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THE CHANGE OF THE MICROBIAL ECOSYSTEM IN A CHERNOZEM AFFECTED BY PHENOXYACETICACID DERIVATIVES

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2,4-D is one of the oldest, most studied [3] and very common herbicide, from which more than 300 000 tons/year are produced throughout the world at the present time. It has been produced and applied in large quantities in Hungarian agricultural practice since 1954. The study of the interaction of phenoxyacetic derivatives and soil microorganisms is important not only for this reason but also as a comparison with other phenoxy derivatives as well as for the chlorine decreasing tendency in agricultural practice (protection of the environment). Some of our results are presented in this paper.

MATERIAL AND METHOD

Herbicides:

I — 2,4-D = dichloro-phenoxyaceticacid,
II — 2,4,5-T = trichloro-phenoxyaceticacid,
III — 2,4,5-TE = trichloro-phenoxy ethanol,
IV — Dikonirt D = 2,4,D-Na salt — carier material,
V — Trifenoxin 80 = 2,4,5,-T-iso-amylaesther — carrier material.

The chemically pure, (I, II, III) and commercial herbicides (IV, V) were tested on 75 strains of *Rhizobium, Bacillus* and *Pseudomonas* species and one strain of *Agrobacterium tumefaciens* in liquid culture and with agar gel diffusion technique on YMA [4] and nutrient agar media [3]. 2,4-D and Dikonirt were added to the samples of chernozem with forest residues [5] (originating from the Research Station of the Institute of Soil Science and Agricultural Chemistry of the Hungarian

Academy of Sciences at Martonvásár) in the amount of 0.1, 1.0, 5.0 and 10% and incubated at optimal temperature (26-29°C) and moisture (50% of the maximal water capacity) for 58 days. The change of microbial number was recorded on YMA, according to Fehér [1].

The 2,4-D Dikonirt residues of different soils were measured by the spectrophotometer and gas chromatograph [6].

RESULTS AND DISCUSSION

We did not find 2,4-D residues in 113 soil samples taken from 26 agricultural and 10 experimental plots of 10 sites from 6 regions in Hungary representing different soil types [2] in spite of the fact that Dikonirt had been applied in these plots for 1-13 years.

When 2,4-D and 2,4-D-Na (Dikonirt) were added in model experiments to the chernozem with forest residues, they were decomposed by the microorganisms in different ratios for 62 days (Figs 1 and 2). The chemically pure 2,4-D was decomposed more readily (58, 26, 78, 94%) than Dikonirt (51, 20, 43, 22%) having the carrier material, used in agricultural practice.

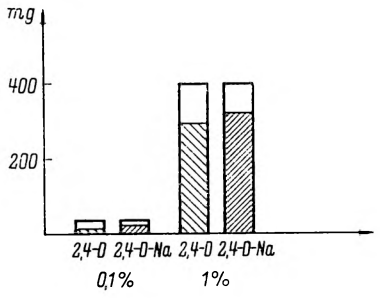

Fig. 1. Residues in soil samples treated with different doses I

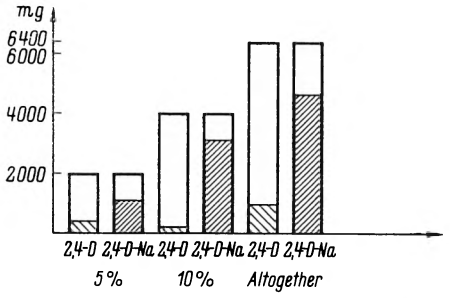

Fig. 2. Residues in soil samples treated with different doses II

The growth of bacteria (Fig. 3) presumably the specialized bacterial flora, was generally stimulated by the 0.1 and 1.0% of 2,4-D and 0.1 Dikonirt treatments. Though this stimulative effect of 0.1 and 1.0% doses was much higher from the 8th day until the 25th day of incubation, in the case of 2,4-D than with 0.1% Dikonirt, the effect of the latter on the number of bacteria increased gradually until the 43rd day of incubation. Although we intended to study only the change of bacterial number, we recorded the changes of ray fungi and microscopic fungi too. The number of ray fungi was slightly depressed by the 0.1% and markedly by treatments with both preparations.

The 0.1 and 1.0% treatments of the investigated herbicides stimulated the multiplication of microscopic fungi after 2 and 4 weeks but especially after 43 days. Higher doses (5.0 and 10.0%) had a marked reducing effect.

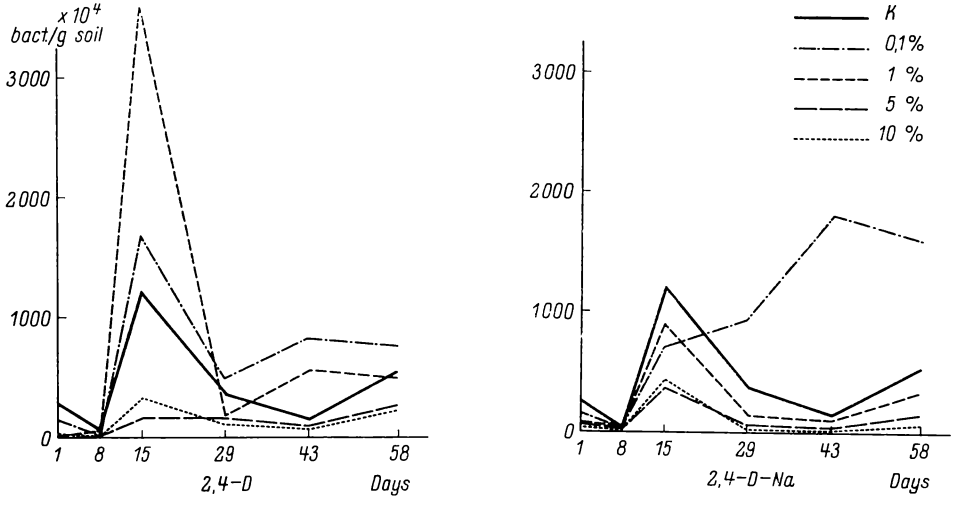

Fig. 3. The change of number of bacteria in chernozem with forest residues affected, by 2,4-D and 2,4-D-Na

We found *Bacillus* strains in the treated soil samples described above during 62 days and these strains of *Bacillus pasteurii, Bacillus subtilis* var. *niger* and *Bacillus cereus* var. *mycoides* isolated by us (Fig. 4) were relatively more sensitive to Dikonirt D than to chemically pure 2,4-D which is supported by the data obtained with 26 tested strains of *Rhizobium* species (Fig. 6).

The comparative sensitivity tests of 41 *Rhizobium* strains to Dikonirt and Trifenoxin (Fig. 5) revealed a relatively higher toxicity of Trifenoxin than that of Dikonirt.

As it is apparent from Fig. 5 the chemically pure compounds (2,4,5-TE, 2,4,5-T, 2,4-D) proved to be less toxic to the different strains of species belonging to *Pseudomonas, Bacillus* and *Rhizobium genera.* The investigated strain of *Agrobacterium tumefaciens* behaved similarly.

According to the test of 104 herbicides on 26 *Rhizobium* strains (Fig. 7) Dikonirt could be listed as a moderately inhibitory herbicide as well as Trifenoxin, though the latter was relatively more toxic to rhizobia both in the rate of inhibition and percentage of inhibited strains.

We did not find 2,4-D residues in many samples of different Hungarian soil types to which Dikonirt was applied earlier. Since it is

known to move down quickly and to be readily decomposed under aerobic conditions we suggest on the basis of our experience with other pesticides e.g. gamma BHC [3] a more intensive survey of the pesticide contamination of soils with the introduction of the biologically microbiologically and chemically controlled "Pesticide cadastrial".

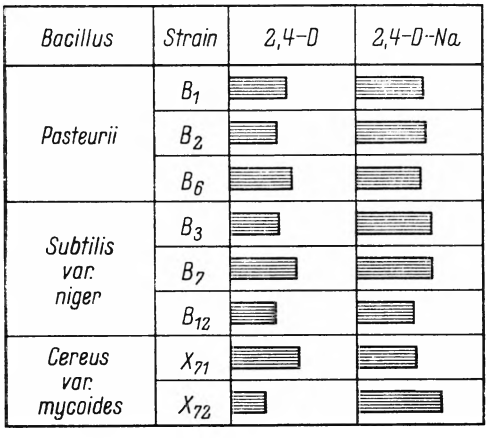

Fig. 4. 2,4-D and 2,4-D-Na sensitivity of bacilli

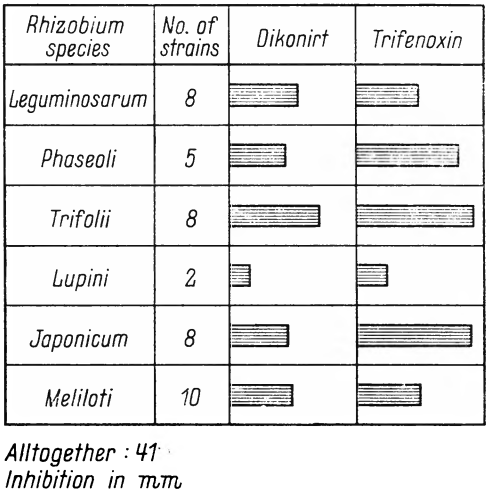

Fig. 5. Sensitivity of rhizobia to Dikonirt and Trifenoxin

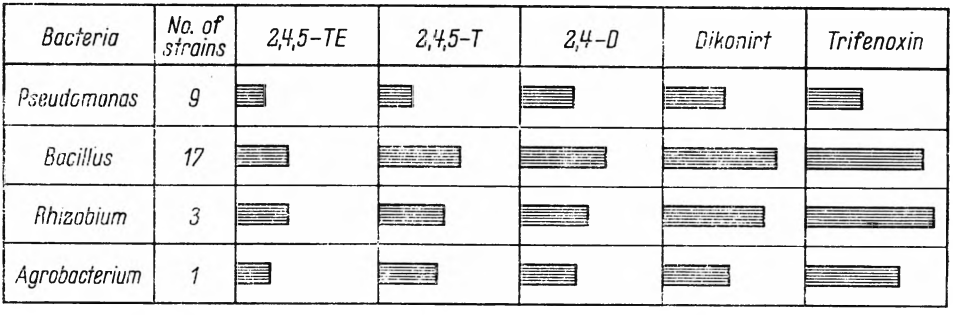

Fig. 6. Phenoxyaceticacid sensitivity of bacterial strains

In this system a combination of the above mentioned methods is recommended to detect the persistence of pesticides not only when the danger is great or the damage already inevitable, but also for prognostic and preventive purposes.

We are convinced that the above outlined "Pesticide cadastrial"

Inhibition	Herbicides	Inhibitory zone mm	Inhibited strains %
Slight	Kloben	14,1	65,0
	Gesatop 50	14,2	77,0
Moderate	Dikonirt	28,0	92,0
	Trifenoxin	36,1	100,0
Strong	Aretit	80,0	100,0
	Basamid	88,1	100,0

Fig. 7. Herbicides sensitivity of 26 *Rhizobium* strains

a survey of pesticides in the soil and the tests with micro- and macroorganisms showing the soil contamination degree with pesticides are an absolute necessity.

CONCLUSIONS

1. The chemically pure 2,4-D was decomposed much more than the commercial herbicide preparation Dikonirt D in the different treatments during 62 days, in a chernozem with forest residues soil.

2. The smaller doses of 2,4-D and occasionally Dikonirt stimulated the multiplication of the specialized bacterial flora with higher doses reducing the number of bacteria, (ray fungi) and microscopic fungi.

3. The decreasing toxicity order of phenoxyaceticacids and derivatives tested on bacilli isolated from chernozem with forest residues soil treated with 2,4-D and Dikonirt D and different strains of species of *Rhizobium*, *Bacillus* and *Pseudomonas* genera was as follows: the chemicaly pure 2,4-D Dikonirt, Dikonirt Trifenoxin; the chemically pure 2,4,5-TE, 2,4,5-T, 2,4-D Dikonirt and Trifenoxin commercial preparations.

4. Though 2,4-D residues were not found in 113 samples of different soil types, microbiological and plant tests as well as chemical analyses of other soil pesticides in the soil call for a more increased control of the pesticide content in soils, with the introduction of "Pesticide cadastrial" system based on quick biological microbiological and chemical tests.

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ZMIANA W EKOSYSTEMIE DROBNOUSTROJÓW POD WPŁYWEM POCHODNYCH KWASÓW FENOXYOCTOWYCH W CZARNOZIEMIE

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Streszczenie

Badano rozkład kwasów fenoxyoctowych i ich pochodnych w glebie oraz ich toksyczność w stosunku do drobnoustrojów glebowych. 2,4-D był rozkładany w glebie (w 113 próbkach różnych gleb) szybciej niż Dikonirt. 2,4-D w małych dawkach zwiększał, a w dużych dawkach zmniejszał ilość mikroorganizmów. Zbadano toksyczność herbicydów rodzaju *Bacillus*, *Pseudomonas* i *Rhizobium*.

Zasugerowano wprowadzenie do praktyki rolniczej lepiej sprawdzonego pod względem biologicznym i chemicznym katastralnego systemu stosowania pestycydów.

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ИЗМЕНЕНИЯ В ЭКОСИСТЕМЕ МИКРООРГАНИЗМОВ ПОД ВЛИЯНИЕМ ПРОИЗВОДНЫХ ФЕНОКСИУКСУСНЫХ КИСЛОТ В ЧЕРНОЗЕМЕ

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Резюме

Исследовали разложение феноксиуксусных кислот и их производных в почве, а также их токсичность по отношению к почвенным микроорганизмам. 2,4-D разлагался в почве (в 113 образцах разных почв) быстрее, чем диконирт. 2,4-D в низких дозах повышал а в высоких дозах сокращал число микроорганизмов. Исследовали токсичность гербицидов на видах *Bacillus*, *Pseudomonas* и *Rhizobium*.

Выдвинуто предложение внедрения в сельскохозяйственное производство лучше испытанной в биологическом и химическом отношении кадастральной системы применения пестицидов.