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Matrix solid-phase dispersion associated to gas chromatography for the assessment in honey bee of a group of pesticides of concern in the apicultural field

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ABSTRACT

A method based on matrix solid-phase dispersion (MSPD) associated to gas chromatography-flame photometric detection (GC-FPD), GC-electron capture detection (GC-ECD) and GC-mass spectrometry (GC–MS) for confirmation purposes, was developed for the determination of a representative group of twelve pesticides in honeybee with particular concern in the apicultural field (fipronil, thiamethoxam, acetamiprid, acrinathrin, metamidophos, dimetoathe, diazinon, chlorpyrifos, methidathion, profenophos, azinphos methyl and coumaphos). Factors influencing the extraction efficiency of MSPD were investigated and optimized through response surface method. The use of octadecylsilyl (C18) sorbent combined with a florisil clean-up and acetonitrile-methanol (99:1) elution was the optimal condition for the extraction of the selected pesticides. Under this condition the recovery of pesticides at the limit of quantification of the method (0.007 to 0.050 $\mu g\,g^{-1}$) ranged from 68 to 102% with RSDs for within-laboratory reproducibility ≤20%. The proposed method was applied to the analysis of honeybees collected in 68 field hives from areas of great apicultural and agricultural development in central Chile. In 65% of these samples eight different pesticides were detected. Pesticides most frequently found were chlorpyrifos (34% of the samples, <0.017–0.067 $\mu g\,g^{-1}$), acrinathrin (32% of the samples, <0.020–0.026 $\mu g\,g^{-1}$) and diazinon (10% of the samples at values <0.015 $\mu g\,g^{-1}$). The incidence of these pesticides in bees can be related to their high employ in central Chile, use to combat the varroosis in hives and hydrophobicity.

1. Introduction

Honeybees (*Apis mellifera*) are the natural and economically most important group of pollinators worldwide; 35% of the world food crop production depends on pollinators [1]. The decline of pollinating species, which has grown over the last decades, may lead to a parallel decrease of plant species or vice versa. More specifically, there is a great concern about the decline of honeybee in several parts of the world. In this sense, the worldwide fact most recently observed is the acute depopulation of hives (a honeybee vanishing), which has been called Čolony Collapse Disorder (CCD)ťhat was first named in 2007 [2]. Along with the decline in honey production, the loss of pollinators has had a negative impact on the reproduction of multiple crops [3]. The possible causes of CCD include parasites, bacteria, fungi, viruses, pesticides, deficient nutrition, management practices and environmental factors. However, recent studies postulate that a combination of these factors could be responsible, principally parasites and the exposure to cocktails of agrochemical as pesticides of different class [4–8]. Although no single pesticide has been shown to cause CCD, the additive and synergistic effects of multiple pesticide exposures may contribute to declining honey bee health [9–12].

It is rare to find exposure to a single pesticide in bees; usually they are exposed to various insecticides, fungicides, and acaricides, among others. Between them, neonicotinoid, organophosphorus pesticides (OPPs) and halogenated pesticides (HPs) are included. In recent years it has been postulated that neonicotinoid pesticides could be a trigger of CCD. Some authors have done a wide overview on the effect of neonicotinoids on bees and their relationship with CCD [1,13,14]. Thiamethoxam and acetamiprid belongs to this group of pesticides where the first one is highly toxic for bees and bumble bee [15,16]; while acetamiprid is less toxic compared to the other neonicotinoids [17]. Recently it has been reported that thiamethoxam significantly reduce the reproductive capacity of male

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honeybees (drones) by decrease of sperm viability and living sperm quantity [18]. On the other hand, fipronil like the neonicotinoids is considered one of the probable causes of CCD by increasing the mortality of honeybees previously infected by *Nosema ceranae* [19,20] and inducing behaviors that reduce foraging efficiency [21]. Otherwise, residues of OPPs have been frequently detected in matrices of honeybee colonies, and their potential risk to the colonies reported [22–25]. While results of some studies suggest that OPPs would not be directly involved with the cause of colony losses [24], they may be interacting with other stressors or in combination with other pesticides. An example is the synergistic effect observed between the acaricide coumaphos and the fungicide chlorothalonil on bee larvae mortality [12]. It has also been observed that bumble bee colonies exposed to chlorothalonil produced fewer workers, lower total bee biomass, and had lighter mother queens than control colonies [26]. As a result, the use of pesticides in agriculture has indubitable repercussions on the environment and a highly probable negative impact on the sustainability of bee colonies, which has become a serious environmental concern that need a continuous monitoring through adequate analytical method.

The determination of volatile pesticides in honey bees has been performed by gas chromatography (GC) coupled to tandem mass spectrometry (GC–MS/MS) [27,28] or to single mass spectrometry (GC–MS) [29] for qualitative and quantitative purposes. Although these GC detection techniques are widely used for the analysis of pesticide residues in bees, the selective detection systems are used to evaluate the performance of new methods, including nitrogen-phosphorus (NPD) [30–33] and electron capture (ECD) [30,33]. On the other hand, although the hyphenated techniques based on mass spectrometry have high selectivity, the fatty matrices require sample extraction and purification to remove partially or totally the lipidic components co-extracted with the target compound. Thus, the determination of residues of pesticides in bees by chromatographic methods is a challenging analytical problem because they have a high content of fats, waxes, pigments and other compounds of varying polarity; which can be co-extracted with analytes and cause problems in the chromatographic detection, particularly by blocking active sites in liners and columns.

The reported process for the extraction of pesticides from honeybees involves a solid-liquid extraction with acetonitrile [27,28], diethylether [29], acetone [32], methylene chloride from sample adsorbed on diatomaceous earth [33] followed by a cleaning step with dispersive solid phase extraction (dSPE) based on the QuEChERS method [27,28,34,35], gel permeation chromatography [29,33], solid phase extraction [29] or solid phase micro-extraction [32]. All these approaches involve two separate steps (extraction followed by cleaning) which is time consuming and increases the handling of samples. On the other hand, matrix solid phase dispersion (MSPD) permits to carry out the extraction and clean-up in one step simplifying the treatment of samples. However, this method has been scarcely proposed for the extraction of pesticides from honeybees before chromatographic analysis [30,31,36,37].

In this study we have optimized a MSPD method for the extraction, determination by gas chromatography-with selective detectors (FPD and ECD) and confirmation by GC–MS; of twelve pesticides of particular relevance for honeybee and/or a wide use in crop protection; with the aim of obtaining good recoveries and decreasing as much as possible the potential matrix effect onto detection. Pesticides included were fipronil, thiamethoxam, acetamiprid, acrinathrin, metamidophos, dimetoathe, diazinon, chlorpiryphos, methidathion, profenophos, azinphos methyl and coumaphos. In this manner we proposed a common sample treatment together with different chromatographic systems, considering the different types of instruments that a laboratory can have depending on the availability of resources. To our knowledge, this is the first report on the determination of thiamethoxam and acetamiprid, together with organophosphorus and halogenated pesticides by gas chromatography in honeybee. Residues of the selected pesticides were determined in honeybees collected in field hives from central Chile characterized by its great apicultural and agricultural development.

Table 1
Log K_{ow}, solubility in water at 20 °C and vapor pressure at 25 °C of the studied pesticides [39].

Compound	log K_{ow}	Sol. water (mg/l)	Vapor pressure (mPa)
Thiamethoxam	−0.13	4,100	6.6×10^{-6}
Fipronil	3.8	3.78	0.002
Acetamiprid	0.8	2,950	1.7×10^{-4}
Acrinathrin	6.3	0.002	4.4×10^{-5}
Methamidophos	−0.8	200,000	2.3
Dimethoate	0.7	39,800	0.25
Diazinon	3.7	60	12
Chlorpyriphos	4.7	1.05	1.43
Methidathion	2.6	240	0.25
Profenophos	4.8	28	2.53
Azinphos methyl	3.0	28	5.0×10^{-4}
Coumaphos	4.1	1.5	0.013

2. Experimental

2.1. Chemicals and reagents

The pesticides used (fipronil, thiamethoxam, acetamiprid, acrinathrin, metamidophos, dimetoathe, diazinon, chlorpyrifos, methidathion, profenophos, azinphos methyl and coumaphos) had a purity of ≥98% (Sigma-Aldrich ®, St. Louis, MO, USA). Table 1 summarized some relevant properties of these compounds. All solvents used were residue analysis grade (Merck ®, Darmstadt, Germany). Triphenylphosphate (TPP) and pentachloronitrobenzene (PCNB) (Sigma-Aldrich ®, St. Louis, MO, USA) were used as internal standard for GC-FPD and GC-ECD determinations, respectively. Stock solutions were prepared in acetonitrile at 1 g L^{-1}. Working standard solutions were diluted with acetonitrilie for spiking purposes. Clean-up® unbonded silica (15 mL, 2 g); Clean-up® carbon graphitized non-porous (6 mL, 0.5 g) and Enviro-clean® florisil (15 mL, 2 g) solid phase extraction (SPE) cartridges and Selectrasorb® bulk sorbent end-capped C18 for matrix solid phase dispersion (MSPD) were provided by UCT ® (Bristol. PA, USA).

2.2. Chromatographic analysis

2.2.1. GC-FPD

A Hewlett Packard (Agilent; Little Falls, DE, USA) model 5890 Series II ® gas chromatograph equipped with split/splitless injector and FPD was employed. A BPX5 (SEG Analytical Science, Australia) capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness) was used. Helium and nitrogen (99.995%) were selected as carrier and auxiliary gas, respectively. Pesticides were separated and determined under the following conditions: injector temperature, 250 °C; detector temperature, 280 °C; column temperature program: 70 °C, held for 2 min; increased at a rate of 25°/min up to 250 °C; increased at a rate of 50 °C/min up to 320 °C; held for 7 min A 1-μL volume of the extract was injected in the splitless mode (1.5 min purge). Carrier gas flow in the column was 1.8 mL/min. Hydrogen, 75 mL/min and air, 100 mL/min were used as combustion gases. Under these conditions, the mixture of eight pesticides and internal standard (TPP) was well resolved in a run time of 18 min. Since the matrix-induced chromatographic response enhanced effect previously reported [31,38], all the chromatographic analysis were performed using spiked extracts of free residues honeybee as matrix-matched standard.

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