SUPPLEMENTARY MATERIAL

Monitoring of neonicotinoid pesticides in bee-keeping.

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ABSTRACT

The decline of pollinating species is correlated to the extensive use of neonicotinoids against pest insects for crop protection. In this study, the concentrations of neonicotinoid insecticides were determined in honeybees, honeycomb and honey samples, collected in Spring 2015 (blooming period) from different areas in Sicily (IT), to carry out an evaluation of bees-products safety and an overview of neonicotinoids contamination in bee-keeping.

The results obtained showed only the presence of clothianidin in bees samples and these concentrations don't represent a risk for bees vitality and safety. The absence of residue in all honey samples, instead, showed the quality of bees-products.

KEYWORDS

Neonicotinoids; honeybees; bee products; LC -MS/MS

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Reagents

Standard solution of Imidacloprid (99.4%), Clothianidin (99.6%), Thiamethoxam (99.2%), Acetamiprid (99.5%), Thiacloprid (99.3%) were purchased by Sigma Aldrich, Amsterdam. Acetonitrile and ultrapure water for LC/MS analysis were used as solvent.

Sampling

50 samples of dead bees, 50 sample of honeycomb and 50 samples of honey were collected from different bee-keeping farms of Sicily (Marsala, Vittora, Palermo, Scillato, Gela, Castelvetro, Pachino, Sciacca, Cassibile, Milazzo) during spring 2015 in the blooming time. The sampling plan was designed to have a balanced number of samples by area and type, and it was carried out in the same week. The samples were stored at + 4 °C.

Analysis

For each samples, 5 g were homogenized and diluted in water (100 ml); from this solution, 15 ml were collected, spiked with standards solution, at different concentration, vortexed and after 15 minutes added with 10 mL of acetonitrile and centrifuged for 10 minutes at 3000 rpm. The organic supernatant was then purified using a SPE (Solid Phase Extraction) system: from each were collected 6 ml, put in cartridge (Supelco PSA SPE Cleanup Tube), centrifuged for 10 minutes at 3000 rpm. Then, 2ml of eluates were collected, dried under nitrogen, recovered with water and acetic acid (0.1%), vortexed for 30 minutes and finally submitted to chromatographic analysis. The analysis in LC was performed using a triple quadrupole, model Thermo ACCELA (Thermo Fisher, Germany), with ESI source. The column used was an Agilent Eclipse XDB-C18 (2,1x100mm), at T. 30 °C. The mobile phase consisted of deionized water (with 0.1% of formic acid) (60%) and acetonitrile (40%) for 3 min, programmed to 80% and 20% until 3,5 minutes, finally maintained to 60% and 40% until to 6 minutes. The flow rate was of 0.4 mL/min and injection volume of 15 µL.

For validation method detection limit (LOD), limit of quantification (LOQ), uncertainty and repeatability were calculated. Recovery was measured using fortified samples (25, 50, 75 µg/kg for thiacloprid acetamiprid and imidacloprid (5, 10, 15 µg/kg for clothianidin and thiamethoxam; 100, 200, 300 µg/kg for thiacloprid). All specific parameters are reported in Table 1S.

For mass analysis the *Selected Reaction Monitoring* was used for acquisition of data of selected ion (Table 2S). The parameters used for the acquisition in MS in positive mode were: source ESI+; spray voltage 4500; Temperature of vaporization 300°C; capillary voltage 310 °C; sheath gas pressure 40 bar; auxiliary gas pressure 15 bar; peak resolution per Q1 e Q3: 0,70 FWHM. The specific mass spectra for each analite were reported in Figure 2S.

Table S1: Parameters for validation method.

<i>Standard</i>	<i>conc.</i> ($\mu\text{g/Kg}$)	<i>R</i> ²	<i>LOD</i> ($\mu\text{g/Kg}$)	<i>LOQ</i> ($\mu\text{g/Kg}$)	<i>Repeatability</i> ($\mu\text{g/Kg}$)	<i>Uncertainty</i> ($\mu\text{g/Kg}$)	<i>Recovery</i> (%)
ACETAMIPRID	25 - 75	0.9997	4.55	4.85	1.2 - 5.3	4.2 - 9.4	70-75
CLOTHIANIDIN	5 - 15	0.9975	1.20	1.30	0.8 - 1.3	1.8 - 2.7	82-95
IMIDACLOPRID	25 - 75	0.9996	7.6	7.9	1.1 - 2.2	2.9 - 6.3	95-110
THIACLOPRID	100 - 300	0.9981	33	39	14 - 39	32 - 66	85-110
THIAMETHOXAM	5 - 15	0.9926	2.7	3.4	0.75 - 1.1	2.0 - 4.3	92-110

Table S2: Parameters for SRM acquisition of selected ions.

	Parent ion	Product ion 1	CE*	Product ion 2	CE**
Acetamiprid	223.1	126.0	26	56.1	20
Clothianidin	250.0	169.0	16	132.0	22
Clothianidin D3	253.0	131.9	22	172.0	17
Thiacloprid	253.0	90.0	54	126.0	26
Imidacloprid	256.0	175.1	28	209.1	21
Imidacloprid D4	260.1	179.1	24	213.0	21
Thiamethoxam	292.3	132.0	23	211.3	15
Thiamethoxam D3	295.0	184.0	24	214.1	17

*Collision energy for product ion 1; **Collision energy for product ion 2

Figure S1. Chemical structures of the main members of neonicotinoid insecticides

Figure S2. Massa spectra of single neonicotinoids studied: clothianidin (a), thiametoxam (b), imidacloprid (c), acetamiprid (d) and thiacloprid (e).

(a)

(b)

(c)

(d)

(e)