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A case of acute intoxication with deltamethrin in bee colonies in Romania

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ABSTRACT

In May 2002, in a mellifera zone situated in the east part of Romania a high mortality of bees colonies from all apiaries has been observed.

The damages situated at 90% from colonies were registered during and 24 hours after, when phytosanitary treatments were effectuated to rape plantations.

Examination of all apiaries has relived many colonies organized by an average of 7-8 brood frames on different stages of development, dead worker bees and drones with no pathological modifications, massive grouped in the front of hive entrance and on whole harvested zone. Hive moribund bees had normal chitin, but presented uncoordinated movements, ventral exposition, own axe rotations, flying incapacity, wings extension, posterior feet paralyses, intermittent needle and oral system expulsion.

Usual bacteriological and parasitical exams and toxicological determinations regarding pesticides were effectuated on bees, brood and mellifera flora harvested from affected area.

The results of bacteriological and parasitical exams were negative. Using gas chromatographic method deltamethrin was identified both in mellifera flora and bees.

The cause of mortality was an acute deltamethrin intoxication.

Key words: intoxication, deltamethrin, bees

INTRODUCTION

Deltamethrin [(*S*) - cyano (3 - phenoxyphenyl) methyl (1*R*, 3*R*) - 3 - (2, 2 - dibromoethenyl)-2, 2 - dimethylcyclopropanecarboxylate] (Fig. 1) is a pyrethroid insecticide used for agricultural, public health and livestock applications.

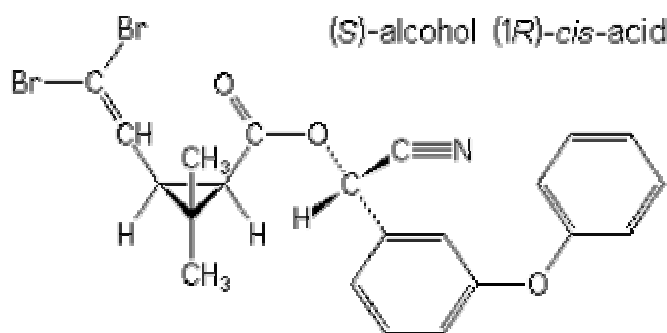


Figure 1: Structural formula of deltamethrin

Usage. It is used to control apple and pear suckers, plum fruit moth, caterpillars on brassicas, pea moth, aphids (apples, plums, hops), winter moth (apples and plums), codling and tortrix moths (apples). Control of aphids, mealy bugs, scale insects, and whitefly on glasshouse cucumbers, tomatoes, peppers, potted plants, and ornamentals. It also controls numerous insect pests of field crops.

Mode of action. Deltamethrin is a synthetic insecticide based structurally on natural pyrethrins, which kills insects on contact and through digestion and rapidly paralyze the insect nervous system giving a quick knockdown effect.

Deltamethrin has a rapidly disabling effect on feeding insects and for this reason there is hope that it may be useful to control the vectors of "non-persistent" viruses (viruses that can be passed on by the vector within a few minutes of starting to feed on the plant). Deltamethrin's mode of action is thought to be mainly central in action, or at least originate in higher nerve centers of the brain. Death of insects seems to be due to irreversible damage to the nervous system occurring when poisoning lasts more than a few hours. Deltamethrin poisoning occurs through cuticular penetration or oral uptake. The susceptibility of insects is dependent on a variety of factors and can vary, as with many insecticides, according to the environmental conditions. Flies are most susceptible to pyrethroid poisoning shortly before dawn.

Many pyrethroids are not very active against cattle ticks, but some alpha cyano compounds (of which deltamethrin is one) have higher activity than organophosphates or amidines, the former standard compounds for this purpose. Deltamethrin has very good residual activity for outdoor uses (field crops, cattle dip, tsetse) and for indoor uses (mosquitoes, stable flies, horseflies, fleas, cockroaches, stored product insects).

Formulations include emulsifiable concentrates, wettable powders, ULV and flowable formulations and granules.

Environmental fate.

Soil and Groundwater: In soil, degradation occurs within 1-2 weeks.

Surface Water: Deltamethrin in pond water was rapidly adsorbed, mostly by sediment, in addition to uptake by plants and evaporation into the air.

Vegetation: About 10 days after use, there are no deltamethrin residues observed on plants. There is no known phytotoxicity to crops.

Toxicity

Deltamethrin is classed as a WHO Class II moderately hazardous pesticide.

Mammalian toxicology. The acute oral LD50 in male rats ranged from 128 mg/kg to greater than 5,000 mg/kg depending on the carrier and conditions of the study; the LD50 for female rats was 52 mg/kg and other published values range from 31 to 139 mg/kg. Values ranging from 21 to 34 mg/kg were obtained for mice; while dogs had a reported LD50 of 300 mg/kg. The intravenous LD50 in rats and dogs was 2 to 2.6 mg/kg, and the dermal LD50 was greater than 2,940 mg/kg. The acute percutaneous LD50 for rats was reported to be greater than 2,000 mg/kg; greater than 10,000 mg/kg for quail; and greater than 4,640 mg/kg for ducks. The acute dermal LD50 for rabbits was greater than 2,000 mg/kg.

Human. Studies have shown many cases of dermal deltamethrin poisoning after agricultural use with inadequate handling precautions, and many cases of accidental or suicidal poisoning by the oral route at doses estimated to be 2-250 mg/kg. Oral ingestion caused epigastric pain, nausea, vomiting and coarse muscular fasciculations. With doses of 100-250 mg/kg, coma was caused within 15-20 minutes.

Effects on Birds: The reported 8-day LC50 for ducks was greater than 4,640 mg/kg diet; and greater than 10,000 mg/kg diet for quail.

Effects on Aquatic Organisms: As is common with many pyrethroids, deltamethrin has a high toxicity to fish under laboratory conditions. However, in field conditions under normal conditions of use, fish are not harmed. Deltamethrin had an impact on aquatic herbivorous insects. This impact led to an increase of algae. Although the fish (fathead minnows) accumulated the deltamethrin, no mortality could be observed. In laboratory trials, the LC50 for fish was 1-10 micrograms/l. Aquatic fauna, particularly crustacea, may be affected, but fish are not harmed under normal conditions of use.

Effects on Other Animals (Nontarget species): Deltamethrin is considered toxic to bees. The LD50 oral is 0.079 micrograms/bee and the LD50 contact is 0.051 micrograms/bee.

In this paper we present an acute deltamethrin intoxication in bees colonies in Romania.

In May 2002, in a mellifera zone situated in the east part of Romania a high mortality of bees colonies from all apiaries has been observed.

The damages situated at 90% from colonies were registered during and 24 hours after, when phytosanitary treatments were effectuated to fruit trees plantations.

Examination of all apiaries has relived many colonies organized by an average of 7-8 brood frames on different stages of development, dead worker bees and drones with no pathological modifications, massive grouped in the front of hive entrance and on whole harvested zone. Hive moribund bees had normal chitin, but presented uncoordinated movements, ventral exposition, own axe rotations, flying incapacity, wings extension, posterior feet paralyses, intermittent needle and oral system expulsion.

Based on these observations our research has the aim to investigate pesticides from affected apiaries.

MATERIALS AND METHODS

CLINICAL SIGNS AND PATHOLOGICAL EXAM

To all colonies, only a few dead worker bees with black chitin (Fig. 2) and all the other hive dying bees had normal chitin (Fig. 3), but presented uncoordinated movements, ventral exposition, own axis rotations, flying incapacity, wings extension, posterior feet paralysis, intermittent needle and oral system expulsion.



Figure 2: Some death worker bees with black chitin



Figure 3: Dying worker bees in the hive

Brood examination revealed sealed and unsealed cells, brood died in the cells and all young larvae were killed. There were dead bees during the emerge process and dying young bees on

the hive, too. These young bees were in the interior of the frame cell with nervous syndrome and many other adult hive worker bees become trembly, and presented uncoordinated movements.

Bacteriological exam

We made bacteriological exam on dying bees, from hemolymph and gut contents. This exam didn't reveal specific pathogenic bacteria.

Parasitological exam

Parasitological exam was negative for the identification of the following mite species: *Varroa destructor*, *Braula coeca*, *Acarapis woodi*, *Nosema apis* and *Malpighamoeba mellificae*.

Toxicological exam

Having information about the commercial product used for fitosanitary treatment, we determined the concentration of deltamethrin in dead bees and rape samples.

Analytical procedure:

- rape samples: homogenize 50 g sample with 10 g Celite 545 and 200 ml hexane-acetone mixture (8:2) for 3 min.; suction-filter the homogenate and make up the organic phase of the filtrate to a total volume of 250 ml, washing the homogenate with the same solvent mixture; shake 50 ml of the organic phase twice with acetonitrile; combine the acetonitrile phases, add sodium chloride 5% in deionized water and shake with hexane; dry the combined hexane phases on sodium sulphate and filter in a 500 ml flask; wash the sodium sulphate with hexane and rotary-evaporate the combined filtrates to near dryness with 30°C bath temperature; the extract is cleaned using a florisil/sodium sulphate column and eluate with hexane-toluene mixture (2:8); evaporate this eluate to dryness, dissolve the residue in hexane and inject an aliquot of this solution into the gas chromatograph.
- honey bees samples: homogenize 25 g sample with 5 g Celite 545 and 100 ml hexane-acetone mixture (8:2); suction-filter the homogenate through a fast flow-rate filter paper in a Buchner porcelain funnel and wash the filter cake with the same solvent mixture: the organic phase is dried on sodium sulphate and then rotary-evaporate near dryness with 30°C bath temperature. The residue is transferred on a florisil/sodium sulphate column prewashed with hexane: the column is rinsed with eluting mixture hexane-toluene 8:2 and discard the eluate: then eluate pyrethroid from column with eluting mixture hexane-toluene 2:8 : collect this eluate and rotary-evaporate to dryness with 40°C bath temperature. Dissolve the residue in hexane and inject an aliquot of this solution into the gas chromatograph.

Instruments:

- gas chromatograph system consists of a Thermo Quest Instruments TRACE GC 2000 equipped with autosampler, electron capture detector (ECD) and split/splitless injector; the equipment is controlled by PC using Chrom-Card soft.
- column: DB-5, 0,25 mm i.d., 30 m long, film thickness 0,25 µm.

Operation conditions:

- Injector temperature: 250°C
- Detector temperature: 290°C
- Column temperature: programmed to rise at 60°C/min. from 70 to 180°C, and at 3°C/min. from 180 to 290°C, then isothermal at 290°C for 5 min.
- Carrier gas: helium, 1 ml/min.

- Make-up: nitrogen, 50 ml/min.
- Injection volumn: 1 μ l (splitless)
- Standard concentration: deltamethrin 1 μ g/ml in hexane (Fig. 4)

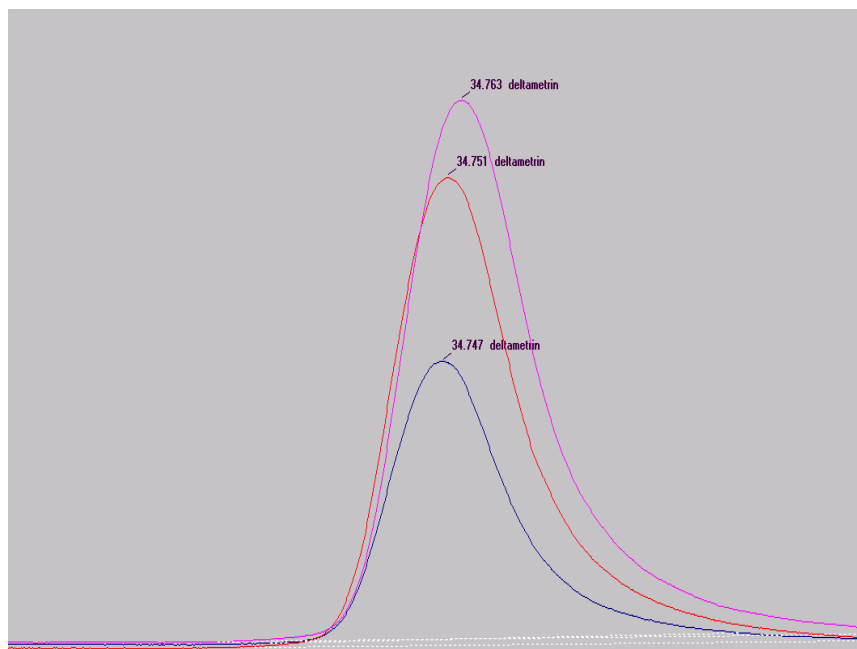


Figure 4: CG chromatogram of the deltamethrin standard, bee and rape sample (between 34,4213 and 35,3153 min.)

- bee sample (retention time = 34,763 min.)
- rape sample (retention time = 34,747 min.)
- standard (retention time = 34,751 min.)

RESULTS AND DISSCUTIONS

In our laboratory we obtained the following results:

Samples	Concentration of deltamethrin (μ g/g)	Concentration of deltamethrin (μ g/bee)*
Rape	2,621	-
Rape	1,785	-
Rape	6,331	-
Bees	2,591	0,3239
Bees	2,338	0,2923
Bees	0,859	0,1074
Bees	1,580	0,1975
Bees	1,517	0,1893
Bees	2,414	0,3081

* We considered that a bee weights 0.125 g

As we presented, deltamethrin may be toxic to bees in certain conditions. Comparing the concentrations of deltamethrin in bees samples we observed that these are between 1.3 and 4.1 times higher than toxic level so that the mortality of bees was caused by an acute deltamethrin intoxication.

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