HONEY AND WAX ANALYSIS FOR ACRINATHRIN RESIDUES

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Abstracts

In the last decades considerable economic damages were caused by Asian bee mite (Varroa destructor) in European and also in Hungarian apiaries. For the control fumigant strips, solutions and aerosols containing acaricide active ingredients were introduced. Plastic and wooden strips impregnated with synthetic pyrethroid active ingredients show a high efficacy againts the mites. Strips are continuously present for 3-4 weeks in the beehives so they are effective in different developmental phases of mites. These treatments, however may leave residues in apicultural products (honey, propolis, wax).

The Gabon PA 92 strips (Czech product) contain a synthetic pyrethroid active ingredient, acrinathrin. There is an increasing awareness among consumers of the hazard of chemical contamination of food. With this in view experimental treatments were carried out to determine achrinathrin residue levels in honey and beewax.

Five bee-colonies were treated by hanging 2 Gabon PA 92 strips in every beehive for 25 days in the apiary of the Institute for Small Animal Research. Two weeks after removing the strips from the treated hives (at the time of honey extraction) honey and wax samples were taken. Untreated (control) samples were also taken at the same time.

The appearance of the lipophilic active ingredient was expected first of all in the beewax. Therefore honey and wax samples were processed separately, using different extraction and sample extract clean up methods. Acrinatrin residues were determined by gaschromatography, using electron-capture detector.

Parallel to the treated samples we examined control honey and wax samples from untreated hives and samples spiked with active ingredient. Achrinathrin was added to the honey samples in 0.05-0.20 mg/kg and to the wax samples 1.00-5.00 mg/kg amounts, and subsequently the recovery rates (%) were determined. The main recovery was 96.7 % for honey samples and 88.3 % for beewax samples. Blanks of the chemicals involved did not show any cross-contamination. Acrinathrin residues were not detected in the extracts of control samples.

The residue levels of active ingredient were less than 0.01 mg/kg in honey and less than 0.10 mg/kg in wax taken from hives treated with Gabon PA 92 wooden strips. From the food-hygienic point of view it was favorable that acrinathrin residues were not detectable in any of the processed honey and beewax samples. During the experimental treatment the honey did not become contaminated with acrinathrin.

The results of this residue determination trial served as a basis for the registration and marketing authorisation of this veterinary product in Hungary. In the future, the investigations will be extended to residue tests of acrinathrin in samples taken from different locations and at different intervals after treatment. For health protection

purposes and maintain the exportability of Hungarian honey, further tests will be performed with acrinathrin and other active ingredients of bee acaricides (fluvalinate, flumethrin, amitraz).

Introduction

The continuous damage done by the Asian bee mite *(Varroa destructor)* since 1978 made it necessary to hold an international symposium in Prague in 1993 to discuss methods for the control of this parasite. At that symposium different biological, mechanical and chemical tools were presented.

In Hungary, the living conditions of bees, the short distance between apiaries as well as the wandering of bee-keepers make it impossible to eradicate the mite completely. The damage of this parasite varies as a result of the dissimilar and continuously changing conditions. Its presence is, therefore, always presumable and should be considered. At the moment we cannot protect our colonies either by biological-breeding methods (Woyke 1989, Bienefeld and Pritsch 1992, Büchler 1993, Hoffmann 1993, Rosenkranz et al. 1990) or by applying materials of natural origin (Wallner, 1993). As a complement to other methods, the application of synthetic chemicals with different active ingredients is accepted in Hungary (Tóth, 1998).

The prolonged application of these chemicals may lead to the developement of resistance (Hillesheim et al. 1996) or cross-resistance (Milani et al. 1995) and to the accumulation of the compounds in beewax (Wallner, 1997).

Our aim was to find a method providing acceptable protection against the Varroa-mite with minimal exposure of chemicals. Therefore, we started experiments with the application of Gabon PA 92 wooden strips. This veterinary product proved to be effective. Our earlier trials (lasting several years) showed that a single treatment performed in autumn was sufficient for maintaining the bee colonies in healthy condition and keeping the number of mites on an acceptably low level.

There is increasing awareness among consumers of the hazard of chemical contamination of food. With this view, an experimental treatment was carried out to determine the levels of acrinathrin residues in apicultural products.

Comparative experimental treatment was performed in the apiary of Institute for Small Animal Research aimed to test Gabon PA 92 strips. In this experiment the efficiency of Gabon PA 92 was compared with Apistan (with fluvalinate active ingredient) and a home made wooden plate impregnated with Klartan (also containing fluvalinate). The experiment involved laboratory and open field tests of both brood and adult bees (Szalai et al. 1998).

Materials and methods

Gabon PA 92 strips contain acrinathrin in a quantity of 1.2-1.7 mg/strip.
Figure 1 shows the structure of active ingredient, which belongs to the group of synthetic pyrethroids.

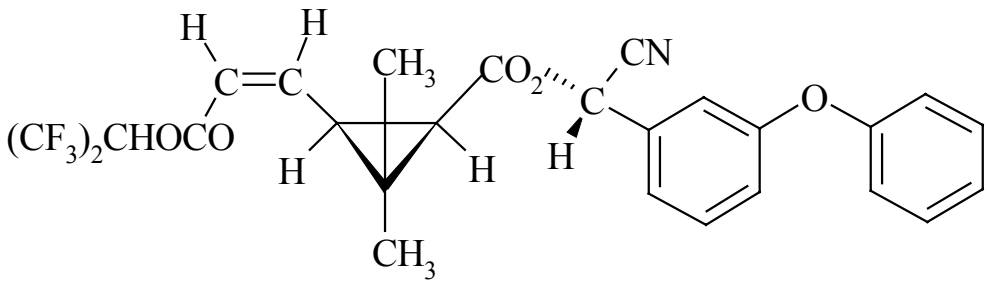

Figure 1

Structure of acrinathrin

Active ingredient: (S)-α-cyano-3-phenoxybenzyl (Z)-(1R,3S)-2,2-dimethyl-3-[2-(2,2,2-trifluoro-1-trifluoromethylethoxycarbonyl) vinyl] cyclopropanecarboxylate (IUPAC)

Experimental treatments:

Five bee-colonies were treated by hanging two GABON PA 92 strips into every beehive for 25 days.

After this treatment the amount of active ingredient residue in honey and beewax samples taken from beehives treated with GABON PA 92 was determined.

Two weeks after removing the strips from the treated beehives (at the time of honey extraction) honey and wax samples were taken. Untreated (control) samples were also taken at the same time. For avoid the degradation of the active ingredient, the samples were stored at -18 °C.

The appearance of the lipophilic active ingredient was expected first of all in the beewax. Therefore honey and bees wax samples were analysed for residue content separately, according to the sample preparation and analytical method presented in Figure 2 and 3.

Analysis contained the following stages: sampling, extraction, clean up, gas chromatographic determination with external standardisation.

Sample preparation was based on the method introduced by Vesely et al. (1995). The acrinathrin residue was determined by gas chromatography with electron capture detection. The parameters of gas chromatography are shown in Table 1.

Parallel to the treated samples we examined control honey and beewax samples from an untreated beehive and samples spiked with active ingredient. Acrinathrin was added to the honey and beewax in 0.05-5.0 mg/kg amount, and subsequently the recovery rates (%) were determined.

Mean recovery rate was 96.7 % for honey samples and 88.3 % for beewax samples (Table 2).

Table 1: Parameters of gas chromatographic determination

	I. for honey extracts		II. for beewax extracts
instrument	Chrompack 9000		Perkin Elmer Sigma 3B
detector	ECD (Ni 63)		ECD (Ni 63)
column	25 m x 0.32 mm i.d.∅ CP Sil PAH CB d_f=0.12 μm	25 m x 0.25 mm i.d.∅ CP Sil 5CB d_f=0.25 μm	1 m x 2 mm i.d. ∅ glass 2% OV 101/GasChrom Q 100-120 mesh
carrier gas	nitrogen of high purity 3 cm^3/min	1.8 cm^3/min	nitrogen of high purity 45 cm^3/min
make up gas	nitrogen of high purity 30 cm^3/ min		nitrogen of high purity 30 cm^3/min
split proportion	5:1	-	-
temperatures			
injector	240 °C	on column	240 °C
column	220 °C	80 °C 2 min ,15 °C/min 240 °C hold 20 min	220 °C
detector	280 °C	300 °C	280 °C
acrinathrin retention time	3.89 min	19.51 min	3.00 min
detection limit:			
from honey	0.01 mg/kg	0.01 mg/kg	
from beewax	0.10 mg/kg		0.10 mg/kg

Table 2:Recovery rates in the course of the determination of acrinathrin residues

Sample	Added acrinathrin (mg/kg)	n	Mean recovery (%)	SD	CV (%)
control honey	0.05	5	94.8	3.62	3.82
	0.20	5	98.6	2.27	2.30
control beewax	1.0	5	87.6	5.37	6.13
	5.0	5	89.0	4.02	4.52

Table 3: Residue content of honey and beewax samples taken from beehives treated with GABON PA 92 strips against Varroa mites

Sample	(Nr. of bee colony)	Acrinathrin residue content (mg/kg)
HONEY	22.	< 0.01
	34.	< 0.01
	46.	< 0.01
	56.	< 0.01
	60.	< 0.01
WAX	22.	< 0.10
	34.	< 0.10
	46.	< 0.10
	56.	< 0.10
	60.	< 0.10

Results

Results are summarized in **Table 3.** The data represent the means of the results of two parallel measurements each.

Blanks of the chemicals involved did not show any cross-contamination. Acrinathrin residues were not detected in the extracts of control honey and control wax samples, which indicates residue levels of < 0.01 mg/kg for honey and < 0.10 mg/kg for beewax

Discussion

The analytical results proved that the residue levels of active ingredient were less than 0.01 mg/kg in honey and less than 0.10 mg/kg in beewax.

From food-hygienic point of view it is favourable that acrinathrin residues were not detectable in any of the processed honey and wax samples. During the experimental treatment honey did not become "contaminated" with acrinathrin.

The results of this residue determination trial serve as a basis for the registration and marketing authorisation of veterinary product GABON PA 92 in Hungary. In the future, the investigations will be extended to residue tests of acrinathrin in samples taken from different locations and at different intervals after treatment. For health protection purposes and to maintain the exportability of Hungarian honey, further tests will be performed with acrinathrin and other active ingredients of bee acaricides (fluvalinate, flumethrin, amitraz).

References

1. Bienefeld, K. and Pritsch, G. (1992): *Kooperation zwischen Züchtern und zentrale Auswertung: Ansatze für eine erfolgreiche Zucht der Honigbiene.* Die Biene, 128, 443-447.
2. Büchler, R. (1993): *Rate of damaged mites (Varroa jacobsoni) in the natural mite fall with regard to seasonal effects and infestation development.* Apidologie, 24 , 492-493.
3. Hillesheim, E., Ritter, W., Bassand, D. (1996): *First data on resistance mechanisms of Varroa jacobsoni (Oud.) against tau-fluvalinate.* Experimental and Applied Acarology, 20, 283-296.
4. Hoffmann, S. (1993): *The occurence of damaged mites (Varroa jacobsoni) in cage test and under field conditions in hybrids of different Carniolan lines.* Apidologie, 24, 493-495.
5. Milani, N., Vedona, G. della, Greatti, M. (1995): *A bioassay to test the susceptibility of Varroa jacobsoni to pyrethroids.* APIMONDIA Verlag, Bucharest, p. 192.
6. Rosenkranz, P., Rachinsky, A., Strambi, C., Röstorf, P. (1990): *Juvenil hormon titer in capped worker brood of Apis mellifera and reproduction in the bee mite Varroa jacobsoni.* General and Comparative Endocrinology, 78, 189 - 193.
7. Szalai, M. E., Pacs, Zs., Molnár, E. (1998*): Examination of Gabon PA 92 in Varroa control (in Hungarian).* Méhészújság, 11, 21-22.
8. Szerletics Túri, M., Szalai Mátray, E. (1998): *Analysis of Achrinathrin residues from honey and beewax samples in colonies treated with Gabon PA 92*. ″Neue Anforderungen an die Bienenzucht für das 21. Jahrhundert″, IV. Polnish-Deutches Symposium, Berlin, Sept. 22-24, Book of Abstracts pp. 81-83.
9. Szerletics Túri, M., Szalai Mátray, E. (1999): *Determination of achrinathrin residues in honey and beewax*. Acta Veterinaria, 47(2), 173-179.
10. Tóth, Gy. (1998): *Chemicals available for Varroa control (in Hungarian)* Méhészet, 4,4-5.
11. Vesely, V., Malonova, D., Titera, D. (1995): *Acrinathrin, an effective varroacide and its residues in stores, honey and wax.* Apidologie, 26, 321-322.
12. Wallner. A. (1993): *Mein Weg in der Varroaresistenzzüchtung*. Bienenvater, 114, 107-108.
13. Wallner, K. (1997): *Der Weg zur rückstandfreien Imkerei Strategien aus der Sackgasse.* Bienenvater, 6, 9-13.
14. Woyke, J. (1989): *Breeding of honey bees resistant to Varroa j.* American Bee Journal, 129, 11-23.