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Effect of Some Pesticides on Microorganisms Isolated from Honeybees

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New pesticides are often introduced through agricultural practice into the bioenvironment without a thorough check of their fate and side-effects. It has been demonstrated that rapid and accurate tracing of pesticide residues is a limiting factor in this regard. Very precise physico-chemical methods need expensive and very complicated procedures. However, for many purposes, microbiological methods were suggested as sufficiently accurate and inexpensive for preliminary experiments (MOWAT 1976, BABICH & STOTZKY 1977, TREVORS & BASARABA 1980, LIU 1981) since some pesticides exhibit inhibitory effect on many microorganisms (ALEXANDER 1973, EHLE 1973, VOETS et al. 1975).

The object of this contribution is to study the effect of several pesticides on some representative microorganisms of the alimentary tract of honeybees and the possibility to use the analysis of this microflora for biological monitoring of pesticide contamination. Pesticides which have not been previously studied in this regard were selected for our testing.

MATERIALS AND METHODS

Microorganisms used in all experiments were isolated by DROBNÍKOVÁ (1976) and BACÍLEK (1976) from the alimentary tract of worker bees and queens: Bacillus brevis, Bacillus macerans, Micrococcus varians, Acinetobacter sp., Schizosaccharomyces sp., Torulopsis versatilis, Candida shehatae and three strains of Bacillus larvae (Nos. 5356a, 5356b and 3007), a causative agent of American foulbrood. All these microbes were from the collection of cultures of Apicultural Research Institute, Dol.

Pesticides:
fenitrothion, VÚ AgT Bratislava, Czechoslovakia
Actellic® EC 50 (50% pirimiphos-methyl), ICI-PP, United Kingdom
phosalon, Rhône-Poulenc, Lyon, France
bromophos-ethyl, CelaMerck, Ingelheim, German Federal Republik
Evisekt® SP 90 (90% thiocyklamhydrogenoxalate) Sandoz, Basel, Swiss
Dimilin 25 DP (25% diflubenzuron) Duphar B.V., Amsterdam, Holland
pirimicarb, ICI-PP, United Kingdom
folpet (Chimica GmBH, Wien, Austria) and its hydrolysis product phthalimide (Lachema Brno, Czechoslovakia)

Doses were calculated in grams of active ingredients. Each substance was dissolved in dimethylsulphoxide except pirimicarb and

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fenitrothion which were diluted in ethanol to the concentration 0-300 µg substance per mL (0-300 ppm). Tested solutions were applied in the 7 mm holes in agar medium preliminary seeded by indicator microorganisms.

Medium: Tryptone - yeast extract agar plates (J agar, GORDON et al. 1973) were prepared 24 h before the experiment. This medium was suitable for both pathogenic B. larvae and the other saprophytic microorganisms. Inoculated plates were incubated at 36C and inhibition zones were measured in 24 h intervals. Results were evaluated and calculated according to STOLEJDA & HRDLIČKA (1978).

At the same time we measured the effect of pesticides on growth rate of microorganisms in J broth at 36C. Optical density of liquid media was measured at 600 nm with pure solvent as the control.

In the next experiment, we tried to assess the presence of pesticides in poisoned honeybees. The bees were killed by fenitrothion and extracted by the 1:1 mixture of acetonitrile and petroleum ether in the presence of anhydrous sodium sulphate (BACÍLEK 1976). Extract of untreated bees and ethanol were used as control.

RESULTS AND DISCUSSION

The detection of pesticide contamination by microbiological methods has been described by several authors. EHLE (1973) successfully applied this method for pesticide-treated seeds. KRUGLOV & KVIATKOVSKÁ (1973) detected herbicide residues by some algae. The effect of biocides on the metabolism of Escherichia coli cells was described by DAUBNER & JEŽOVÁ (1976).

Also the composition of microbial population in alimentary tract of bees has been influenced by some pesticides (GILLIAM et al. 1975, BACILEK 1976)

BACILEK (1976) found that folpet introduced to caged worker honeybees prolonged LT_{50} of the control and proved that this increase of the life span is due to the inhibition of yeasts in the digestive tract of honeybees by folpet. This phenomenon encouraged us to check if different pesticides even less toxic than fenitrothion could affect honeybee microflora.

Pure dimethylsulfoxide or ethanol had no effect on microorganisms in the experiment.

Folpet (Table 1) showed with all tested bacteria inhibition zone 5 mm and 10 mm at 100 ppm and 200 ppm, respectively. The zones were quite clear with sharp contours. Schizosaccharomyces sp., Candida shehatae and Torulopsis versatilis were more sensitive to folpet than tested bacteria, as their inhibition zones were double. BACILEK's finding (1976) concerning folpet inhibition of bee-alimentary tract yeasts was confirmed.

Table 1. Influence of folpet and phthalimide on alimentary tract microorganisms of honeybees given as a zone of inhibition in mm after 24 h incubation.

Microorganisms	Folpet, ppm		Phthalimide, ppm	
	100	200	100	200
Bacillus brevis	5	10	2	4
Bacillus macerans	5	10	2	5
Micrococcus varians	5	10	0	0
Acinetobacter sp.	5	10	2	5
Schizosaccharomyces sp.	10	20	0	0
Torulopsis versatilis	10	20	0	0
Candida shehatae	10	20	0	0
Bacillus larvae	5	10	(1-2)+	(3-5)+

+ blurred zone

Phthalimide (Table 1) exerted the bacteriostatic inhibition only on B. brevis, B. macerans, Acinetobacter sp. and B. larvae, but much smaller than folpet, especially with B. larvae where zones were blurred. Yeasts and Micrococcus varians were not affected by phthalimide.

Fenitrothion which is considered to be very harmful to bees and fenitrothion-contaminated bee extract showed only very weak bacteriostatic effect to Acinetobacter sp. and B. larvae at the concentration of 15 ppm. Fenitrothion at 5 and 15 ppm in liquid medium slowly reduced the growth rate of B. larvae only. Nevertheless, it is improbable that accidental contact with fenitrothion in sublethal doses could influence the dissemination of American foulbrood disease.

The increase of fenitrothion concentration to 200 ppm provoked the retardation of the growth rate of all species except for B. macerans after 26 h incubation. This phenomenon was reversible as after 24 h there was no difference with the control in all species except for Micrococcus varians where faster growth was observed.

The growth rate of microorganisms (Table 2) was not negatively influenced by the fenitrothion-killed bee extract containing 5 and 15 ppm of fenitrothion. On the contrary, the latter dose stimulated the growth by 30-40%. This stimulation can be attributed to some acetonitrile-petroleum ether coextractive substances. Practical applicability of microbiological detection of pesticides seems to be highly dependent on biological material and the cleaning of the extracts should be adjusted for every material.

Actellic which belongs also to the group of organophosphorous insecticides showed inhibitory effect on honeybee bacteria only, not on yeasts. It may be that the additives in commercial product are responsible for its action. Then, it is possible that in treated bees, Actellic breaks the microbial equilibrium in alimentary tract and the number of yeasts in it may fluctuate as described GILLIAM et al.

(1975) and BACÍLEK (1976) for phenoxycarboxylic acids and folpet.

Table 2. Influence of fenitrothion on growth rate of microorganisms. Changes in OD after 16 h incubation in percent of the control grown only with the solvent.

Microorganisms	Fenitrothion (ppm)				
	Pure matter			Extracted from bees	
	15	200	200/24+	5	15
Bacillus macerans	100	100	100	100	140
Bacillus brevis	100	70	100	100	130
Acinetobacter sp.	100	70	100	100	130
Micrococcus sp.	100	70	120	100	140
Schizosaccharomyces sp.	100	70	100	100	130
Bacillus larvae	80	80	100	100	130

+ After 24 h incubation

Stimulation nor inhibition was observed with other organophosphorous insecticides, phosalone and bromophos-ethyl.

Table 3. Influence of Actellic on microorganisms of alimentary tract of honeybees given as a zone of inhibition in mm. after 24 h incubation.

Microorganisms	Actellic (ppm)		
	50	150	200
Bacillus macerans	5	11	15
Bacillus brevis	5	10	15
Acinetobacter sp.	-	4	8
Micrococcus varians	5	6	7
Schizosaccharomyces sp.	0	0	0
Torulopsis versatilis	0	0	0
Candida shehatae	0	0	0
Bacillus larvae	0	5	7

Pirimicarb belonging to carbamate insecticides was tested at 10, 30, 100 and 300 ppm. Only Micrococcus varians showed inhibition starting from 30 ppm. The growth rates were not affected at all. Evisekt SP 90 and Dimilin 25 DP demonstrated no effect on microorganism under study up to 200 ppm. Pirimicarb, phosalone, bromophos-ethyl, Evisect and Dimilin have low toxicity to adult honeybees and our results support their suitability for integrated pest control.

The results show that it is hard to predict the inhibitory action of an organophosphorous insecticide on bee microflora from its structure. General physiological or microbiological effects of products showing low toxicity to bees should be studied.

Only few pesticides are shown to be significantly effective inhibitors of microorganisms so that they can be detected by microbiological residue analysis. However, this method can reveal important side-effects of so-called harmless pesticides for honeybees.

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