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Effect of the Insecticide Nerametrine EK-15 on the Activity of Soil Microorganisms

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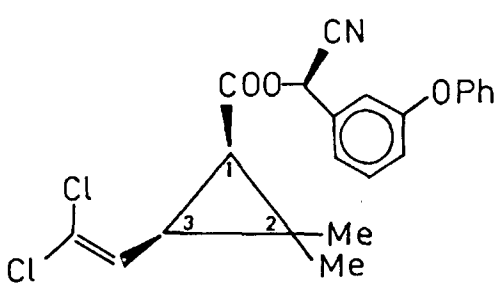
ABSTRACT. The effect of the insecticide Nerametrine EK-15 (containing an active supercypermetrine component) on nitrification, nitrogen fixation, CO_2 production and cellulase activity of soil microorganisms was investigated. Four soil types were sampled from various localities. Supercypermetrine at 31 pmol/kg soil affected remarkably the metabolic activity of all soil samples tested by producing CO_2 after a 1-d exposure. After a 14-d exposure no difference in the metabolic activity related to CO_2 production was noticed in the case of garden soil where the insecticide at 31 pmol/kg soil and the unaffected control were used. As far as other samples are concerned, the supercypermetrine concentration amounting to 31 pmol/kg soil explicitly inhibited the metabolic activity of soil microorganisms. On the other hand, concentrations of 0.61 and 6.1 pmol/kg soil stimulated the metabolic activity of soil in the locality of Senica. The soil samples enriched with nutrients (organic nitrogen in urea) manifested an evident inhibition at 31 pmol/kg soil. The nitrification activity of all soil types was interrupted at 61 pmol/kg soil. Supercypermetrine 0.12 pmol/L stopped completely nitrogen fixation with *A. chroococcum* and that corresponding to 0.3 pmol/L stopped aerobic cellulase decomposition.

Nerametrine EK-15 is an insecticide containing, as the efficient component, supercypermetrine, $C_{22}H_{19}O_3NCl_2$, (*S*)-α-cyano-3-phenoxybenzyl-(1*R*,3*R*)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate, M = 416.32 g/mol, with 15 % active material. The substance is water-insoluble and it dissolves in dimethyl sulfoxide. Its application in agriculture has been known under the commercial name Permethrin I produced as an insecticidal spray. Its high effect is caused by the dichlorovinyl group which binds actively to biological systems. Supercypermetrine is the insecticide synthesized on the basis of natural, biologically efficient substances produced by plants for their protection against various pests (insects, fungi, bacteria, *etc.*). In nature, the plants produce these substances at very low concentrations tolerated by animals. The concentrations may be substantially increased by plant-affecting shocks, such as extreme temperatures, pesticide sprays, *etc.*



Pesticides can get into the food chain through plant and animal products. The risk is great especially in the case of insecticides based on chlorinated hydrocarbons. Chlorinated pesticides are characterized by the high persistence, ability to be accumulated in animal and human organisms and by the mutagenic activity (Gilbert *et al.* 1980; Rannug and Beije 1979). In nature, the problem of environmental protection by detoxication of the chlorinated pesticides is solved by soil and water microorganisms (Mohilev 1982). Simon (1973) pursued the effect of chlorinated aliphatic herbicides on ammonization, nitrification, biological activity and on aerobic decomposition of cellulose in the four soil types in Slovakia. The doses of herbicides slightly stimulated the CO_2 production. During detoxication, environmental humidity plays also an important role. With 80 % water capacity (FWC) the toxic effect will last 40 d and with 40 % humidity the time of toxicity will be prolonged up to 55 d.

MATERIALS AND METHODS

Insecticide. Nerametrine EK-15 (*Research Institute of Chemical Technology*, Bratislava, Slovakia) was used.

Four soil types from localities Bodorová, Báhoň, Senica and from a garden plot were tested. The soils had not been previously treated with pesticides. Their characteristics are given in Table I.

Estimation of CO_2 production by titration was carried out after 1 and 14 d, respectively, without adding the nutrients; after 1 d it was done by adding urea (Bernát and Seifert 1955). The tested soils were enriched with 60 mg nitrogen per kg soil. 2.2 mg are required for the neutralization of 1 mL of 0.1 mol/L KOH. CO_2 was entrapped in 0.1 mol/L KOH.

Table I. The results of agrochemical analyses of soils

Soil (type)	Kind	pH_{KCl}	pH_{H2O}	Nutrients, ppm		Locality
				P	K	
Medium heavy brown clay	medium	6.0	6.4	160	263	Senica
Brown glued	medium	5.8	6.0	113	201	Bodorová
Medium heavy sandy-clay loam, brown	medium	5.0	5.3	89	46	Báhoň
Garden	light	6.5	7.0	54	177	garden

Locality	Grain distribution in % and frequency in μm, %						Organic carbon[a] % C_{ox}	Total nitrogen[b] %	Humus[c] % (C_{ox} 1.724	Specific conductance μS/cm
	10	1	1–10	10–50	50–250	250–2000				
Senica	95.2	16.2	19.0	49.1	14.3	1.4	1.24	0.115	2.14	180
Bodorová	52.0	30.0	22.0	42.0	0.0	0.8	2.16	0.254	3.72	150
Báhoň	41.6	23.6	18.0	41.8	15.6	1.0	1.49	0.15	2.57	90
Garden	38.4	10.6	27.8	40.7	17.1	3.8	13.26	1.33	22.86	1450

[a]All organic nitrogen is oxidizable with chromosulfuric acid; excess of acid titrated with Mohr's salt.
[b]The total nitrogen determined by Kjehldal.
[c]Organic carbon, % × empirical factor 1.724 indicates the content of humus in %.

The nitrification intensity in soil samples was determined according to the nitrate content ($N\text{-}NO_3$), being established spectrophotometrically by using 2,4-phenoldisulfonic acid after a 14-d incubation of the soil (Drobník *et al.* 1957). Both an inorganic and an organic source of nitrogen was added to the soil. An increase of the nitrate concentration was determined in the soil extract. The following variants were tested: soil; soil + $(NH_4)_2SO_4$; soil + $(NH_4)_2SO_4$ + insecticide; soil + urea; and soil + urea + insecticide. The tested soils were enriched with 60 mg of nitrogen per kg soil.

Estimation of nitrogen fixation by an *Azotobacter chroococcum* culture was done according to Kopčanová *et al.* (1979). The microorganism was isolated from the soil on an Ashby agar medium and cultivated for 14 d at 28 °C. The bacterial colonies were first translucent and then became white. After a 14-d cultivation on nitrogen-free soil containing glucose as carbon source (10 g/L) the increase of nitrogen and the decrease of saccharides were monitored.

The aerobic decomposition of cellulose was evaluated qualitatively after a week's interval at laboratory temperature in the presence of the insecticide examined (Čerňáková *et al.* 1990). The total cellulose activity, C_x, was tested on hydroxyethylcellulose cross-linked by 2-chloromethyloxirane. In the positive case the cross-linked cellulose was liquefied.

RESULTS AND DISCUSSION

An influence of the insecticide on the biological activity of soil monitored by CO_2 production was observed during 1 and 14 d, respectively. A sequence of the effectiveness related to CO_2 production in 1 d was as follows: garden soil > soil of Bodorová > soil of Senica > soil of Báhoň. The effectiveness observed after 14 d was as follows: garden soil > soil of Senica > soil of Bodorová > soil of Báhoň. Supercypermetrine at 0.31 pmol/kg soil inhibited the metabolic activity except for the garden soil where the inhibition became manifest after 1 d and corresponded to the control after 14 d. After adding urea at 60 mg nitrogen per kg soil the inhibition continued for 24 h. A moderate inhibition was recorded already with 6.1 pmol/kg soil (Table II). The best metabolic activity was found in the garden soil which is, in comparison with the other soils, light and alkaline; it contains a 3-fold greater amount of potassium than phosphorus, it has a much higher conductivity than the other tested soils and ten

times more humus, nitrogen and carbon. No substantial differences among the other soil types were noticed. The results of agrochemical analyses are shown in Table I. It can be generally stated that the garden soil itself contains more nutrients.

Table II. Effect of supercypermetrine measured by CO_2 production in mmol/kg soil

Time d	Soil	Control	Supercypermetrine, pmol/kg		mmol CO_2 per kg soil
			0.61	6.1	
	Without urea				
1	Garden	390	400	410	320
	Bodorová	340	320	300	150
	Senica	230	220	220	200
	Báhoň	220	220	220	150
14	Garden	>1300	>1300	>1300	>1300
	Bodorová	810	920	750	530
	Senica	900	1300	1180	690
	Báhoň	840	690	640	460
	With urea (60 mg nitrogen per kg soil)				
1	Garden	1300	1290	1270	1100
	Bodorová	840	830	810	660
	Senica	840	810	760	570
	Báhoň	750	700	680	560

Nitrification proceeded most effectively on using an inorganic source in the garden soil at the locality of Báhoň. Supercypermetrine at a concentration of 61 pmol/kg soil stopped completely the nitrification in all soil types (Table III).

Table III. Effect of supercypermetrine (61 pmol/kg soil) on nitrification intensity in soil samples after 14 d

Soil	Nitrates before cultivation mg/kg soil	Additional nutrients[a]	Nitrates, mg/kg soil	
			after cultivation	increase[b]
Garden	20	$(NH_4)_2SO_4$	500	480
Bodorová	80		270	190
Senica	120		180	60
Báhoň	80		90	10
Garden	20	urea	180	160
Bodorová	80		290	210
Senica	120		310	190
Báhoň	80		810	730

[a]60 mg nitrogen per kg soil.
[b]After supercypermetrine addition at a concentration of 61 pmol/kg soil are all values zero.

The effect of supercypermetrine on nitrogen fixation by *Azotobacter chroococcum* yielded the results shown in Table IV. The concentration of 12 fmol/L led to an 18.4 % decrease in nitrogen fixation compared with the control. Pesticide concentration of 0.12 pmol/L resulted in a total inhibition. To monitor the effect on pure cultures, a lower concentration of the pesticide was necessary in comparison with soil samples (Čerňáková *et al.* 1991, 1992). It is likely that the soil complex will bind the

pesticide up to its saturation and only afterwards the microorganisms will be affected by the next free fraction (Čerňáková *et al.* 1991*a,b*). Geller and Chariton (1961) claim that the activity of ammonization microorganisms and of nitrogen fixers, such as *A. chroococcum*, is inhibited by TCA more intensely than that of nitrification bacteria.

Table IV. Effect of supercypermetrine on nitrogen fixation by a culture *A. chroococcum*

Quantity	Concentration, fmol/L		
	0	12	120
Glucose utilized, g/L	9.6	9.5	0
Nitrogen bound, g/L	6.6	4.3	0
Nitrogen binding, mg N/g glucose	680	450	0
Decrease of nitrogen fixation, %	0	34	100

On the basis of a qualitative evaluation of aerobic decomposition of cellulose it can be concluded that the control was positive after 4 d and the samples after 7 d. The concentration (amounting to 0.3 pmol/L) of the insecticide tested gave a 100 % inhibition which persisted even after a one-month period. The results obtained at supercypermetrine concentrations of 6 and 12 fmol/L in the medium showed to be positive like in the unaffected control.

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