Science of the Total Environment 605–606 (2017) 745–754

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Occurrence of pesticide residues in Spanish beeswax

Pau Calatayud-Vernich [a,*], Fernando Calatayud [b], Enrique Simó [b], Yolanda Picó [a]

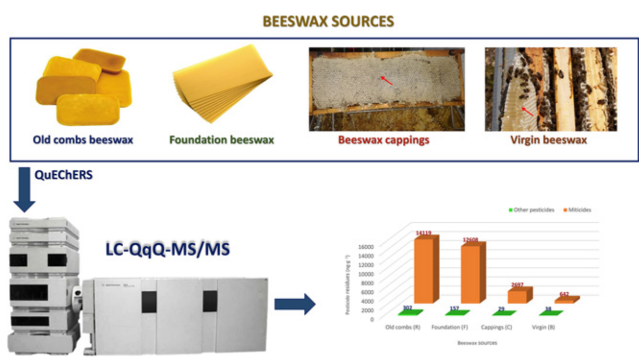
[a] Environmental and Food Safety Research Group (SAMA-UV), Research Center on Desertification (CIDE, UV-CSIC-GV), Faculty of Pharmacy, University of Valencia, Av. Vicent Andrés Estellés s/n, 46100 Burjassot, Valencia, Spain

[b] Agrupación de Defensa Sanitaria Apícola (apiADS), Ctra. Montroi-Turís, 46193 Montroi, Valencia, Spain

HIGHLIGHTS

- Pesticide levels in foundation, old combs, cappings or virgin wax were compared.
- QuEChERS extracts screened for 58 pesticides by LC-QqQ-MS/MS.
- Acaricides were the main source of beeswax contamination, >95%.
- Insecticides and fungicides were less frequent and at lower concentrations.
- Cappings and virgin wax were markedly less contaminated.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 4 April 2017
Received in revised form 21 June 2017
Accepted 21 June 2017
Available online 2 July 2017

Editor: D. Barcelo

Keywords:
Beeswax
Pesticides
Miticides
Insecticides
LC-MS/MS
Spain

ABSTRACT

Beeswax from Spain was collected during 2016 to determine pesticide residues incidence. The 35 samples were divided in foundation, old combs, cappings or virgin beeswax to compare pesticide content between groups. Wax was screened for 58 pesticides or their degradation products by QuEChERS extraction and liquid chromatography mass spectrometry (LC-MS/MS). Beeswax was uniformly contaminated with acaricides and, to a much lesser extent, with insecticide and fungicide residues. Virgin followed by cappings were less contaminated than foundation and old combs beeswax. The miticides applied in-hive had a contribution to average pesticide load higher than 95%. Compounds widely used as acaricides, as coumaphos (100%), fluvalinate (86%) and amitraz (83%), were the pesticides most frequently detected with maximum concentrations of 26,858, 3593 and 6884 $ng \cdot g^{-1}$, respectively. Chlorfenvinphos, acrinathrin and flumethrin, also acaricides, were detected in 77, 71 and 54%, respectively. Frequencies of pesticides used in crops were 40% for chlorpyrifos, 29% for dichlofenthion, 9% for malathion, 6% for fenthion-sulfoxide and 3% for azinphos-methyl, carbendazim, ethion, hexythiazox, imazalil and pyriproxyfen. Pesticide assessment in beeswax could be an excellent monitoring tool to establish veterinary treatments applied by beekeepers and environmental contaminants exposure of honey bees.

1. Introduction

Beeswax is the comb architecture element manufactured by honey bees (*Apis mellifera* L.) themselves that is literally the walls, home, nursery, pharmacy, storage pantry and dance floor for the numerous inhabitants of the colony (Schmidt and Buchmann, 1992). When visiting flowers, honey bees collect nectar rich in carbohydrates (i.e. the honey sugars fructose, glucose and sucrose) and utilize them for wax formation into their specialized wax-secreting epidermal glands found on the ventral side of the worker bees' abdomen in a high energy demanding process (Bogdanov, 2004).

* Corresponding author.
E-mail address: pau.calatayud@uv.es (P. Calatayud-Vernich).

http://dx.doi.org/10.1016/j.scitotenv.2017.06.174

Beeswax is a very complex mixture of lipophilic compounds which major components are hydrocarbons and lipids up to an 80% (Tulloch, 1980). Of all beehive products, beeswax has the lowest replacement rate and can remain in the hive for many years, thus leading to a greater accumulation of different non-polar xenobiotics applied in beekeeping and agriculture (Chauzat and Faucon, 2007; Mullin et al., 2010; Lambert et al., 2013). Due to that, beeswax is the most contaminated beehive product and has already been used as a bioindicator of environmental pollution (Porrini et al., 2003; Tsigouri et al., 2004; Lodesani et al., 2003; Orantes-Bermejo et al., 2010; Johnson et al., 2010).

Since the worldwide spread of the parasite *Varroa destructor* (Anderson & Trueman), beekeepers started to use acaricides to control mite population, avoiding damage threshold to the colonies. Nowadays, acaricides (e.g. coumaphos and fluvalinate) applied against varroa mite are the main source of beeswax contamination in both, frequency and concentration. In USA, coumaphos and fluvalinate residues showed the highest frequency (98.1% for both pesticides) and the highest average levels in beeswax samples, 3300 and 7474 $ng \cdot g^{-1}$, respectively (Mullin et al., 2010). Europe surveys have also revealed the extensive use of these acaricides. In France, coumaphos and fluvalinate were found in 46.7% and 52.2% of the samples, and reached average levels of 648 and 220 $ng \cdot g^{-1}$, respectively (Chauzat et al., 2011). In Italy, coumaphos (83%) and fluvalinate (75%) were also the most frequently detected pesticide residues in beeswax samples (Lodesani et al., 2003). Belgium beeswax results also confirms the high presence of these two acaricides (Ravoet et al., 2015). In Spain, one of the EU largest honey producer and the European country with the highest beehives census (Agriculture and rural development - European Commission, 2017), results showed a high incidence of fluvalinate (>93%) (Orantes-Bermejo et al., 2010; Serra-Bonvehi and Orantes-Bermejo, 2010; García et al., 2017). Among insecticides residues found in beeswax samples, the organophosphate chlorpyrifos was the most frequently detected in North American apiaries (63.2%) (Mullin et al., 2010). Other frequently detected contaminants were the pyrethroids cypermethrin, fenpropathrin, esfenvalerate and bifenthrin (12–18%) together with some fungicides (Mullin et al., 2010; Chauzat et al., 2011).

The use of veterinary agricultural treatments in beehives and its environment implies a risk of contamination of the honey bees and related apicultural matrices (wax, honey, pollen, royal jelly and propolis) and their analysis have also shown a widespread contamination (Mullin et al., 2010; Chauzat et al., 2011; Lambert et al., 2013; Kasiotis et al., 2014). In addition of recycled beeswax used in beekeeping, beeswax is found in myriad products: lipsticks, facial creams, pill coatings, salves, chewing gum, candles, floor and furniture polishes, and waterproofing materials. As beeswax is used in cosmetics and pharmaceuticals it should contain minimal amounts of contaminants (Bogdanov, 2004). Therefore, studying residues in beeswax is relevant not only to beekeeping issues but also to economic, environment and to public health purposes.

Recently, advances in analytical methods have improved sensitivity and sample throughput that were the problems of previous studies to tackle this subject. Long and tedious solid liquid extraction procedures involving a great number of additional clean-up steps that takes several days have been progressively replaced by simple, generic and rapid QuEChERS platforms. As well, application of liquid chromatography-mass spectrometry (LC-MS/MS) has ensure optimum sensitivity and selectivity to analyze pesticide residues in a complicated matrix because its apolar character and the high hydrocarbons content (Niell et al., 2014; Calatayud-Vernich et al., 2016a; Calatayud-Vernich et al., 2016b; Herrera López et al., 2016).

In view of these concerns, this study aimed at comparing pesticide residues in foundation, old combs, cappings and virgin wax, to discuss implications for the beekeeping management practices and health of the honey bee colonies taken into account the pesticide residues levels and frequency, as well as whether they come from veterinary treatment or the surrounding environment. Among the 58 pesticides included in this study, the most relevant were the pyrethroids achrinathrin, cyhalothrin, flumethrin, and tau-fluvalinate, the organophosphates chlorpyrifos, coumaphos and chlorfenvinphos, and acaricide amitraz. The target analytes were chosen based on their potential toxicity to honey bees and/or their widespread use in plant protection or in the beehive against varroosis. The sample preparation method was based on QuEChERS extraction with subsequent determination by liquid chromatography coupled to a triple quadrupole mass spectrometry (LC-MS/MS).

2. Materials and methods

2.1. Chemicals

High purity (98–99.9%) standards of the 55 selected pesticides together with the transformation products of amitraz; 2,4-dimethylaniline (DMA), 2,4-dimethylphenylformamide (DMF) and N-(2,4-dimethylphenyl)-*N′*-methylformamidine (DMPF) were acquired from Sigma-Aldrich (Steinheim, Germany) (listed in Table 1). Individual standard solutions were prepared in methanol at a concentration of 1000 $mg \cdot L^{-1}$. The working standard solutions were prepared by mixing the appropriate amounts of individual standard solutions and diluting with methanol to a final concentration of 1 and 10 $mg \cdot L^{-1}$. All solutions were stored in 15 mL vials at 4 °C in the dark.

Magnesium sulfate was obtained from Alfa Aesar (Karlsruhe, Germany), ammonium formate, sodium hydroxide, sodium chloride, acetonitrile and formic acid were purchased from Sigma-Aldrich (Steinheim, Germany). Methanol was obtained from VWR chemicals (Radnor, Pennsylvania). PSA and C18 sorbents, and PTFE (13 mm × 0.22 μm) filters were purchased from Análisis Vínicos S.L. (Tomelloso, Spain). High purity water was prepared using a Milli-Q water purification system (Millipore, Milford, MA, USA). Milli-Q water and methanol, both with ammonium formate 10 mM, were used as mobile phase in LC-MS/MS.

2.2. Origin and characterization of the samples

A total of 35 beeswax samples were collected from different relevant beekeeping areas in Spain during 2016 (Fig. 1). Four different beeswax sources were analyzed: beeswax foundation from commercial suppliers as a mixture of beeswax from many beekeepers (F1–F11); beeswax cappings (virgin wax covering on sealed honeycombs) rendered by particular beekeepers (C1–C12); beeswax from recycled old combs from the brood chamber of commercial hives from particular beekeepers (R1–R10); and virgin wax combs recently built (<7 days) by honey bees in empty spaces of commercial beehives were used as an assumed contrast less contaminated beeswax reference (B1–B2) (Fig. S1 Supplementary material). Except foundation, beeswax samples were acquired from migratory beekeepers that alternate wild flowering plants as rosemary, thyme and heather, with crops blooming, principally citrus and sunflower, but also canola, almond, plums and other fruit trees orchards that require entomophilous pollination.

Method for rendering the beeswax of R group was the centrifugal extraction, in which old combs, placed into a metal basket, are melted by steam (over 70 °C) in a centrifugal wax extractor spinning at >1500 rpm. Metal basket perforated walls eliminate solid impurities while liquid phase containing melted beeswax flows into the lower part of the tank. After solidification, pieces of beeswax blocks from particular beekeepers were collected as R source samples. C group samples were obtained during honey extraction process, when wax cappings are removed from ripe honeycombs. After that, beeswax is subjected to a melting and cleaning procedure similar to the process for rendering R beeswax. Combined steam and press extraction manufacturing method is usually used in suppliers companies (F samples) and it consists on a tank of boiling water where old combs are placed and melted. Afterwards, a piston exerts pressure for about an hour to separate solid