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Bee pollen as a bioindicator of environmental pesticide contamination

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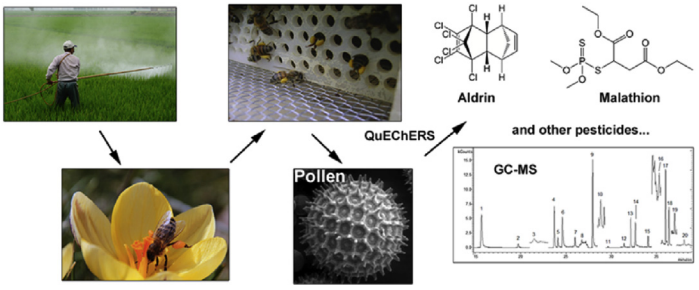
HIGHLIGHTS

- Bee pollen samples were used for monitoring pesticide residues in the environment.
- Bee pollen is a bioindicator of environmental pesticide contamination.
- A multiresidue method was developed for analysis of pesticides in bee pollen.
- Pesticide sorption on bee pollen was evaluated.
- Pesticides were quantified by ion trap mass spectrometry.

GRAPHICAL ABSTRACT



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ABSTRACT

Honeybees and bee products are potential bioindicators of the presence of contaminants in the environment, enabling monitoring of large areas due to the long distances travelled by bees. This work evaluates the use of bee pollen as a bioindicator of environmental contamination by pesticides. A GC-MS/MS analytical method for multiresidue determination of 26 different pesticides in pollen was developed and validated in accordance with the recommendations of the European Union SANCO guide. Environmental monitoring was conducted using the analysis of 145 pollen samples collected from ten beehives in the experimental apiary of Embrapa in Jaguariúna (São Paulo State, Brazil). Bioallethrin and pendimethalin were identified in four and eighteen samples, respectively, at concentrations below the LOQ of the method (25 ng g^{-1}). Passive sampling with polyurethane foam discs was used as a control, and no pesticides were found. The detection of pesticide residues in seven samples (33%) from commercial apiaries in Ribeirão Preto (São Paulo State) confirmed the efficiency of the analytical method and the need for environmental monitoring for the presence of pesticide residues. The results demonstrated the potential of bee pollen as a bioindicator of environmental contamination by pesticides.

1. Introduction

The use of pesticides is essential in agriculture as it provides pest control, increases productivity, and reduces costs. However, constant application of pesticides poses risks to human health, due to exposure to contaminated water, soil, air, animals, and plants. Furthermore, pesticide use can lead to the disappearance of beneficial insects (bees and wild pollinators, for instance) and may promote the appearance of new pests and the evolution of pesticide resistance among the pest population (Fernández et al., 2001; Tomita and Beyruth, 2002; Koifman and Hatagima, 2003; Koifman and Koifman, 2003; Magalhães, 2005; Veiga, 2007).

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Although honeybees (*Apis mellifera*) are not the target of pesticides, they are highly vulnerable to contamination, since they are exposed to these substances while collecting nectar, pollen, and water for maintenance of the colony. In cases of acute toxicity, honeybees can die rapidly after the application of pesticides, while chronic exposure to sub-lethal doses can injure their foraging behavior and affect colony health and development (Johnston et al., 2010; Gregorc et al., 2012; Pettis et al., 2012; Di Prisco et al., 2013; Williamson and Wright, 2013). The declining number of pollinating insects, usually due to the abuse of pesticides, is of concern in Brazil and in many other countries, due to the damage it can cause to agricultural production (Bacandritsos et al., 2010; Pettis and Delaplane, 2010; Burkle et al., 2013; Castilho and González, 2013; Tylianakis, 2013a, b; Tomazela, 2014).

Bees cover a wide area (up to 7 km^2) in their search for nectar and pollen, and are also numerous and easily kept by humans (Celli and Maccagnani, 2003). For these reasons, the use of honeybees and bee products (pollen and honey) as bioindicators of environmental contamination has been of great interest in recent years. Studies have shown that the level of contamination of hives by pesticides is closely related to the proximity of the contamination source and the duration of exposure (García-Chao et al., 2010; Mullin et al., 2010; Chauzat et al., 2011; Panseri et al., 2014; Malhat et al., 2015). Among bee matrices, pollen has been indicated as the best for assessment of the presence of environmental pesticide residues, as it is easy to collect and is frequently contaminated (Chauzat et al., 2006, 2011). Furthermore, Chiesa et al., 2016 verified that the presence of persistent organic pollutants in organic honey, affected by the geographical area, confirms that honey bee and beehive matrices are appropriate sentinels for monitoring environmental contamination.

In order to use bee pollen as a bioindicator, it is necessary to develop a sensitive and selective analytical method with an appropriate sample preparation step, as this matrix is highly complex. Solid-liquid extraction and the QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) approach have been widely employed for this purpose. Regarding analytical techniques, gas chromatography coupled to mass spectrometry (GC-MS) and liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) are the techniques most commonly used for multiresidue analysis of pesticides at low levels of detection (Berrada et al., 2010; García-Chao et al., 2010; Mullin et al., 2010; Wiest et al., 2011; Kasiotis et al., 2014).

The main objective of the present study was to evaluate the potential of bee pollen as a bioindicator of environmental contamination by pesticides. Sorption of pesticides on bee pollen was performed in order to assess the affinity between the pesticides and the matrix. The presence of 26 pesticides was monitored by GC-MS/MS using bee pollen samples, with passive samplers as controls.

2. Material and methods

2.1. Chemicals and reagents

Certified analytical standards of acetochlor, alachlor, disulfoton, alpha-endosulfan, beta-endosulfan, etrinfos, fempropatrim, phosalone, heptachlor epoxide, hexachlorobenzene, oxifluorfem, ethyl parathion, methyl parathion, pendimethalin, and terbufos were purchased from Dr. Ehrenstorfer (Augsburg, Germany). Aldrin, bifenthrin, bioallethrin, methyl chlorpyrifos, DDT (dichlorodiphenyl-trichloroethane), phenthoate, fluazifop, lindane, malathion, permethrin, pirimiphos-ethyl, and trifluralin were obtained from Fluka (USA). Abamectin were purchased from Chem Service Inc. (USA). All these compounds had purity of at least 98%. Acetonitrile (residue analysis grade) was purchased from Tedia (Brazil). Anhydrous magnesium sulfate, sodium chloride, sodium citrate, and sodium hydrogen citrate sesquihydrate (all analytical grade) were purchased from J. T. Baker (USA). Primary-secondary amine (PSA, 40 μm Bondesil) sorbent was purchased from Varian (USA).

2.2. Standard solutions

For preliminary studies, standard stock solutions (1000 μg mL^{-1}) of alachlor, aldrin, bifenthrin, bioallethrin, etrinfos, fluazifop, heptachlor epoxide, malathion, oxyfluorfen, pendimethalin, terbufos, and trifluralin were prepared in acetone. DDT, alpha endosulfan, beta endosulfan, phenthoate, ethyl parathion, methyl parathion, permethrin, pirimiphos-ethyl, and fempropatrim were prepared in hexane. Hexachlorobenzene was prepared in chloroform. Abamectin, used in the sorption studies, was prepared in methanol. All the stock standard solutions were stored in dark glass vials at −16 °C. Individual intermediate standard solutions containing 10.0 μg mL^{-1} of these pesticides and the internal standard lindane were prepared daily by diluting 0.10 mL of the stock standard solution in 10 mL of acetonitrile.

Method validation employed two working standard solutions. Mixture I contained aldrin, bifenthrin, bioallethrin, chlorpyrifos-methyl, disulfoton, alpha-endosulfan, beta-endosulfan, fempropatrin, o'p'DDT, oxifluorfem, ethyl parathion, methyl parathion, pendimethalin, trifluralin, and the lindane internal standard. Mixture II contained acetochlor, alachlor, etrinfos, phenthoate, fluazifop, fosalone, heptachlor epoxide, hexachlorobenzene, malathion, permethrin, pirimiphos-ethyl, terbufos, and the lindane internal standard. Mixture I, containing fifteen standards (including the internal standard), was analyzed using Method I, while Mixture II, containing thirteen standards (including the internal standard), was analyzed using Method II. All standard solutions were kept in a freezer at −16 °C for up to six months.

2.3. Environmental monitoring

An apiary containing 10 beehives was installed in a bushland region of a protected natural reserve. This field station, in Tanquinho Velho (municipality of Jaguariúna, São Paulo State), is located between latitudes 22°42′44″ and 22°42′55″ S and longitudes 47°00′53″ and 47°01′05″ W, near km 127.5 of the SP340 highway (Campinas-Mogi Mirim), between the Atibaia and Jaguari rivers, and has a total area of 131.0 ha.

The main crops in and around the experimental field were sugar cane (*Saccharum officinarum* L.) pasture hay (several grass species), coffee (*Coffea arabica* L.), citrus (mainly *Citrus sinensis*), corn (*Zea mays*), sunflower (*Helianthus annus*), eucalyptus (*Eucalyptus* spp.), and green manure (several species). There were also areas of protected native forest.

Collection of 145 pollen samples was made in the experimental apiary between May 2012 and May 2013. The collection of the pollen samples was performed directly in the ten hives using front porch pollen traps. This type of collector has an entrance screen with several 4.5 mm holes that allow the passage of worker bees but are small enough to retain the pollen grains attached to their hind legs. Subsequently, the pollen falls into a collection drawer. The collection period with the screen installed was continued for three successive days, after which the bees were given free access to the hives for approximately 30 days, in order to prevent any negative impacts on colony development.

As a control, the presence of pesticides in the environment was also monitored using passive samplers containing polyurethane foam (PUF) discs, as described by Shoeib and Harner (2002). The