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Occurrence of agrochemical residues in beeswax samples collected in Italy during 2013–2015

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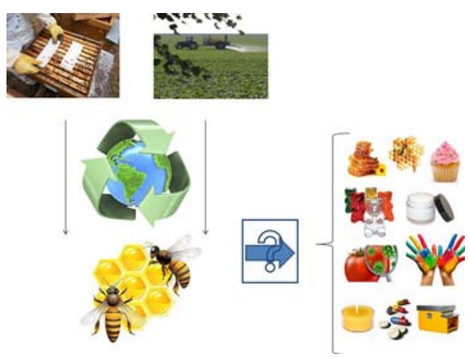
HIGHLIGHTS

- Most beeswax samples were contaminated by pesticides residues.
- Coumaphos was the most frequently detected pesticide.
- Samples were also contaminated by compounds used for agricultural purposes.
- The occurrence of DDT isomers is probably due to the use of African beeswax.
- Data confirm the presence of unauthorized compounds in Italian beeswax samples.

GRAPHICAL ABSTRACT



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ABSTRACT

Considering the importance of honey bees for pollination activity and biodiversity preservation different studies have dealt with the impact of pesticides on honeybee health. Within the hive, beeswax is a matrix of particular interest because most of the chemicals used in agricultural and beekeeping activities can easily accumulate and persist in this matrix long after treatment has ceased, affecting honeybees survival. However, chemical analyses of pesticide residues in beeswax are not mandatory, so there are no residue limits, for beeswax. The present study was carried out with the aim of investigating the beeswax residue pattern in the Italian hives. 178 beeswax samples collected from 2013 to 2015 were analyzed for 247 pesticides. 73.6% of the analyzed samples reported the presence of one or more pesticides. On average every single sample showed a mean of three different pesticides each with a maximum of fourteen compounds, some of which were banned in Europe or not authorized in Italy. The higher frequencies were associated with three acaricides: coumaphos (60.7% of samples), *tau*-fluvalinate (50%) and chlorphenvinphos (35.4%), but the higher concentrations were associated to pyrethrins and piperonil butoxide.

1. Introduction

Insect pollination is important for most of the flowering plants all over the world (Ollerton et al., 2011). The latest estimate of the benefits of pollination in the world reach about 153 thousand million euros

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(Gallai et al., 2009) and nearly 80% can be attributed directly or indirectly to honey bees (Calderone, 2012; Calatayud-Vernich et al., 2016). With a serious decline in wild honey and solitary bees, the importance of beekeeping in sustaining biodiversity and crop pollination is increasing and more investigations are dedicated to the role of pesticides on honeybee preservation (Calderone, 2012; Moritz et al., 2010). Recent surveys show that honey bees are being exposed to high levels of agricultural pesticides and acaricides applied by the beekeepers in the hives to control of pest mites (Kiljianek et al., 2016; Krupke et al., 2012). Honey bees are exposed to sub-lethal doses of chemical compounds used in agriculture during their foraging activity and over long-time periods in the hive due to the contamination of all hive elements (Bogdanov, 2006; Chauzat et al., 2011; Rortais et al., 2005). Some authors showed that several persistent and lipophilic acaricides such as coumaphos and *tau*-fluvalinate can be found in the hive, even long after treatment has ceased (Chauzat and Faucon, 2007; Johnson et al., 2009; Serra-Bonvehí and Orantes-Bermejo, 2010). The determination of acaricides and other pesticides has been undertaken in several bee products such as honey, pollen, royal jelly, and beeswax (Chauzat et al., 2011; Mullin et al., 2010; Tanner and Czerwenka, 2011).

Beeswax, consisting primarily of a mixture of esters of fatty acids and fatty alcohols, paraffinic hydrocarbons, and free fatty acids, is a matrix of particular interest because most of the chemicals used in agricultural and beekeeping activities are lipophilic, low-volatile, and persistent (Tulloch, 1980). So, these substances can accumulate and persist in this matrix long after treatment has ceased and beeswax can become a source of subsequent contamination for bees and beehives matrices (Mullin et al., 2010; Johnson et al., 2010; Serra-Bonvehí and Orantes-Bermejo, 2010).

A very common beekeeping practice involves the collection of old combs to produce new foundations. This cyclic process causes a continuous accumulation and re-circulation of all lipophilic contaminants resistant to the wax melting temperature and leads to increasing amounts of residues in beeswax (Ravoet et al., 2015). Beeswax finds also important applications in the food, cosmetic, pharmaceutical industries and can become a source of possible exposure to toxic substances for humans. Beeswax is listed in the Pharmacopeia of different countries and in Europe is authorized as a food preservative under the name of E 901 (Coggshall and Morse, 1984; Directive EU No. 95/2/EC, 1995; EFSA, 2007; Mitrowska and Antczak, 2015). The food applications of beeswax include its use as a component in dietary food supplements (soft gelatin capsules and tablets), glazing and coating, chewing gum, and as a carrier for food additives (including flavors and colors). Any toxic substance dissolved or incorporated in beeswax can be released much later when the beeswax is used in the production of cosmetics and other products, consumed as food or given to bees in the form of wax foundation sheets (Mitrowska and Antczak, 2015).

Over the last decades, information about beeswax contamination and the possible consequences of chronic exposure to pesticide residues on honeybee health, caused major interest in wax residue determination. However, in Europe, official quality controls on beeswax do not exist and the chemical risks associated to its use are not monitored. In Italy, the Technical Guidelines RT-16 of 2009 (RT-16 rev.04, 2009) set the limits for five acaricides as the restrictive condition for the use of wax foundation sheets in organic beekeeping. These limits were expressed as sum of amitraz, coumaphos, chlorfenvinphos, *tau*-fluvalinate and cymiazole concentrations and were set individually only for coumaphos, chlorfenvinphos and *tau*-fluvalinate. Although several toxic pesticides for honeybees have been banned or never authorized in Europe, their presence has a long term duration in beeswax which remains the best matrix to study historical contamination coming from agricultural practices or, such as highlighted by other authors, derived from the illegal use of some forbidden compounds in home-made preparations (Chauzat et al., 2011; Ravoet et al., 2015).

Considering that long-term accumulation of lipophilic pesticides in wax can become a source of contamination for bees and hive products, the aim of the present study was to obtain an overview of pesticides in Italian beeswax. We looked for the presence of 247 compounds among acaricides and/or plant protection products to understand the pattern and trend of these chemicals. We also investigated the presence in beeswax of the new generation products authorized in agricultural practice to prove the presence of an indirect contamination in beeswax. To monitor beeswax comb samples coming from different Italian regions we validated and use a multiresidue method for pesticide analysis. The majority of target insecticides, acaricides, fungicides, and herbicides were selected because of their common use in Europe for the agricultural and beekeeping practices (Commission Implementing Regulation (EU) No. 595/2015, 2015).

2. Materials and methods

2.1. Beeswax sampling

Samples (178) over the entire study were collected at the Pungiglione, Onlus Cooperative Society, an active wax factory located in Mulazzo, Tuscany (Italy), dedicated to the manipulation of the rough raw beeswax, on behalf of Italian beekeepers, for organic and conventional foundation sheets production. Over 3 years, from January 2013 to December 2015, 10 g of wax foundations were taken randomly from different batches of wax. At least one beeswax sample for the main Italian regions was collected every year. The origin of beeswax samples is reported in Table 1. This method of sampling was useful for monitoring approximately 60 Italian apiaries each year. Each sample was transported to the laboratory in a plastic container, then stored at −20 °C until the analysis.

2.2. Multiresidue pesticide analysis

Samples were analyzed for 247 chemicals belonging to herbicide, insecticide, acaricide and fungicide toxicological classes (Table 2).

Analytical-grade standards of pesticides were purchased from Dr. Ehrenstorfer (Augsburg, Germany) and Sigma-Aldrich (Steinheim, Germany). Pesticide-grade solvents, Supel™ QuE Citrate Extraction Tube and Supel™ QuE PSA/C18 Cleanup Tube were used for the extraction analysis and were supplied by Sigma-Aldrich (Steinheim, Germany).

The frozen samples were cut by a crushing mill (IKA, Wilmington, NC). The extraction was performed on 5 g of sample adding 5 mL of hexane and shaking for 1 min by vortex. To this mixture, 10 mL of water and 10 mL of acetonitrile were added and all solvents were mixed by vortex as above. At the end of this step Supel™ QuE Citrate extraction salt-

Table 1
Total samples analyzed according to the origin and year of collection.

Italian regions	2013	2014	2015	2013	2014	2015	Total samples
	Foundation			Capping			
Abruzzi	2	5	–	–	–	–	7
Aosta Valley	1	–	1	–	–	–	2
Apulia	2	1	2	–	–	1	6
Campania	1	1	–	2	–	1	5
Emilia-Romagna	1	3	5	1	5	2	17
Latium	2	3	–	1	–	–	6
Ligury	–	4	2	–	1	–	7
Lombardy	5	6	–	1	4	1	17
Marches	2	–	–	1	–	1	4
Piedmont	5	8	3	3	4	2	25
Sardinia	2	1	1	–	–	–	4
Sicily	1	1	–	–	–	–	2
Trentino-Alto Adige	1	1	–	–	–	1	3
Tuscany	17	23	11	7	7	6	71
Umbria	–	1	–	–	–	–	1
Veneto	1	–	–	–	–	–	1
Total samples	43	58	25	16	21	15	178