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Influence of pesticide use in fruit orchards during blooming on honeybee mortality in 4 experimental apiaries

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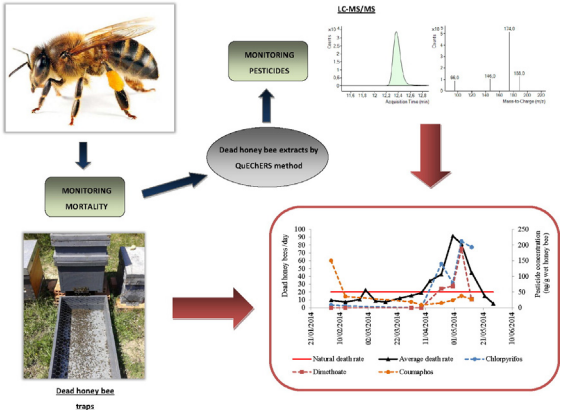
HIGHLIGHTS

- Pesticide residues and bee mortality were monitored in four apiaries for six months.
- QuEChERS extracts of bees were screened for 58 pesticides using LC–MS/MS.
- Honey bee mortality increased in blooming season until highest levels.
- Coumaphos at a residual concentration (50 ng/g) was not related to bee mortality.
- Chlorpyrifos and dimethoate concentrations were highly related to mortality peaks.

GRAPHICAL ABSTRACT



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ABSTRACT

Samples of dead honey bees (*Apis mellifera* L.) were collected periodically from 4 different locations during citrus and stone fruit trees blooming season to evaluate the potential impact of agrochemicals on honey bee death rate. For the determination of mortality, dead honey bee traps were placed in front of the experimental hives entrance located in areas of intensive agriculture in Valencian Community (Spain). A total of 34 bee samples, obtained along the monitoring period, were analyzed by means of QuEChERS extraction method and screened for 58 pesticides or their degradation products by LC–MS/MS. An average of four pesticides per honey bee sample was detected. Coumaphos, an organophosphate acaricide used against varroosis in the experimental hives, was detected in 94% of the samples. However, this acaricide was unlikely to be responsible for honey bee mortality because its constantly low concentration during all the monitoring period, even before and after acute mortality episodes. The organophosphates chlorpyrifos and dimethoate, as well as the neonicotinoid imidacloprid, were the most frequently detected agrochemicals. Almost 80% of the samples had chlorpyrifos, 68% dimethoate, and 32% imidacloprid. Maximum concentrations for these three compounds were 751, 403, 223 ng/g respectively.

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Influence of these pesticides on acute honey bee mortality was demonstrated by comparing coincidence between death rate and concentrations of chlorpyrifos, dimethoate and imidacloprid.

1. Introduction

Most of the flowering plants all over the world need animal pollination to survive (Ollerton et al., 2011). Insects pollinate more than a third of all crops and honey bees are usually the most abundant pollinators in cultivated areas, carrying out 85% of the effective insect pollination (Barclay and Moffett, 1984; Robinson et al., 1989). Latest estimates of the benefit of pollination in the world reach about 153 thousand million euros (Gallai et al., 2009) and nearly 80% can be attributed directly or indirectly to honey bees (Robinson et al., 1989). With a serious decline in wild honey and solitary bees, the importance of beekeeping and managed hives in sustaining biodiversity and crop pollination is increasing (Moritz et al., 2010; Calderone, 2012). Therefore, colonies of beekeepers in developed countries are assuming a strategic function to society and environment.

Beekeeping is living a murky panorama that many beekeepers and scientists have tried to clear up during last decade. Annual mortality of honey bee colonies is increasing in many developed countries and hives reach a weak state that often is hard to overcome (vanEngelsdorp and Meixner, 2010; Potts et al., 2010). Up to now, there is an agreement in considering honey bee decline as a result of multiple factors combination (Mullin et al., 2010; Moritz et al., 2010). Global effects of varroa parasite and associated viruses, impact of pesticides applied to cropland and deficient nutrition of honey bee colonies caused by lack of plant diversity, are the main factors implicated (vanEngelsdorp and Meixner, 2010; Spivak et al., 2011; Sanchez-Bayo and Goka, 2014).

Regarding pesticides, recent surveys show that honey bees are being exposed to high levels of pesticides used in crops and acaricides applied in hives. The most frequent residues of agrochemicals that honey bee acquire from treated crops are organophosphates and pyrethroids insecticides followed by fungicides (Johnson et al., 2010). Among miticides used against varroosis and detected in the honey bee samples, fluvalinate, amitraz degradation products, and coumaphos have been frequently detected (Ghini et al., 2004; Mullin et al., 2010; Lambert et al., 2013). Although neonicotinoids are not the main insecticides detected, they have become the subject of scientific debate for their impact on honeybees. These new insecticides — extensively used all over the world in the last two decades — are among the most toxic pesticides to bees. They are systemic and persistent, can be absorbed and transported throughout the plant, and remain toxic in vegetal tissues for months or even years (Krupke et al., 2012). Consequently, honey bees can experience chronic exposure over long-time periods (Johnson et al., 2010), coming into contact with sublethal doses when collect pollen, nectar, and other plant secretions. These sublethal doses can impair orientation abilities of honey bees, causing loss of foragers in the field that compromise colony viability (Henry et al., 2012; Blacquiere et al., 2012; Schneider et al., 2012; Fischer et al., 2014).

In general, the first sign of acute pesticide poisoning of honey bees is the appearance of large numbers of dead or dying bees at the colony entrances throughout the apiary. Honey bee is extremely sensitive to pesticides compared to other insects, because its noticeable deficiency in the number of genes encoding detoxification enzymes (Atkins, 1992). Forager honey bees with toxic and non-toxic contaminants return to the colony and if they die inside the hive, they are evacuated by cleaner honey bees and are susceptible of being collected in honey bee traps located in front of the hive entrance. With monitoring and chemical analysis, we can obtain the residues profile of dead honey bees (Porrini et al., 2003a).

This study aimed at establishing the occurrence of pesticide residues in honey bees and relating the concentrations to honey bee mortality rates. To analyze the impact of pesticides on mortality of honey bees, a rigorous counting of dead honey bees was made during blooming season of citrus and stone fruit trees. The QuECheRS technique was used for the extractions of pesticides and liquid chromatography–mass spectrometry (LC–MS/MS) for their analysis (Kasiotis et al., 2014). In the present study, four different locations from Valencian Community (Spain) surrounded mainly by citrus crops were monitored from January to June 2014 to detect pesticides presence in the dead honey bee samples. Acute mortality peaks were related to honey bee poisoning due to high concentrations of several pesticides in the samples.

2. Materials and methods

2.1. Chemicals

High purity (98–99.9%) standards of desired pesticides, namely, acetamiprid, acetochlor, alachlor, atrazine, atrazine-desethyl, atrazine-desisopropyl, azinphos-ethyl, azinphos-methyl, buprofezin, carbendazim, carbofuran, carbofuran-3-hydroxy, chlorfenvinphos, chlorpyrifos, coumaphos, diazinon, dichlofenthion, dimethoate, diuron, DMA, DMF, DMPF, ethion, fenitrothion, fenthion, fenthion-sulfone, fenthion-sulfoxide, fipronil, flumethrin, fluvalinate, hexythiazox, imazalil, imidacloprid, isoproturon, malathion, methiocarb, metolachlor, molinate, omethoate, parathion-ethyl, parathion-methyl, prochloraz, propanil, propazine, pyriproxyfen, simazine, tebuconazole, terbumeton, terbumeton-desethyl, terbuthylazine, terbuthylazine-desethyl, terbuthylazine-2-hydroxy, terbutryn, thiabendazole, thiamethoxam and tolclofos-methyl were acquired from Sigma-Aldrich (Steinheim, Germany). Fenoxon-sulfoxide and fenoxon-sulfone as 1 mL solution at a concentration of 10 $\mu g \cdot mL^{-1}$ in acetonitrile were from Dr. Ehrenstorfer (Augsburg, Germany).

Individual standard solutions were prepared in methanol at a concentration of 1000 $mg \cdot L^{-1}$. The working standard solution was prepared by mixing the appropriate amounts of individual standard solutions and diluting with methanol to a final concentration of 0.5 $mg \cdot L^{-1}$. All solutions were stored in 10 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). Dichloromethane and methanol (gradient grade for liquid chromatography) were obtained from Panreac (Darmstadt, Germany). PSA, C18, 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. Samples collection

2.2.1. Area and season of study

Sampling apiaries (AP1 to AP4) were located in four settlements from Valencian Community in eastern Spain: Chiva, Montroi, Barxeta and Carcaixent (Fig. 1). Apiaries were situated in rural-cultivated areas where pesticides are extensively used. Apiary 2, where agricultural surface represents a 70% of the total area, was surrounded mainly by citrus and peach orchards together with dry farming lands. In the apiaries 1, 3 and 4, there was a clear predominance of citrus, scattered fruit trees orchards with khaki fruits or plums and natural vegetation, a