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THE USE OF CILIATES (CILIOPHORA) FOR BIOASSAY OF THE TOXICITY OF INSECTICIDES

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The Use of Ciliates (Ciliophora) for Bioassay of the Toxicity of Insecticides. Mamedova, V. F., Alekperov, I. Kh. — Field and laboratory experiments have been carried out to determine the degree of toxicity in the soil community of ciliates of two insecticides: "Confidor Extra" and "Decis Profi" at concentrations of 80 mg/l, 50mg/l and 25mg/l. Laboratory experiments were performed with the soil ciliates species *Colpoda cucullus* (Müller, 1773). Changes in phagocytosis and osmoregulation were used as indicators of the survival and physiological parameters. In addition to the laboratory experiments, we carried out field experiments on experimental plots (50 x 50 cm) of the virgin soil. Field experiments were carried out in 5 replications. The results showed that the modification of physiological parameters is thinner exponent toxicity than survival.

Key words: ciliates, bioassay, insecticides.

Introduction

Soil ciliates take an active part in the biological processes occurring in the soil biogenesis. Ciliates are of great importance in the process of production and destruction of organic matter thereby actively participating in the improvement of soil fertility.

As it is known the use of various insecticides for the control of insect pests of crops is still applied on a large scale in many countries. That is why carrying out a research on the impact of bioassay of various insecticides on soil fauna, determining the period of the exposure to the community of pedobionts is of great scientific and practical importance. Especially such studies are urgent today when various companies offer dozens of names of their products.

It should be noted that similar studies of pesticides influence on the soil fauna are still practically lack. As far as we know, the study of the impact of three insecticides (Karate, Nurel-D, Pegas) in various concentrations and their biological testing on cell organism level on changes of physiological parameters of ciliates indicators have been carried out previously only in Azerbaijan (Alekperov, Akhmedova, 2002).

Our experimental work was carried out to identify the toxicity for soil fauna of the two most popular insecticides in the farms of Azerbaijan.

Material and methods

In the period of 2015–2016 we have carried out 75 experiments on the identification of two widely used in insecticides farms, "Confidor Extra" and "Decis Profi", degree of toxicity for soil ciliates. For the use of these drugs produced by the manufacturer working concentration of 100 mg per 1 liter of water is recommended. After application to the soil, the drug concentration gets weaker, for experiments we used concentrations of 80 mg/l — 50 mg/l and 25 mg/l for each of the preparations. The soil ciliates *Colpoda cucullus* Müll. were used for toxicological experiments on cell-organism level. In all experiments, in parallel with the control, the concentrations "Confidor Extra" and "Decis Profi" were used on 80 mg/l and 25 mg/l of each drug. Survival and physiological parameters such as fission and intensity rate of phagocytosis were used as test criteria in experiments on cellular-organism level.

Survival monocultures of ciliates were evaluated taking into account living and dead animals in the experiments compared with the control and they were expressed as a percentage.

The fission rate of ciliates in the experiments and the control was determined by the conventional method: an individual cell was placed in the hole of the immunoassay plate in medium with a certain concentration of the insecticide. After a day the total number of

multiplying ciliates was determined and one specimen was placed again in fresh medium with the toxicant. At the same time, the control experiments were carried out without insecticides. Doubling ciliates time was determined according to the formula where, A and B are cell numbers on the 1st and 2nd day, respectively.

$$R = \log.B - \log.A / \log.2$$

The intensity of phagocytosis has been determined by the Boikova method (Boikova, 1989). The criterion for the intensity of phagocytosis was used as the number of formed vacuoles with the food object model (activated carbon dust or carmine powder) in a certain period of time. The intensity of phagocytosis was calculated for both ciliates in control and in cells exposed to media containing various concentrations of insecticide. Before experiments for the release of ciliates from food vacuoles they were kept in an environment without food. After a few hours they were transferred for 10 min. into the culture medium (with various concentrations of the toxicant) with coal or carmine. Then ciliates were fixed by sublimate and the amount of food vacuoles was calculated under a microscope. All experiments have been carried out on ten surfaces, i. e. only 80 experiments with two insecticides.

In addition to the laboratory experiments we carried out field experiments on experimental plots (50 x 50 cm) of the virgin soil. Field experiments were carried out in 5 replications.

Results and discussion

It is known that the application of various insecticides to some extent takes place in almost all farms. Typically, manufacturing companies provide specific data about each formulation (the active principle, the spectrum of action and mechanism of the impact on pests, etc.). In many cases, even the decomposition time of the drug after its application is indicated. However, previous studies (Alekperov, Akhmedova, 2002) showed that, as a rule, this indicator often has a significantly higher exposure time, and in addition, there is no data on the effect of substance characteristics on soil fauna after the processing of plant, as one or another part of the substance gets the soil surface.

The results of the application impact of two investigated insecticides (“Confidor Extra” and “Decis Profi”) on soil ciliates are shown on fig. 1. For these experiments, we used only the surface layers of soil. In all cases species richness in virgin soils has been evaluated at 100 or more species on the control plots (without the addition of insecticides).

When comparing the data presented on fig. 1 *A* and *B*, it is clear that in all cases a sharp decline is observed in species richness on the first day of the exposure. For example, at a concentration of “Confidor Extra” 80 mg/l a decline in species richness was noted in the first two days of exposure and we found only 20 species from the initial 100 ones. Further, from 3 to 7 days species richness remained stable at a low level and amounted to 18–17 species. Thus, at the concentration of 80 mg/l species richness of soil ciliates is not recovered even after 7 days of the exposure.

The results of the application of 50 mg/l and 25 mg/l concentrations of the “Confidor Extra” are similar to each other. In both cases, on the first and second day a decline in species richness of soil ciliates comes to 40–48 species, the minimum value that we observed on the 4th day was 32–36 species. The main difference between the impact of a weaker concentration “Confidor Extra” and a maximum of 80 mg/l was a well-marked on 6 and 7 days of the exposure, the process of rehabilitation of the soil community of ciliates reflected in an increase to the end of the experiment on species richness to 68 species, and to 81 species at a minimum concentration of 25 mg/l.

The results of similar experiments with the drug “Decis Profi” are presented on the figure 1, *B*. The initial (control) number of species was 60 for these experiments.

As it can be seen from figure 1, *B*, the species richness falls abruptly from 60 to 20–

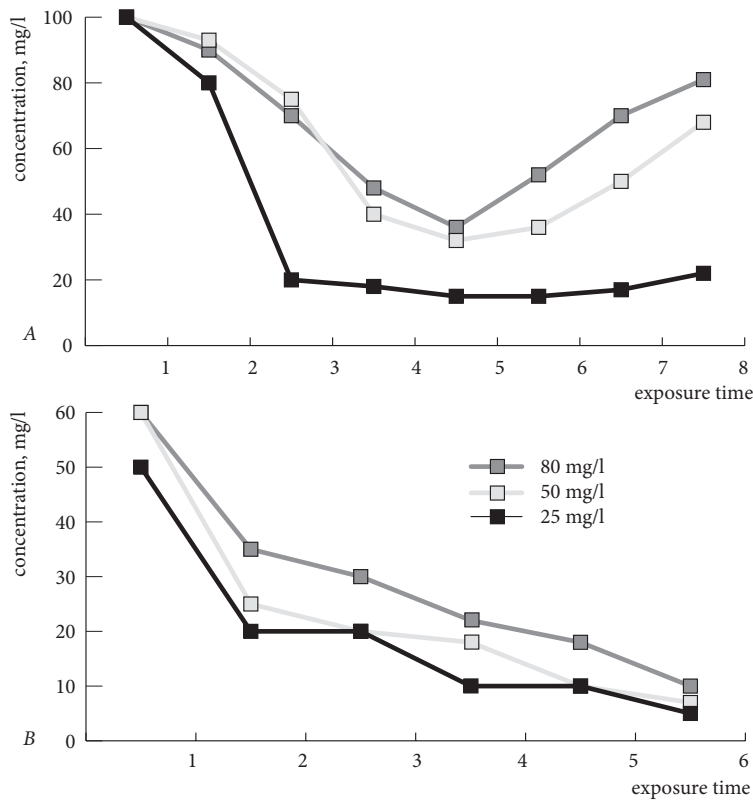


Fig. 1. Changes in the species richness of soil ciliates after application of insecticides “Confidor Extra” (A) and “Decis Profi” (B) (exposure time 7 days).

35 species, depending on the concentration used, already on the second day of the exposure. However, the main difference between the experiments with “Decis Profi” and previous experiments with “Confidor Extra” is absence of any recovery process of soil ciliates community by the end of exposure. Thus, a comparison of the results of experiments with both insecticides indicate a significantly higher and prolonged toxicity of “Decis Profi” product, although working concentrations of both drugs and general characteristics were similar by the parameters.

Table 1. The impact of the concentrations of insecticides “Confidor Extra” and “Decis Profi” on the survival and physiological parameters of ciliates *Colpoda cucullus*, in to control group, %

Insecticide concentration	Survival				Phagocytosis				Osmoregulation			
	Exposure time on days											
	5	10	15	20	5	10	15	20	5	10	15	20
“Confidor Extra”												
80 mg/l	32±1.05	35±2.3	40±2.6	47±1.12	37±0.18	41±1.02	66±1.17	67±2.01	42±0.18	44±1.81	49±2.03	50±2.04
50 mg/l	37±1.54	39±2.03	43±2.05	48±2.02	37±1.2	49±2.12	55±1.11	67±2.03	43±1.09	43±2.01	45±1.82	52±1.34
25 mg/l	42±2.03	44±1.08	44±2.03	50±2.12	45±1.08	54±1.25	66±2.02	67±1.12	46±1.03	46±2.01	47±2.01	53±2.08
“Decis Profi”												
80 mg/l	30±1.08	31±1.15	32±1.18	38±1.03	21±1.06	30±1.18	32±1.18	35±2.15	20±1.05	23±2.15	25±1.07	
50 mg/l	32±2.01	31±2.19	34±2.09	41±2.01	22±1.12	27±1.53	30±1.32	33±1.18	22±2.15	23±1.18	27±2.12	
25 mg/l	37±1.07	38±1.51	41±1.32	48±1.15	24±2.03	34±0.12	37±1.12	37±2.08	25±1.03	25±2.05	28±1.18	

For more correct results, we have carried out some experiments in parallel to determine the influence of these two insecticides on the physiological parameters and survival of soil ciliate species *Colpoda cucullus*. The obtained average results are presented in table 1.

As it is seen from the table, after the impact of two insecticides, each one at a concentration of 80 mg/l–50 mg/l, the survival rate of ciliates ranged from 30 to 42 % of the control in all cases during the first 5 days. During the experiment, the survival rate was gradually increasing, but even on day 20 of the exposure it did not exceed 50 % of the control. In the early stages of the exposure, the data on phagocytosis approximately corresponded to the results of survival. However, the difference between “Confidor Extra” and “Decis Profi” in the toxicity degree was observed at the physiological level. In the first case, even if phagocytosis increased from 37 % to 67 % relatively to control at the maximum concentration of 80 mg/l, during “Decis Profi” testing phagocytosis in *Colpoda cucullus* at all three concentrations used in the experiment ranged from 21%–37 % relatively to control.

Significant differences were observed in the analysis of the osmoregulation process. If the function of contractile vacuole in the experiments with “Confidor Extra” ranged from 42 % to 53 % relative to control at all times of the exposure, then osmoregulation function virtually remained within the 20–28 % in experiments with “Decis Profi” and the cells lost their shape, and their movement became chaotic. Thus, in our opinion, bioassay of toxicity influence on the changes of the physiological parameters of the ciliates, i. e. on cell-organism level, reveals more accurate results than a simple accounting of organisms survival.

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