Coumaphos and amitraz residues in Slovenian honey

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Abstract

Varroa mites were more or less resistant against pyrethroid drugs in Slovenia in the last few years. Consequently, Perizin (active substance coumaphos) was used more frequently. Beekeepers are facing the problem of the drug residues in honey. In order to study the real size of the problem, a series of 12 samples of honey was analysed in 2000, and a series of 19 samples in 2002 for the coumaphos residues. The first series was sampled from the honey on store shelves, the second series was sampled directly from the honey at beekeepers. The samples of honey were analysed using gas chromatography with electron capture detector (GC-ECD). Gas chromatography with mass- selective detector (GC-MS) was used to confirm the positive findings in samples. Seven samples of honey from the first series were found coumaphos free (<0,005 mg/kg), the maximum coumaphos content of 0,025mg/kg was found in the blossom honey from the coastal region. More favourable results were obtained in the second series. 14 samples could be declared as coumaphos free. Coumaphos traces were found in 5 samples with the maximum of 0,022 mg coumaphos /kg in two samples which originated from the eastern part of Slovenia. It could be concluded that, according to the European standards (0,100 mg/kg), all the samples of honey could be declared as appropriate for human consumption.

Key words: honey, honeybee, coumaphos, amitraz

Introduction

Varroa mite *(Varroa jacobsoni* Oud.) has caused serious disease problems and damage in honey bee population in Slovenia since 1980. The beekeepers were slightly confused in the first years. They were warned by beekeepers from other parts of former Yugoslavia who had bad experience with the disease, and they conducted a number of experiments on use of chemical bee protection. This was also the time of the first experiments with coumaphos and amitraz medications (Poklukar, 1999). Some years later, powerful pyretroides, Fluvalinate, Fluomethtrine and Acrinacrile were introduced into practice. However, their effectiveness was limited on time. The first report of pyretroid resistant varroa mites dates back to 1992 (Loglio, 1992, Milani and Della Vedova, 1996). The problem became serious in 1996. The beekeepers were faced again to more frequent bee colony mortality during the winters (Trouiller, 1998). At the same time the protection of bees by amitraz medication (fumigation of Hemovar®) with all positive and negative consequences became the most often mean of varroa mite disease prevention (Jenko-Rogelj M, Matavž J, 2000). The beekeepers were forced to find the optimum between the survival of bees, the risk of drug residues in honey, and last but not at least, between the risk of own health. Perizin® with active substance coumaphos was registered by Slovenian veterinarian authorities in the year 2000. The

registration should prevent any misuse of other coumaphos preparations at the varroa mite prevention.

Coumaphos represents the most frequently detectable varroacide in honey (Wallner 1999). Only 24 % of the applied coumaphos drip solution were found to reach the alimentary canal of bees, the rest remained in the bee hive. Fat–soluble ingredients are distributed throughout the hive by the bee workers. All inner surfaces are coated with a very thin layer of wax. Lipophilic substances are stored there and may at least pass into honey deposited in honey cells (Van Buren et al, 1992). Fortunatelly, coumaphos is less lipophilic than other fat-soluble acaricides used for the prevention of varroa disease (Wallner, 1995).

The control of honey in the first series was performed by honey inspector in the year 2000 (12 samples from store shelves). In the second series it was repeated by the Commission for Economy and Trade Marks at the Slovenian Beekeepers' Association. It sampled 4 samples at one of the larger honey houses in Slovenia, and 15 random samples of honey yielded at 15 beekeepers in Slovenia in the year 2001. The main goal of all samplings is to analyse and to scan the actual level of amitraz and coumaphos residues in the honey produced in Slovenia.

Material and Methods

The method used was the simple in-house developed method. The honey was dissolved in water with ultrasonication and transferred to separatory funnel with Milli-Q water which was already saturated with Na_2SO_4. Hexane with diethyl ether was added and shaken for 2min. The water phase was transferred to another funnel after 2 hours, and the extraction was repeated again.

The organic phases were collected, separatory funnels were rinsed with aceton and extracts were dried with Na_2SO_4, concentrated and injected into gas chromatograph with electron capture detector (GC-ECD)) and gas chromatograph with mass- selective detector (GC-MS).

The quantification of coumaphos in samples of honey was made by gas chromatography with electron capture detector (GC-ECD). The positive findings in samples were confirmed with mass-selective detector (GC-MS).

The amitraz contents was determined by GC-MS system.

The recoveries of coumaphos and amitraz contents in fortified samples in different spiking levels were good (90%-105% for coumaphos and 85%-95% for amitraz).

The limit of quantification was 0.005mg/kg for coumaphos and 0.02 mg/kg for amitraz.

Results and Discussion

Each treatment with acaracides leaves behind residues in the bee hive. Residues of those synthetic compounds can be clearly identified. Independent of the residue values, these synthetics damage the image of "pure", "healthy" honey. The risk of residue accumulation is correlated with the amount of ingredients used and the number of applications during the year.

Amitraz analyses

Amitraz is a fat-soluble compound which is not very stable in honey. It degrades almost completely degrades into several decomposed metabolites after 3–4 weeks. As

expected before, the amitraz residues in all our samples were found under the quantification limit 0,02 mg/kg. This is much below the level of 0,2 mg/kg, allowed by the EU regulation 2393/99. Fortunately, the use of amitraz over many years leaves no risk of accumulation of its residues in wax (Wallner, 1999). Assuming that the use of amitraz medication will be illegal in Slovenia after the end of the year 2002, the beekeepers will be forced to use other alternative varroacides.

Coumaphos analyses

Seven samples of honey from the first series were found coumaphos-free (<0,005 mg/kg), two of them were found on moderate detectable level between 0,005 and 0,01. Two samples of blossom honey exceed the value of 0,01 mg/kg.

Similar results were found in the second series. Two samples, originating from honey house, had coumaphos contents of 0,022 mg/kg. Both samples were produced at two larger honey producers who protected bees by Perizin® some years before. Two samples were moderately detectable, one sample exceeded the value of 0,01 mg/kg.

The minimum residue limit for Italy and Germany is 0,01 mg/kg. The limit was exceeded in two samples of blossom honey from the first series. The maximum value of all analyses of 0,025mg /kg was found in blossom honey produced in spring 2000 in the Slovenian coastal region.

All the samples express less coumaphos residues than the minimum residue limit for Switzerland (0,05 mg/kg) (Bogdanov et al. 1998). At the same time, all the samples are within the standard of 0,1 mg/kg allowed by the EU regulation (2377/90, 1931/99).

Perizin® could be legally used by the middle of the year 2003. Because of some limited effectiveness it must be soon replaced by other chemical means, mainly by organic acids. Nevertheless, the beekeepers were already warned about the coumaphos treatment in order to avoid any risk of residues in honey.

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