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SCREENING AND STUDYING THE DESTRUCTIVE ACTIVITY OF PERSISTENT ORGANOCHLORINE PESTICIDES

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ANNOTATION
Screening of bacterial strains isolated from soils contaminated with organochlorine pesticides was carried out. Bacterial strains have been identified that are capable of growth on a mineral medium with organochlorine pesticides - the γ-isomer of hexachlorocyclohexane and dichlorodiphenyltrichloroethane as the only carbon source. The processes of hexachlorocyclohexane degradation by monocultures and associations of isolated bacterial strains were studied. A monoculture of bacterial strains and a consortium created on the basis of the four most active strains are capable of destroying hexachlorocyclohexane when contained in a nutrient medium at a concentration of 100 μg/ml. It was shown that monocultures of four bacterial strains almost completely degraded hexachlorocyclohexane within one month. The consortium of destructor strains contributed to a decrease in the initial concentration of the drug from 100 μg to 45.55 μg in 12 days. The species identity of the active strains of destructor bacteria was determined.
KEYWORDS: *pesticides, bacteria, hexachlorocyclohexane, lindane, dichlorodiphenyltrichloroethane, destruction.*

RELEVANCE

The world scientific literature has accumulated numerous data on the negative consequences of using pesticides [6, 8]. It should be noted that pesticides are used to protect plants and animals from pests and diseases, despite the fact that they are one of the leading chemical compounds in terms of their harmful effects. Scientists especially note the role of organochlorine pesticides in the biosphere [2, 11].

Based on the results of a study by a number of scientists, the general pattern of migration of pesticides from sources of pollution into the environment through water and the atmosphere has been clarified [3, 10]. By contaminating drinking water, crop and livestock products, they enter the human body, which is very dangerous for human health.

In world literature and practice, soil microbiological degradation of persistent compounds is recognized as one of the most promising [1,4,5,7]. Methods are being developed to clean natural objects from pesticides by microbiological means [9].

In the Republic of Uzbekistan, special attention is paid to the development and implementation of measures in the field of conservation and sustainable development of biodiversity, and in this regard, certain results are being achieved in the detection of soil degrading bacteria for cleaning soil contaminated with organochlorine pesticides. For this reason, determining the degree of degradation of organochlorine pesticides under the influence of degrading bacteria in soils and developing the effectiveness of biological soil restoration is of great practical importance. In particular, the isolation of active pesticide degrading microorganisms from the soil, the identification of degrading microorganisms that are resistant to extreme temperatures, salinity, high and low pH values, increasing the degree and rate of decomposition of pesticides by microorganisms, the creation of biological products that decompose pesticides based on various taxonomic groups of microorganisms are promising.

The purpose of this work is to search for and screen strains of soil bacteria that actively degrade the organochlorine pesticide hexachlorocyclohexane.

MATERIALS AND METHODS OF RESEARCH

A model site located in the village of Kyzyluy, Kegeyli district of the Republic of Karakalpakstan, was selected as the object of study. The model site includes a former pesticide storage site and nearby cultivated fields. The search for destructive bacteria was

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carried out in saline soil, contaminated for a long time with organochlorine pesticides. When conducting the study, microbiological, chemical, chromatographic, radiological and statistical methods were used.

RESEARCH RESULTS AND DISCUSSION

According to the data obtained, the soils of the model area are saline and have a neutral or slightly alkaline reaction. The degree of soil salinity in cotton and rice fields was 0.8 and 1.2%, respectively. The soil of the contaminated area contained about 3% salt, while in the halophytic substrate the salt content reached a maximum value of 3-4%. Analysis of the chemical properties of the studied soils showed that the model site was characterized by a low humus content. The lowest amount of humus was observed in the halophytic area (0.1%), a relatively higher content of humus was observed in the rice field (1.4%). The data obtained indicate that long-term pollution with pesticides and soil salinity has led to a decrease in humus content.

Currently, a large amount of pesticides is still concentrated on the territory of former agricultural airfields, pesticide warehouses and poison burial grounds. Without a doubt, pesticides migrate from these areas with wind and groundwater, which leads to their contamination of nearby soil and water bodies. In this regard, the level of contamination of soils and bottom sediments of the model site with residual quantities of pesticides was determined.

The results obtained indicate a high concentration of organochlorine pesticides in the study area; their content accounted for 90% of the total volume of detected pesticides. Among them, persistent organochlorine insecticides predominated, namely dichlorodiphenyltrichloroethane (DDT) and hexachlorocyclohexane (HCCH) and their derivatives.

Thus, the continued detection of organochlorine pesticide residues in the environment of the Republic of Karakalpakstan does not eliminate the problem of contamination of natural substrates with persistent organochlorine pesticides, such as DDT and HCH, despite the cessation of their use. The data obtained indicate the need for constant monitoring of the state of the environment.

In the environment of Karakalpakstan for decades, the most frequently detected pesticide was HCH. The toxicity of HCH is determined by the γ-isomer - lindane, which is effective in agriculture as an insecticide.

The search and isolation of HCH degrading bacteria were carried out in soil contaminated with pesticides for a long time. From HCH-contaminated soil areas, 10 indigenous cultures of microorganisms were isolated by direct seeding on meat-extract agar containing HCH at a concentration of 100 mg/l, which differed from each other in a set of cultural characteristics. The selected cultures were conventionally numbered from 1 to 10.

All isolated cultures of microorganisms were tested for the ability to use lindane as the sole carbon source by sown on synthetic M-9 medium with the addition of lindane at a concentration of 20 μg/ml. The research results showed that on the synthetic medium, on the 3rd day of the experiment, growth was observed in three cultures No. 2, 7 and 9. On the 5th day, growth was observed in the remaining cultures No. 3, 4, 5, 6, 8 and 10, except for culture No. 1, the growth of which was not recorded during the month.

It is known that microorganisms successfully degrade compounds that are closer in chemical structure. In this regard, it was advisable to study the effect of the organochlorine insecticide DDT on the growth of the test crops, since the most detected pesticide in the model area was DDT and its metabolites.

Experiments conducted to study the ability of crops to grow on a mineral medium with DDT at a concentration of 20 μg/ml showed that 72 hours after sowing, growth of cultures No. 5, 6 and 7 was observed. On the 5th day, growth of cultures No. 3 and 4 was recorded on the 7th day of cultivation; the growth of cultures No. 2 and 10 was detected. During the experiment, the development of cultures No. 1, 8 and 9 on a synthetic medium with DDT was not observed.

Thus, the results obtained indicate that the growth of cultures numbered 2, 3, 4, 5, 6, 7 and 10 on a synthetic medium in the presence of both γ-HCH and DDT as the sole source of nutrition implies their ability to assimilate and transform these compounds without additional organic substrate. Apparently, the studied crops, isolated from a site heavily contaminated with pesticides, acquired resistance to chemical compounds, in this case hexachlorocyclohexane and DDT.

Microbiological destruction of HCH by pure bacterial cultures. At the next stage of research, the degradative activity of the isolated crops was studied. Chromatographic analysis of the study showed (Fig. 1) that out of ten cultures, only four caused active destruction of lindane in the nutrient medium. By the end of the first month, this pesticide was almost completely degraded by culture No. 4, 5, 7 and 10 at its initial concentration in the medium of 100 μg/mg. The destructive activity of the remaining crops (cultures No. 1, 2, 3, 6, 8 and 9) was low

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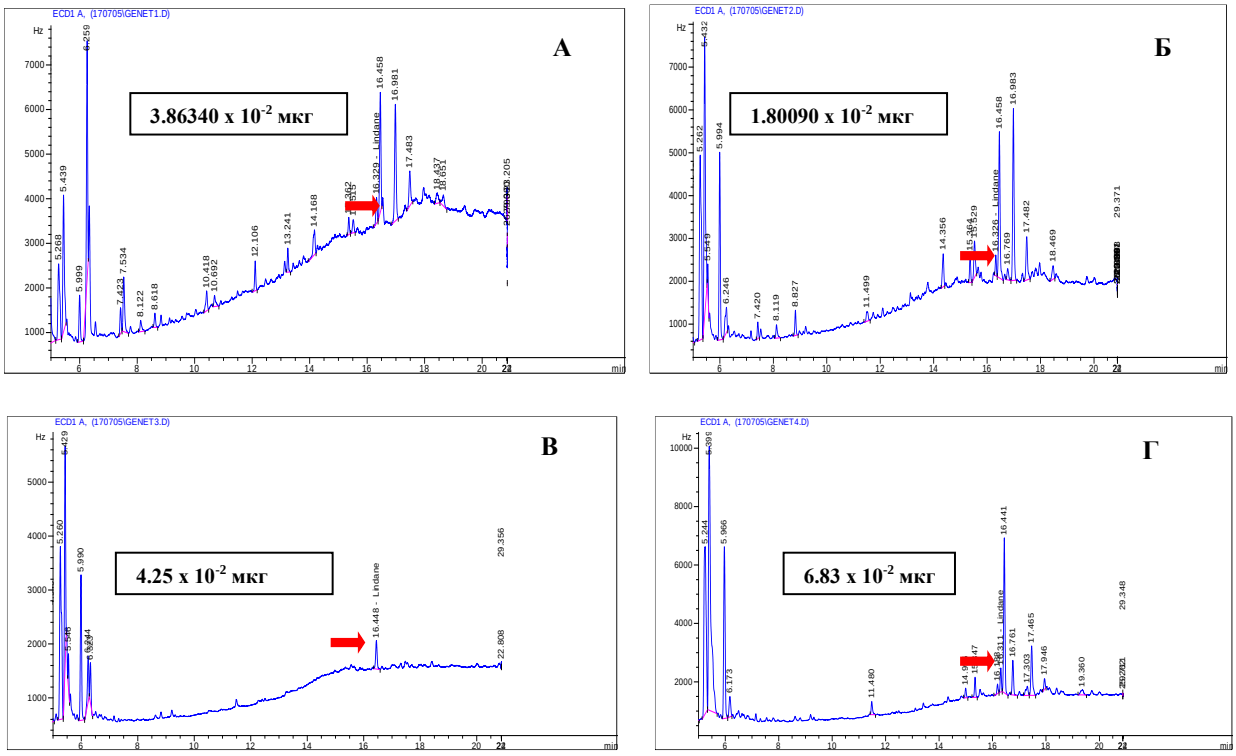

Rice. 1. Chromatographic analysis of the destruction of lindane in the nutrient medium of cultures 4 (A), 5 (B), 7 (C) and 10 (D)

It is known that in nature microorganisms occur in associations and their combined activity can lead to complete mineralization of any organic compounds, while the population of one type of microorganism is not always capable of such destruction. We conducted a study to determine the ability of mixed bacterial cultures to actively degrade lindane in a nutrient medium. For this purpose, a method was used that made it possible to evaluate the distribution of the radioactive label between the culture liquid and bacterial cells.

As can be seen from Table 1, the association of the studied crops had high destructive activity towards lindane, as indicated by the percentage of remaining radioactivity.

Table 1
Content of tritium-labeled lindane in bacterial cells and culture fluid

	Bacteria	Culture medium	Total remaining radioactivity, count 100 µl, pulse/10 sec (% of injected radioactivity)
	count 100 µl, pulse/10 sec (% of remaining radioactivity)		
Control	-	17872	17872 (100%)
Association of Cultures	2180 (26,78%)	5960 (73,22%)	8140 (45,55%)

The data obtained showed that by the end of the 12th day of incubation there was a decrease in the initial concentration of tritium-labeled lindane from 100 µg to 45.55 µg. Of these, 26.78% of the radioactive label was found in bacterial cells, which indicates partial sorption by the surface structures of the cells of the studied cultures. The presence of most of the tritium label - 73.22% in the supernatant allowed us to conclude that the destruction of lindane mainly occurred through the extracellular route. At that time, in the control variant, no loss of radioactive label was observed, as evidenced by the 100% detection of radioactivity in the culture fluid.

Determination of the species of destructor bacteria. For further studies, cultures of destructor bacteria numbered 4, 5, 7 and 10 were selected. The identification of the studied bacteria was carried out on the basis of classical and modern studies. As a result, it was established that culture 4, 5 and 7 belong to the genus Bacillus, species Bacillus subtilis, culture 10 belongs to the genus Micrococcus, species Micrococcus roseus.

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Lindane degradation in soil. The next stage of our research was to determine the destructive activity of selected monocultures of B. subtilis 4 and M. roseus 10 and their association in soil samples artificially contaminated with lindane under laboratory conditions. The initial amount of lindane in soil samples was 14.50 μg/g.

In soil samples with an inoculated culture of B. subtilis 4, after a month, a decrease in the concentration of lindane was noted from 14.50 to 8.37 μg/ml, which is 41.9% of the initial concentration of the drug. Monitoring the state of lindane in the soil with the addition of M. roseus 10 over the same period of time showed a decrease in the concentration of the drug to 5.67 μg/ml from the initial concentration, that is, 60.6% of the drug was destroyed.

Subsequently, we studied the degradation of lindane by a consortium of bacteria B. subtilis 4 and M. roseus 10. As a result, it was revealed that the destruction of lindane by an association of cultures is significantly higher than when using pure destructor cultures. These studies showed that the association of the test crops made it possible to reduce the lindane content in the soil from 14.50 μg/ml to 0.08 μg/ml in a month. Consequently, within thirty days, the percentage of lindane destruction in soil with a mixed crop - B. subtilis 4 and M. roseus 10 was almost 100%.

Analysis of the destructive activity of the tested cultures indicates that at the end of the test period, in samples with the inoculated culture of *B.subtilis* 4, the amount of lindane is 57.8%, and in the case of the culture of *M.roseus* 10 - 39.4%. The introduction of an association of cultures B. subtilis 4 and *M roseus* 10 showed an increase in the degree of destruction of lindane, which amounted to 99.4%.

Thus, the study showed that lindane was effectively utilized by the cultures of B. subtilis 4 and M. roseus 10 in soils under laboratory conditions, and the use of their association would significantly increase the degree of destruction of the pesticide HCH in soil under field conditions.

Based on the conducted research, the following conclusions are presented:

1. Bacterial strains isolated from soil contaminated with organochlorine pesticides were screened for destructive activity towards hexachlorocyclohexane. Cultures No. 4, 5, 7, and 10 showed high destructive activity towards the γ-isomer of HCH in the nutrient medium, both as monocultures and in association.
2. To study taxonomic affiliation, 4 crops were selected based on the study of destructive activity. The results of the study of the phenotypic properties of culture No. 4, 5 and 7 were attributed to the species Bacillus subtilis, culture No. 10 to the species Micrococcus roseus.
3. Based on the synergy between the cultures of B. subtilis 4 and M. roseus 10, an association of these cultures was created for the destruction of organochlorine pesticides. Laboratory experiments have shown that this bacterial association has high HCH-destructive activity in saline soil. It was revealed that on the 30th day of the experiment, the destruction of lindane by the monoculture of B.subtilis 4 was 42%, by the monoculture of M.roseus 10 - 59%, while by the association of 2 degrading bacteria it was 92%.
4. Selected active destructor cultures B.subtilis 4 and M.roseus 10 are recommended to be used to create a new biological product for cleaning soils contaminated with hexachlorocyclohexane under salinity conditions.

LITERATURE

1. *Berezin G.I., Kondakova L.V., Domracheva L.I., Dabakh E.V. Features of microbial groups of soils in the area of the Kilmez burial site for pesticides (Kirov region) // Principles of Ecology. - 2016. - No. 2. - P. 4-15.*
2. *Volgina T.N., Novikov V.T., Reguzova D.V. Paths of distribution of pesticides in environmental objects / Regional problems. 2010. - Volume 13, No. 1. - P. 76–81.*
3. *Galiulin R.V., Bashkin V.N., Galiulina R.A. Agrogeochemistry of persistent pesticides // Bulletin of the Russian Academy of Sciences, 2015. - T. 85, No. 2, - P. 152-154.*
4. *Demidenko G.A., Fomina N.V. Assessment of the influence of herbicides on soil microflora // Bulletin of Kras State Agrarian University. - 2013. - No. 8. – P.49-55.*
5. *Ivantsova E. A. The influence of pesticides on soil microflora and beneficial biota / Bulletin of Volgograd State University. Ser. 11, Natural Sciences. – 2013. – No. 1. – P. 35–40.*
6. *Ivantsova, Yu.V. Kaluzhenkova E.A. Environmental problems of pesticide use // News of the Nizhnevolzhsky Agro-University Complex: science and higher professional education. – 2008. - No. 1 (9). - pp. 41-46.*
7. *Ksenofontova O. Yu., Ivanova E. V. The influence of pesticides on soil microorganisms in the Saratov region / News of Saratov University. Ser. Chemistry. Biology. Ecology, 2012. - T. 12. Issue. 1. - P.75-81.*
8. *Rogozin, M. Yu. Environmental consequences of using pesticides in agriculture//Young scientist. – 2008. - No. 25. (211). - P.39-43.*

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9. *Skorobogatova V.I., Shcherbakova L.F., Ermakova I.T. Rehabilitation of soils contaminated with decomposition products of organophosphorus toxic chemicals / Biology - science of the XXI century: Collection. Abstract 14 Int. Pushchinskaya school Pushchino, 2010. - T. 2. - P. 261.*
10. *Kholoptsev A.V., Ryabinin A.I., Klimenko N.P. Modeling and assessment of surface contamination of the Crimea with pesticides that arrive on it with precipitation in the 21st century / Geography. Geology. - 2017. - Volume 3 (69). - No. 3. Part 2. - pp. 276–293.*
11. *Yamborko N.A., Pindrus A.A. Toxic and mutagenic effect of hexachlorocyclohexane and its microbial destruction products on soil microbial cenosis // Mikrobiol. Journal, 2011. - No. 6. - P.50-57.*