4.7±0.2	0.8±0.06	1.6±0.16	8.8±0.7	10.2±0.8	19.0±1.3	54
						28 day					
Control	–	*10.8±0.9*	*1.2±0.06*	*8.9±0.7*	*6.5±0.4*	*1.1±0.04*	*3.4±0.2*	*11.1±0.9*	*13.7±1.1*	*40.9±2.6*	*88*
Prometryn	min	9.8±0.9	1±0.1	8.4±0.6	4.8±0.3	0.9±0.06	2.1±0.15	10.6±0.7	12±0.8	31.3±2.2	64
	max	8.9±0.7	0.9±0.07	8.6±0.7	5.2±0.32	0.95±0.07	1.8±0.1	9.8±0.5	11.8±1	34±2.3	65
	10 max	9.1±0.8	1.05±0.1	8.7±0.7	5±0.3	0.8±0.04	1.5±0.1	10.2±0.6	11.9±1.1	33.2±2.5	66
Cymoxanil + Dimetomorf	min	6.3±0.5	0.39±0.02	6.1±0.4	2.97±0.1	0.46±0.02	0.9±0.06	8.8±0.6	9.9±0.9	17.3±1.5	44
	max	6.2±0.6	0.4±0.03	6.06±0.5	2.64±0.1	0.3±0.02	0.88±0.07	7.8±0.5	9.2±0.8	16.9±1.4	36
	10 max	6.1±0.5	0.46±0.03	6±0.4	2.84±0.1	0.4±0.02	0.9±0.06	8.3±0.6	9.4±0.9	17.3±1.5	45
Chlorpyrifos-methyl	min	7.9±0.7	0.8±0.06	7.3±0.6	4.3±0.3	0.9±0.06	1.5±0.1	9.0±0.7	10.5±0.7	21.6±1.8	56
	max	7.8±0.8	0.75±0.05	7.5±0.5	5.7±0.2	0.8±0.06	1.3±0.1	9±0.8	10.3±0.7	22.5±2	59
	10 max	7.3±0.8	0.8±0.06	7.2±0.5	4.9±0.2	0.8±0.06	1.2±0.2	8.4±0.7	10.2±0.7	19.7±1.7	52

Promethrin and chlorperiphos-methyl did not significantly reduce the number of phosphate-mobilising bacteria compared to controls during the study period. The number of phosphate-mobilising bacteria under control on the 14th day was 1.24 million CFU/g of soil, on the 28th day – 1.11 million CFU/g of soil. When introducing prometryn and chlorperiphos-methyl, regardless of the day of the study, the number of phosphate-mobilising bacteria was noted at the level of 0.8-1 million CFU/g of soil. The application of cymoxanil with dimetomorf reduced the number of phosphate-mobilising bacteria in comparison with the control by 3 times, the number of this trophic group was fixed at 0.3-0.4 million CFU/g of soil. Similar dependencies were observed for oligotrophic microorganisms and pedotrophs. The level of growth of bacteria of the genus *Azotobacter* as an indicator of soil cultivation indicates that the introduction of cymoxanil with dimetomorph into the ecosystem reduced the percentage of fouling of lumps by bacteria of the genus *Azotobacter* by 1.8-2.5 times compared to the control. The introduction of cymoxanil from dimetomorphs compared with prometryn reduced the growth of *Azotobacter* by approximately 1.5 times and compared with chlorperiphos-methyl 1.3 times. Micellar groups of microorganisms, namely micromycetes and streptomycetes, significantly responded to the introduction of test substances.

The introduction of prometryn compared with the control reduced the number of micromycetes by 1.3 times, streptomycetes by 1.5-2 times depending on the concentration of the introduced substance. Chlorperiphos-methyl compared with the control and depending on the concentration caused a decrease in micromycetes by 1.7-2.1 times, a decrease in the number of streptomycetes was recorded at the level of 2-2.5 times. Cymoxanil with dimetomorf reduced the number of micromycetes by 2.5 times and streptomycetes by 3.5 to 4.5 times relative to the control variant.

Thus, the soil microbiocenosis actively responded to the introduction of the studied substances, which is quite indicative of the number of microorganisms of different ecological-trophic and taxonomic groups. The largest decrease in microorganisms of all ecological-trophic and taxonomic groups in the microbiocenosis compared with the control caused the introduction of cymoxanil with dimetomorf on average from 1.5 to 4.5 times; the use of chlorperiphos-methyl caused a decrease in numbers in the range of 1.1 to 2 times, prometryn reduced various ecological and trophic groups of microorganisms to 1.5 times on average.

It was found that the content of microbial biomass depended on the test (studied) substance (Table 4) and did not depend on the concentration of the test substance (min, max, 10 max) and fluctuated in the intervals of statistically significant intervals. It was found that the level of biomass did not depend on the study period (14 or 28 days).

***Table 4**. Biological activity of soil*

The active substance of the pesticide	Concentration a.s.	Microbial biomass, μg C/g of soil		Intensity of CO_2 emission of soil, mg CO_2/kg soil per day	
		14 day	28 day	14 day	28 day
Control	–	*601.3±36.1*	*597.8±29.9*	*246.8±14.8*	*241.4±16.6*
Prometryn	min	536.4±32.2	517.7±25.9	223.3±17.9	192.1±15.3
	max	521.9±20.9	504.2±36	226.7±13.6	195.1±17.6
	10 max	528.3±26.4	536.6±43	217.1±15.2	188.3±13.2
Cymoxanil + Dimetomorf	min	316.6±22.2	322.4±16.1	122.4±7.3	86.3±6.3
	max	336.4±20.2	339.5±27.2	108.5±8.7	82±6.7
	10 max	321.5±22.5	312.8±18.2	114.7±8	73.2±6.5
Chlorpyrifos-methyl	min	454.2±36.4	456.8±22.8	186.5±11.2	167.2±11.4
	max	429.7±25.8	437.2±30.6	197.1±10.8	155±11.1
	10 max	441.1±30.9	438.3±26.3	192.6±8.7	151.4±10.9

The use of prometryn reduced the content of microbial biomass by an average of 10-13%, depending on the concentration of the active substance. Studies of chlorperiphos-methyl showed that the content of microbial biomass in the studied samples ranged from 429.7 to 454.2 μg C/g of soil, which is 25% lower compared to the control version (without the introduction of any substances). The highest level of reduction of microbial biomass content was caused by the introduction of a composition of cymoxanil and dimetomorf into the soil. The obtained data correlate with the indicators of the number of microorganisms of ecological-trophic and taxonomic groups. Thus, for the experimental variants, there was a significant decrease in the content of microbial biomass by an average of 44% compared to the control variant, 36% compared with prometryn, and 25% – chlorperiphos-methyl.

The use of the studied substances affected the state of the microbial coenosis and caused a decrease in the "respiration" of the soil depending on the variant and period of the study. The concentration of the test substance (min, max, 10 max) did not significantly alter the studied indicator. The use of chlorperiphos-methyl caused a decrease in the intensity of carbon dioxide emissions by 20.7% (14 days of the study) and 31.5% (28 days). In variants with the use of prometryn on the 14th day there was a decrease in the intensity of carbon dioxide emissions by 8%, and on the 28th day – by 12%. The composition of cymoxanil with dimetomorf compared with the control variant caused a decrease in the intensity of carbon dioxide emissions by 51.4% (14 days of the study) and 64.8% (28 days of the study). There was a slight decrease in the intensity of carbon dioxide emission for variants using min, max, and 10 max concentrations of prometryn and chlorperiphos on the 14th and 28th days of the study. However, the use of a composition of cymoxanil with dimethomorph also depended on the period of application. Thus, the indicators of carbon dioxide emission intensity on the 14th day were on average 30% higher than on the 28th day of the study.

The study of soil phytotoxicity with the introduction of PPP chemicals is shown in Table 5. It was found

that the highest level of inhibition-test culture growth (from 20 to 24%) was caused by prometryn. Thus, the soil when using prometryn is slightly phytotoxic. In variants using chlorperiphos-methyl, the level of inhibition of the test culture was recorded at the level of 15-18%, at the level of control. The use of a composition of cymoxanil with dimetomorf reduced the level of soil phytotoxicity from 6 to 10%, compared with the control. That is, this composition inhibits the level of development of phytopathogenic microorganisms and the accumulation of their toxic substances in the soil. Thus, the soil of the variant using a composition of cymoxanil with dimetomorf, regardless of the concentration of the study, is not phytotoxic.

***Table 5**. Inhibition of growth of test plants at application of active substances PPP, %*

Concentration a.s.	Prometryn		Cymoxanil + Dimetomorf		Chlorpyrifos-methyl	
	14 day	28 day	14 day	28 day	14 day	28 day
Control	*18*	*16*	*17*	*18*	*16*	*17*
min	20	21	11	8	18	16
max	22	23	9	12	16	16
10 max	22	24	7	9	17	15

Thus, studies of the effect of introduced substances (prometryn, chlorperiphos-methyl and composition of cymoxanil and dimetomorf) on the state of the microbiocenosis show that the microbial group is most indicative and in a short period allows determining the impact of exogenous chemicals on the number of ecotrophic and taxonomic groups and biological activity of the soil.

Any microbiocenosis consists of microorganisms of different functional and taxonomic groups, which differ in requirements for environmental conditions, nutrition, and energy sources. The quantitative ratio between these groups depends entirely on environmental conditions (abiotic and biotic factors), in which microbial coenosis is formed [38]. As a result of the study, it was found that the soil microbiocenosis reacted most significantly to the use of a composition of cymoxanil and dimetomorf. As a result, a decrease in the number of microorganisms of all ecological-trophic and taxonomic groups, biomass content, and intensity of carbon dioxide emissions was observed. Inhibition of physiological and metabolic processes in the microbiocenosis when using a composition of cymoxanil with dimethomorph is explained by the specific effect of the studied substances. Thus, the studied composition of cymoxanil with dimetomorf affects not only phytopathogenic fungi (inhibits their reproduction) but also all groups of soil micromycetes (cellulose-destroying, saprophytic), which predominate in the formation of a pool of soil microorganisms. The use of chlorperiphos-methyl also caused a restructuring of the microbiocenosis, but the inhibition of metabolic reactions was significantly lower compared with a composition of cymoxanil and dimetomorf. The use of prometryn (the active substance of a significant amount of soil herbicides) was characterised by a slight effect on the number of microorganisms of the different ecological, trophic, and taxonomic groups and the overall biological activity of the soil. Soil microbiocenosis is not only a sensitive indicator of the influence of biotic, abiotic, or anthropogenic factors. Microbiocenosis has another function of the ecosystem level – the ability to produce biologically active substances, which have both phytotoxic and phytostimulant effects. Therefore, the state of the microbiocenosis (level of activity, restructuring), the response to exogenous factors can be assessed by phytotoxicity. It is shown that the use of a composition of cymoxanil with dimethomorph reduces the level of soil phytotoxicity to 12% compared to the control variant of 18%. When using chlorperiphos-methyl – the level of phytotoxicity remained at the level of control indicators. The highest level of phytotoxicity as a result of the research was determined for prometryn – 24%. That is, such soil where prometrin was used has phytotoxic properties. It is believed that this is due to the chemical class of the substance (herbicide) and the mechanism of action on the plant organism. Therefore, the influence of PPE chemicals on the microbiocenosis can be shown as follows: *PROMETRYN (the lowest level of influence) → CHLORPYRIFOS-METHYL → CYMOXANIL + DIMETOMORF (the highest level of influence)*.

The present research is consistent with data from other scientists who have studied the effects of pesticides and agrochemicals on microbiocenosis and plant productivity [39-46]. M. Ahmed et al. in the *in vitro* research showed that increasing the concentration of fungicide in the nutrient medium causes not only a decrease in the number of microorganisms of the genus *Rhizobium*, but also a decrease in the diameter of the colony on the nutrient medium [39]. K. Pudełko et al. emphasised that the use of the fungicide Funaben T (148 g/l carbendazine + 332 g/l thiram) with further seed inoculation with a biological product causes nodulation process of soybean plants, which leads to a decrease in qualitative and quantitative yields [40].

The issue of the influence of PPP directly on soil microorganisms was shown in the work of M.N. Filimon

et al. It was found that pre-sowing seed treatment with fungicides cypermethrin and thiamethoxam negatively affects the number of microorganisms of the main ecological-trophic and taxonomic groups. Namely, reduces the number of bacteria of the *Azotobacter* genus and bacteria that use mineral forms of nitrogen (diazotrophs), at least twice [41]. Researchers from the D.K. Zabolotny Institute of Microbiology and Virology of the NASU showed that the treatment of seeds with fungicides (Maxim Star, Vitavax, Kyoto Duo), mainly caused a decrease in the number of microorganisms in the studied groups. However, inoculation of seeds with *Bradyrhizobium japonicum* strains (UKM B-6035 and UKM B-6023) reduced the negative effect of Maxim Star and Kinto Duo fungicides on oligoazotrophic and prototrophic microorganisms. At the same time, Vitavax did not alter the number of microorganisms but inhibited the nitrogen-fixing activity of all industrial strains of inoculants [42; 43].

Results of vegetation research of the formation of the pigment system of pea and soybean plants depending on the use of different PPP in combination with biological products' inoculation showed an increase in the content of photosynthetic pigments. The growth of chlorophyll a was recorded on average by 14-18%, chlorophyll b – 45-63%, the amount of chlorophyll a+b – 20-27%, carotenoids – 9-11% [44; 45]. Scientists emphasise the increase in the activity of the photosynthetic apparatus due to the intensification of metabolic processes in plants and as a result of the more active synthesis of pigments. It is proved that PPP directly affects the condition of plants [46]. The usage of the herbicide Prima Forte 195 (in the recommended and inflated rates) causes oxidative stress in winter wheat plants. But the integrated use of Prima Forte herbicide in combination with a plant growth regulator has contributed to an increase in the rate of detoxification of xenobiotics in winter wheat plants.

Thus, the management of qualitative and quantitative indicators of soil is, first of all, the management of microbiological processes occurring in it. Therefore, a comprehensive study of the basic patterns of development of microbial groups and their functional activity depending on edaphic and agronomic factors remains relevant.

CONCLUSIONS

The soil microbiocenosis actively responded to the introduction of the studied chemicals, which are the basis of known and widely used in agricultural production PPP. In the present research it was determined that the introduction of cymoxanil with dimethomorph significantly affected the number of microorganisms of different ecological-trophic and taxonomic groups. On average, the number of microorganisms decreased by 1.5-4.5 times relative to control; the content of microbial biomass decreased by 44%, and the intensity of carbon dioxide emissions decreased from 51.4 to 64.8%; the level of phytotoxicity of the soil was in the range of 7-12%.

It was found that chlorperiphos-methyl in comparison with the control caused a decrease in the number of microorganisms of the main ecological-trophic and taxonomic groups in 1.1-2 times; reduced the content of microbial biomass by 25%, the intensity of carbon dioxide emissions by 20.7-31.5%; the level of soil phytotoxicity ranged from 15 to 18%.

It is shown that the level of phytotoxicity of the soil increases to 20-24% when using prometryn, but the indicators of the state of the microbiocenosis are at a high level in comparison with other investigated substances. Compared with the control variant, the number of microorganisms decreased by no more than 1.5 depending on the ecological-trophic group; carbon dioxide emission intensity by 8-12%, microbial biomass content – by 10-13%.

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Вплив хімічних речовин засобів захисту рослин на мікробіоценоз ґрунту

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Анотація. З моменту виникнення землеробства перед людиною постають складні завдання: як використати потенціал грунту й при цьому зберегти його якісні та функціональні властивості, як застосовувати в агротехнологіях ефективні та еколого-безпечні для здоров'я людини та біоти ЗЗР і багато інших. Існує низка інформативних і достовірно реєструючих критеріїв і систем показників, які повністю та всебічно описують зміни екологічного стану ґрунту і агроценозів, проте всі ці системи мають один недолік – це час від дії чинника до «реакції» показника. Рання діагностика змін в агроценозі можлива завдяки біологічній компоненті ґрунту, а саме мікробіоценозу, оскільки мікроорганізми мають велику поверхню контакту з середовищем, високу швидкість розмноження в просторі та часі, чутливість до мінливих умов існування. У лабораторних умовах досліджено реакцію мікробіоценозу ґрунту та його активність (чисельність мікроорганізмів еколого-трофічних і таксономічних груп, інтенсивність респірації, вміст мікробної біомаси та фітотоксичність ґрунту) за дії хімічних речовин, які є основою засобів захисту рослин (ЗЗР). Виявлено, що чисельність мікроорганізмів різних еколого-трофічних і таксономічних груп за дії суміші цимоксанілу з діметоморфом зменшувалась у 1,5–4,5 рази відносно контролю, хлорперіфос-метилу в 1,1–2 рази, а прометрину – не вище ніж у 1,5. Вміст мікробної біомаси й інтенсивність емісії диоксиду вуглецю при застосування цимоксанілу з діметоморфом порівняно з контрольним варіантом зменшувався на 44 % та 51,4–64,8 %, відповідно; прометрину – на 10–13 % та на 8-12 %. Найвищий рівень фітотоксичності ґрунту відмічали для варіантів з застосуванням прометрину (20–24 %), найнижчий для суміші цимоксанілу з діметоморфом (7–12 %). Вважаємо, що високий рівень інгібування розвитку тест-культури при застосуванні прометрину пов'язаний з класом ЗЗР і механізмом його впливу на рослинний організм. Низькі показники фітотоксичності грунту та активності мікробіоценозу при застосуванні цимоксанілу з діметоморфом пояснюються впливом досліджуваної суміші не лише на фітопатогенні мікроміцети, а й на усі групи ґрунтових мікроміцетів (целюлозоруйнівних, сапрофітних), які домінують у мікробіоценозі. Отже, вплив хімічних речовин ЗЗР на мікробіоценоз можливо показати у такий спосіб: *ПРОМЕТРИН (найнижчий рівень впливу) → ХЛОРПЕРИФОС-МЕТИЛ → ЦИМОКСАНІЛ + ДІМЕТОМОРФ (найвищий рівень впливу)*

Ключові слова: мікробіоценоз, мікроорганізми основних еколого-трофічних та таксономічних груп, емісія диоксиду вуглецю, мікробна біомаса