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Study of Harmful Effects of Pesticides, Especially Seed Producers, on the Components of Agrocenosis

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ABSTRACT
The paper shows that seed and planting material treatment is the most economical and least dangerous way to use pesticides. The formula of ecotoxicity calculation for comparing the danger level of soil pollution by various substances was given. The analysis of influence of some seed disinfectants: Black Jack (active substances – humic acids (19–21%) and fulvic acid (3–5%); Kelpak (active substances – auxins (11 mg/l), cytoxins (0.03 mg/l); Dalila (imidacloprid 600 g/l); Impact K (flutriafol (117.5 g/l) and carbendazim 250 g/l) on various groups of plants, soils and microorganisms was carried out. It was proven that the use of drugs with fungicidal action causes a certain effect on some soil parameters, mostly due to the effect on the microbiological activity of the soil. The results obtained in the research show that each of the chemical compounds, which is the active substance of the studied pesticides, does not have a significant detrimental effect on the rhizosphere biota when used separately; however, regular use of chemical pesticides can lead to an irreversible disturbance of the natural microbial balance in the soil and a gradual loss of fertility. Much attention is paid to the study of soil organisms, in particular earthworms, which have become the main test species in the tests to assess the toxicity of new xenobiotics, as well as to calculate the risks of adverse effects of pollutants on the environment. It was shown that pesticides of different nature of action have varying effects on soil microbiota and earthworms. Studies have shown that pre-sowing treatment of seeds with pesticides has undeniable economic, organizational and technological advantages over other methods of applying chemical plant protection products. The calculation of the ecological danger of pesticides was carried out and it was concluded that pre-sowing seed treatment can be called an ecologically low-hazardous measure. The paper recommended the main ways to prevent the increase of the negative impact of pesticides (including seed disinfectants) on the environment.

Keywords: pesticides, seed disinfectants, agrochemical parameters of soil, microbiological activity, ecotoxicity.

INTRODUCTION

A significant part of environmental pollution corresponds to chemicals that humanity contributes to ecosystems to protect against pests, diseases and weeds. The impact of some pesticides on the environment has been insufficiently studied due to the complexity of their interaction with the elements of the main components of biocenoses. Therefore, active research is conducted on the consequences of the negative impact of pesticides in agrocenoses [Ashikhmina T.Ya., 2010; Baev N.A., 2014; Manucharova N.A., 2014; Jacobsen C.S., 2014], on pollution and behavior of pesticides in soil systems [Ivantsova E.A., 2013; Rose M.T., 2016], the impact of pesticides on soil microflora [Aripov T.F, 2015; Astaikina A.A., 2020; Ksenofontova O.Yu., 2015; Lo C.-C. 2010]. Attention is paid to the migration of pesticides in soils [Smetnik A.A., 2005; Rossi F., 2018]. The main causes of contamination of biocenoses with pesticides are violations of regulations for their use, the use of persistent drugs [Zinchenko V.A., 2012] and other technological factors.

One of the ways to protect seeds from pests in order to obtain the maximum yield is its pre-sowing treatment, which is carried out by appropriate disinfectants (pesticides), that are harmful to the environment. Thus, the issue of harmful effects of pesticides, in particular seed disinfectants, on the agrocenosis is extremely important, relevant and needs to be investigated.

The aim of the work was to study the influence of pesticides in particular seed disinfectants, on the agrocenosis and its components (agrochemical parameters and microbiological activity of soil and soil fauna). For this purpose, it is necessary to analyze the properties of pesticides and determine their impact on soil microbiological activity and soil fauna; to study the changes in agrochemical parameters of soil with the use of various seed disinfectants; determine the agri-environmental assessment of the use of seed disinfectants; as well as develop the ways to prevent the increase of negative impact of pesticide use on agrocenosis and its components.

MATERIALS AND METHODS

In today's world, more than a thousand different pesticides are used to control pests, the losses from which reach more than 33% of the potential crop. The level of yield largely depends on the phytosanitary condition of crops, which is improved by the pre-sowing seed treatment. Pesticides are most often used for this purpose, which are an effective means of controlling pests and plant diseases, as well as various parasites, weeds, pests of grain and grain products.

Pesticides are classified according to various parameters. By purpose, they are divided into groups: insecticides (including acaricides, aphids, nematicides, limbicides, rodenticides), fungicides (including bactericides) and herbicides (including arboricides, algaecides). According to the method of entry into the body, pesticides are divided into intestinal, contact, systemic and fumigants. According to the hygienic classification, there are potent toxic substances (with LD_{50} to 50 mg/kg), highly toxic (with LD_{50} = 50–200 mg/kg), moderately toxic (with LD_{50} = 200–1000 mg/kg) and slightly toxic (with LD_{50} = more than 1000 mg/kg).

A separate group of drugs by purpose are seed disinfectants. Many researchers identify seed and planting treatment as the most economical and least dangerous way to use pesticides. It has only one significant disadvantage: a small overall time of protective action. On the other hand, plants are most vulnerable to pests and diseases in the early (juvenile) stages of development, and sowing of seeds provides significant savings on seasonal pesticides and precautions for their handling.

Due to the long-term toxic effects of pesticides (which are valued for this type of phytopharmacological agents), they have a certain cumulative property, i,e, they can accumulate in the soil and affect the biological components of the soil (soil animals, insects, microorganisms, etc.).

To date, the possible harmfulness of pesticides has been assessed mainly by the acute toxicity of the drug, but this parameter alone is not enough, as the duration of storage of xenobiotics in the environment and their rate of consumption also have a significant impact. In this regard, the amount of ecotoxicity should be determined by the formula:

$$E = \frac{P \times H}{LD_{50}} \tag{1}$$

where: E – ecotoxicological hazard, in ecotoxins; P – persistence (half-life) in weeks for the preparations applied to the soil, or waiting time in weeks for the preparations applied to green plants; H – rate of application of the drug, kg/ha (l/ha); LD_{50} – lethal dose of pesticide, which causes the death of 50% of experimental animals, mg/kg.

For the unit called "ecotox", the value obtained according to this formula for a well-studied DDT at a rate of 1 kg/ha, toxicity to animals 300 mg/kg and persistence of 312 weeks. "Ecotox" allows comparing the risk of soil contamination with different substances, the greater the value of this indicator, the greater the possible danger of this compound.

Various agrochemicals (fertilizers, growth stimulants, pesticides) affect the agrochemical and physicochemical parameters of the soil. Thus, growth regulators with humic substances (Black Jack) do not have a negative impact on the basic parameters of the soil, because humic acids and fulvic acids, which are part of such drugs, are components of soil humic substances. However, the amounts of such substances that are introduced into the soil with seeds are too small to have a significant positive effect on the soil.

Synthesized growth regulators (auxins, cytokinins, gibberellins, abscisic acid) actively affect plants, but there is an indirect effect on other components of the agrocenosis. Thus, auxins (compounds of predominantly indole nature) are formed in apical meristems and stimulate cellular stretching. There are no data on the effect of auxin on the soil.

Gibberellins are easily biodegradable (using sewage sludge, 76% of mineralization occurred in 28 days); decompose rapidly in air (DT_{50} for GA4 – 1.67 hours; DT_{50} for GA7 – 0.99 hours). Gibberellins are safe for small insectivorous birds living in orchards: the daily dose for a single injection with food is 0.56 mg a.s./kg b.m. These growth regulators are practically non-toxic to aquatic organisms (fish and invertebrates), algae and bees: LD_{50} for rainbow trout > 100 mg a.s./l (exposure 96 hours); LK_{50} for daphnia > 100 mg a.s./l (exposure 48 hours); EbC_{50} for microalgae > 100 mg a.s./l; EbC_{50} for duckweed > 0.96 mg a.s./l; LD_{50} (contact) for honey bees > 100 mg/bee. According to EFSA, for gibberellic acid GA3, the value of ADI (acceptable daily intake) = 0.68 mg/kg, based on the NOEL value for rats - 680 mg/kg in studies of subchronic toxicity and a stock ratio of 1000; for gibberellins GA4/7, the value of ADI = 0.3 mg/kg, based on the NOEL value for rats – 300 mg/kg in studies on reproductive toxicity and stock ratio 1000.

Cytokinins are formed mainly in the roots and move to the aboveground organs through the xylem, affecting metabolism. Nitrogen nutrition enhances the formation of cytokinins, which require a sufficient supply of nutrients to the plant, especially nitrogen. The use of drugs with cytokinins, auxins and gibberellins also affects other plant organisms of the agrocenosis (weeds), causing their growth to increase within certain limits.

Pesticides have a greater impact on soil components and agrocenosis. In Ukraine the use of seed treatment Dalila 600, containing imidacloprid, is widespread. It causes the accumulation of the active substance in the leaves (it practically does not enter the fruit), which indicates the relative safety of the use of drugs based on imidacloprid in drip irrigation of vegetable crops indoors [Ermolova L.V., 2020]. No significant toxic effect of the imidacloprid drug Dalila 600 on the content of humus in the arable layer of ordinary chernozem was noted (Table 1). Since this active substance has a relatively small inhibitory effect on the microorganisms that mineralize humus, there is a tendency to reduce the humus content for 180 days after the application of the drug.

Contamination of the soil with pesticides and their metabolites can sometimes cause an increase in the content of mobile forms of nitrogen and phosphorus in the arable layer, i,,e improve the nutrient regime of plants. The pesticides entering the soil have both a direct effect on the *pH* of the soil solution in the process of their hydrolysis, and an indirect effect by stimulating or inhibiting the activity of microorganisms. According to Table 2, soil contamination with imidacloprid causes an increase in the content of mobile phosphorus in the soil, especially with an increase in the amount of pesticide in the soil (62.3–68.4 mg/kg for 180–360 days against 24.4–31.5 mg/kg on control).

When using the drug Impact K, two active substances and products of their metabolism enter the soil. The half-life of flutriafol in the soil is 65–125 days, carbendazim – up to 6 months. Carbendazim is intensively destroyed by soil microflora.

Thus, the use of drugs with fungicidal action causes a certain effect on some soil parameters, mostly due to the effect on the microbiological activity of the soil.

Microorganisms play an important role in soil formation and participate in the formation of soil structure, humus formation and other important soil processes. Numerous pesticides used provide different mechanisms of action of these substances on prokaryotic and eukaryotic cells of microorganisms, on heterotrophic and photosynthetic microorganisms, and the range of these mechanisms is very wide. For example, carbamate derivatives affect the process of cell division; organic compounds of copper and dithiocarbamates – influence membrane permeability and oxidative phosphorylation; organic mercury compounds react

Table 1. The effect of imidacloprid on the humus content in ordinary chernozem, %

Term of accounting, days	The content of humus in the soil with different amounts of pesticides			$HIP_{0.95}$
	Control	1 MAC	10 MAC	
7	2.87	2.58	2.74	0.72
30	2.85	2.96	2.83	0.34
180	3.18	3.05	2.72	0.28
$HIP_{0.95}$	0.27	0.26	0.25	

Table 2. The effect of imidacloprid on the content of mobile phosphorus in ordinary chernozem, mg/kg

Term of accounting, days	The content of mobile phosphorus in the soil with different amounts of pesticides, mg/kg of dry soil			$HIP_{0.,95}$
	Control	1 MAC	10 MAC	
7	29.0	60.0	38.9	6.4
30	32.3	29.2	30.0	4.6
180	31.5	58.4	62.3	8.7
360	24.4	30.3	68.4	4.9
$HIP_{0.95}$	4.4	6.7	7.5	

with cellular components, reacting with carboxyl, sulfhydryl, amino groups, and metal ions. Very often, the information about the effects of pesticides on microorganisms is contradictory.

Most studies suggest that pesticides have minimal effect on soil microbial activity in the field [Filimon M.N., 2015]. This may be due to the complexity and redundancy of the pool of soil microorganisms, ie the loss of soil functions does not occur due to the high rate of physiological and evolutionary adaptation of microorganisms to such effects. On the other hand, there is a possibility that minor changes in the structure or functioning of the community may still reduce the ability to further adapt or to withstand other stressors [Rose M.T., 2016]. This fact indicates in particular the need for a more careful study of the impact of pesticides on biodiversity indicators of microbial communities.

Therefore, the use of imidacloprid has a greater effect on the grouping of soil micromycetes compared to prokaryotes. Note that the samples with 10-fold rates of pesticide use revealed an increase in the number of representatives of the Basidiomycota division. This causes a short-term stimulating effect on the carbon content of microbial biomass; stimulates nitrogen fixation [Astaikina A.A., 2020]. Carbendazim is moderately toxic *to Pseudomonas fluorescens* and *Bacillus subtilis*, but highly toxic to *Trichoderma harzianum*, which is a potent biocontrol agent against fungal diseases such as fusarium wilt, pityosis and rhizoctonia [Rossi F., 2018]. Flutriafol does not cause retardate and phytotoxic effects if the regulations are used.

As a result of field research, it was noted that during the treatment of corn seeds with pesticides of fungicidal nature (Impact K) in the phase of 3-5 leaves, the number of ammonifiers and microscopic fungi in the rhizosphere of vegetative plants is reduced by one order (Table 3). Such disinfectants had a slightly lower effect on the content of actinomycetes, oligonitrophils and pedotorophs. Compared with the control variant, the number of these microorganisms decreased by 75, 70 and 50%, respectively, for the conditional groups. The content of pedotrophs and oligotrophs did not change under the action of chemicals and was at the level of control.

Under the conditions of use of insecticidal pesticides (Dalila 600), the content of the studied microorganisms was at the level of control, and changes in the number of any group of microbiota were not observed.

The obtained results show that each of the chemical compounds, which are the active substance of the studied pesticides, does not have a

Table 3. The number of different groups of microorganisms in the rhizosphere of corn under the conditions of chemical and biological protection

Version	Microorganisms, million CFU/g of soil					
	Ammonifiers and spores	Actinomy-cetes	Microscopic fungi	Oligo-trophs	Oligoni-trophils	Pedotrophs
1 selection (phase 3-5 leaves)						
Control (without processing)	148.8±17.3	2.9±0.7	5.4±0.6	1.5±0.04	7.6±0.3	3.1±0.5
Dalila seed treatment (imidacloprid)	151.2±12.1	3.2±0.5	5.5±0.4	1.4±0.02	8.8±0.5	3.2±0.4
Seed treatment Impact K (flutriafol + carbendazim)	19.1±3.2	0.8±0.02	0.2±0.03	1.1±0.05	1.8±0.1	2.1±0.3
2 selection (panicle ejection phase)						
Control (without processing)	154.3±15.2	2.6±0.6	6.3±0.6	1.7±0.02	7.8±0.4	4.1±0.6
Dalila seed treatment (imidacloprid)	161.2±11.1	3.1±0.3	5.9±0.4	1.6±0.02	8.2±0.5	4.4±0.5
Seed treatment Impact K (flutriafol + carbendazim)	107.1±13.1	2.7±0.2	4.4±0.6	1.4±0.09	4.7±0.5	3.1±0.5
3 selection (after harvest)						
Control (without processing)	167.3±11.2	3.7±0.6	5.2±0.6	1.7±0.09	7.5±0.4	4.2±0.4
Delilah seed treatment (imidacloprid)	171.2±12.1	4.1±0.3	5.8±0.3	1.9±0.05	7.1±0.5	4.6±0.2
Seed treatment Impact K (flutriafol + carbendazim)	168.1±13.3	3.7±0.1	5.9±0.7	1.7±0.09	7.5±0.8	4.1±0.7

significant detrimental effect on the rhizosphere biota when used separately. However, regular use of chemical pesticides can lead to irreversible disruption of the natural microbial balance in the soil and the gradual loss of fertility.

While analyzing the structure of soil prokaryotic groups, it was noted that the control samples among prokaryotes are dominated by representatives of the phylogenetic groups *Proteobacteria* and *Actinobacteria* (Fig. 1). Representatives of other groups make up no more than 15% of the total number of prokaryotes.

For the use of seed disinfectants with active ingredients Flutriafol, Carbendazim (fungicides) and Imidoclopride (insecticide), an increase in the proportion of actinobacteria was observed with the addition of starch-mineral mixture as a film-forming agent for fixing pesticides on seeds and preventing microfiltration. Intensive development of this group was noted with the use of 10 times the rate of carbendazim. A similar trend is observed throughout the experimental period, which suggests an increased participation of actinobacteria in the decomposition of pesticides.

Changes in the degree of representation of other groups (*Verrucomicrobia*, *Planctomycetes*, *Cyanobacteria*, *Bacteroidetes*, *Gemmatimonadetes*, *Firmicutes*, *Crenarchaeota*, *Acidobacteria* and candidates of groups *AD*3, *WPS*-2) are not statistically significant. During the experiment on the introduction of three pesticides together and without the addition of starch-mineral mixture in all samples there is a dominance of representatives of the phylogenetic group *Proteobacteria*.

Evaluation of the α-diversity of the bacterial community according to the Shannon index (Table 4) revealed that the application of pesticides in both one-time and 10-fold norm Shannon index is the same in both cases.

With the introduction of pesticides in 10 times the α-diversity of the bacterial complex of the soil is reduced. This is due to the development of a specific microbial group capable of decomposing xenobiotics. It was established that the structure of the bacterial community is significantly affected only by the introduction of the starch-mineral mixture. The increase in the presence of the Actinobacteria group in the soil samples on the 56th day of incubation with the addition of starch is due to the

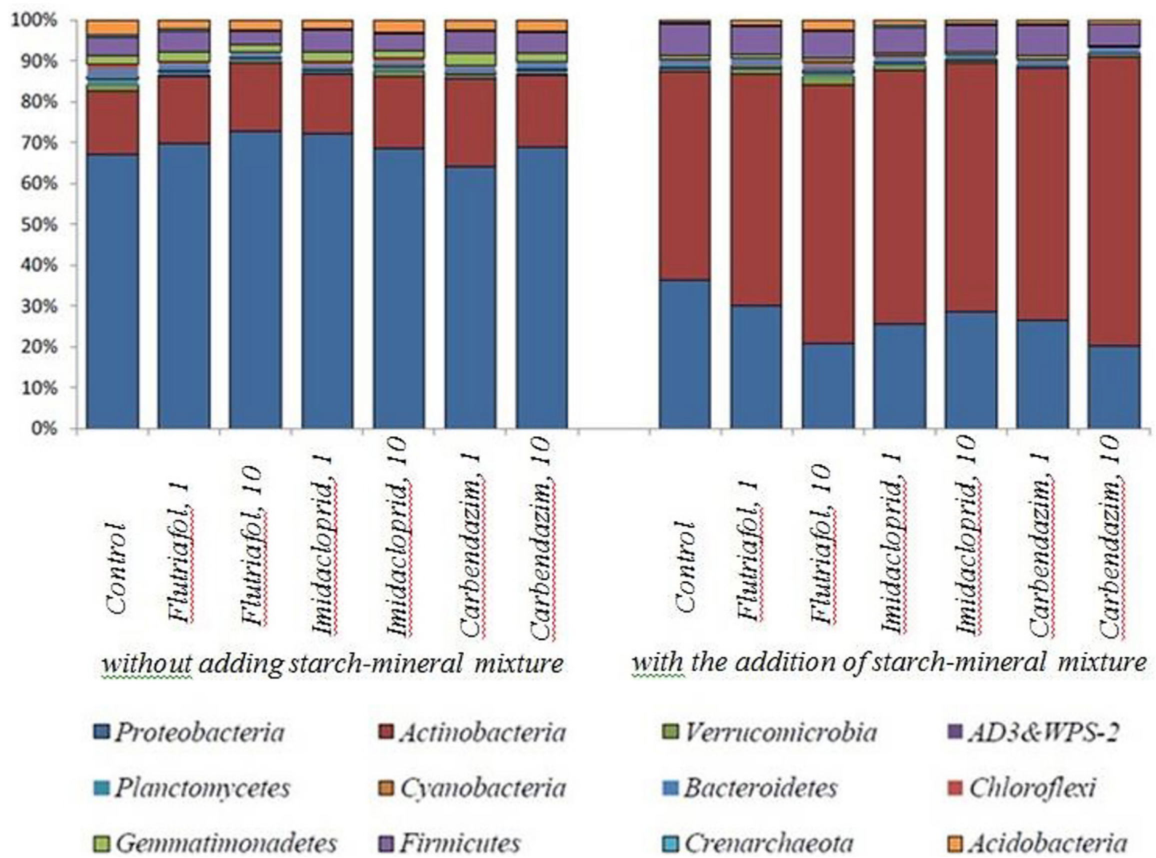

Figure 1. The structure of the prokaryotic grouping in the soil on the 14th day after the use of pesticides (1 - single application rate; 10 - ten-fold application)

Table 4. Indicators of the diversity of the bacterial community of the soil with the use of pesticides

Disinfectant, dosage	Number OTU	Shannon Index	Chao1 index
Without adding starch-mineral mixture			
Control	784	5.20	774
Flutriafol, 1	396	4.29	397
Flutriafol, 10	264	4.00	265
Imidacloprid, 1	368	3.86	369
Imidacloprid, 10	198	3.89	198
Carbendazim, 1	383	4.33	382
Carbendazim, 10	415	4.36	417
With the addition of starch-mineral mixture			
Control	997	4.43	895
Flutriafol, 1	608	4.42	608
Flutriafol, 10	500	4.33	488
Imidacloprid, 1	391	4.08	399
Imidacloprid, 10	420	4.22	418
Carbendazim, 1	428	4.11	426
Carbendazim, 10	391	3.84	395
1 – single application of the drug, 10 – ten-fold application of the drug			

assimilation of carbon of this bacterial group and the ability of this group to intensively decompose highly polymeric compounds [Manucharova N.A., 2014; Alvarez A., 2017].

An adverse consequence of pesticide use is the loss of biological diversity [Jacobsen C.S., 2014]. Due to the specific application of most pesticides, soil organisms are the first to face negative effects.

Earthworms have become the main test species in the tests to assess the toxicity of new xenobiotics, as well as to calculate the risks of adverse effects of pollutants on the environment [Rose M.T., 2016]. They have a number of advantages in comparison with other species-bioindicators: they multiply quickly, are easily cultivated under laboratory conditions and at the same time, they are sensitive to many anthropogenic pollutants, including pesticides and heavy metals.

Of the three groups of pesticides, the most toxic to earthworms are insecticides and fungicides, which are organophosphates, nicotinoids, strobilurins, triazoles and carbamates [Wang Y., 2012]. In small doses, carbendazim does not inhibit earthworms, in large – it significantly reduces the population of *A. caliginosa*. *A. chlorotica* and *O. lacteum* species, especially adults, are more resistant. *Lumbricus terrestris,* living in the surface layer of the soil of populations, is most threatened, so in the areas where this species predominates, the use of carbendazim is excluded.

Due to long-term storage in water, flutriafol is dangerous for aquatic organisms, moderately dangerous for bees, and low toxic for birds and fish; it does not adversely affect the number, weight and species composition of earthworms, as well as microbiological processes in the soil.

Imidacloprid is moderately toxic to warm-blooded animals [Ermolova LV, 2020] and humans (LD_{50} = 480–1000 mg/kg); very dangerous for bees, but it has no adverse effects on earthworms.

Thus, different treatment agents have various effects on soil microbiota and earthworms.

The ability of pesticides and their degradation products to accumulate in soil, plant and animal organisms can lead to local environmental pollution and undesirable environmental, economic and social consequences. In this regard, the data on the magnitude of environmental hazards of pesticides widely used in agricultural production become especially relevant.

In order to calculate the environmental hazard of seed disinfectants, the norms of their application for growing corn with a seeding rate of 20 kg/ha were used. Because there is no toxic effect on living organisms for the Black Jack, s.c. and Kelpak, w.s.c. drugs, the ecotoxicity for them cannot be calculated (Table 5).

Dalila preparations, 60%, FS and Impact K, 36.75% s.c. have a low level of ecotoxicity (0.00065–0.0021). At the same time, it should be noted that the low value of the ecotoxicity index should not weaken the attention to safety for the

Table 5. Environmental hazard assessment of seed disinfectants

The name of the drug	LD_{50} for rats (mg/kg)	Waiting time, (weeks)	Norm, kg/ha	Ecotoxicity, ecotox
Black Jack, s.c.	-	-	0.009–0.027	-
Kelpak, w.s.c.	-	-	0.036–0.090	-
Impact K, 36.75% s.c.	1140–1480	3	0.6–0.8	0.0012–0.0021
Dalila, 60% FS	480–1000	4.3	0.09–0.15	0.00065–0.00081

use of these drugs, as it only characterizes the comparative danger of possible residual amounts of compounds for environmental objects.

The pre-sowing treatment of seeds with pesticides has undeniable economic, organizational, technological advantages over other methods of application of chemical plant protection products: it is cheaper, cost-effective, timed to the periods that are less busy with other agricultural work; moreover, it is limited and is not associated with the need to spray pesticides on thousands of hectares. That is, pre-sowing seed treatment can be called an environmentally friendly measure – when sown with pickled seeds, the zone of contact of the chemical with the soil is 60-200 m^2, when applied to the soil – 500 m^2, and when spraying – 10000 m^2.

The supply of pesticides is in accordance with their inherent physical and chemical properties, and the main causes of pollution are violations of regulations for their use, the use of persistent drugs and other technological factors.

Migration and redistribution of pesticides in the soil profile is carried out due to diffusion in the liquid and gas phases, capillary and gravitational movement of water, absorption and exudation by the root system of plants [Smetnik A.A., 2005]. Detoxification of pesticides in the soil environment significantly depends on soil properties, weather and climatic conditions (precipitation, temperature, insolation), as well as on the microbiological activity of the soil.

The use of pesticides (especially fungicides and herbicides) affects the microbial groups of the soil, their activity and species composition.

The main ways to prevent an increase in the negative impact of pesticides (including seed disinfectants) on the environment are: (1) If possible, replacement of chemicals with biological products, or other technological measures to limit the development of harmful organisms; (2) Preferring less toxic drugs with lower persistence; (3) Compliance with all recommended regulations for the use of drugs (rate and frequency of application, consideration of protected areas, proper disposal of containers and drug residues); (4) Improving the general level of agriculture, replacing chemicals with biological products, compliance with the recommended regulations for the use of drugs, etc. will reduce the negative impact of the use of seed treatment agents on the agrocenosis and its components.

CONCLUSIONS

This study found that pesticides of different nature of action, in particular seed disinfectants, have varying effects on soil microbiological activity and soil fauna; therefore, it is necessary to give preference to the seed disinfectants with the least negative impact on the beneficial inhabitants of the agrocenosis. None of the chemical compounds, which are the active substance of the studied pesticides, has a significant detrimental effect on soil agrochemical parameters, but regular use of chemical pesticides can lead to irreversible disruption of the natural microbial balance in the soil and their gradual loss of fertility. The studied drugs have a low level of ecotoxicity (0.00065–0.0021) or do not have a toxic effect on living organisms, but this fact should not weaken the safety precautions for the use of these drugs, as it only characterizes the relative risk of possible residual amounts of compounds for environmental objects. Improving the general level of agriculture, replacing chemicals with biological products, compliance with the recommended regulations for the use of drugs, etc. will reduce the negative impact of the use of seed treatment agents on the agrocenosis and its components.

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