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Organophosphorus insecticides in honey, pollen and bees (*Apis mellifera* L.) and their potential hazard to bee colonies in Egypt

Yahya Al Naggar [a,b,*], Garry Codling [b], Anja Vogt [b], Elsaied Naiem [a], Mohamed Mona [a], Amal Seif [a], John P. Giesy [b,c,d,e,f,g]

[a] Department of Zoology, Faculty of Science, Tanta University, 31527 Tanta, Egypt
[b] Toxicology Centre, University of Saskatchewan, 44 Campus Drive, Saskatoon, SK S7N 5B3, Canada
[c] Department of Veterinary Biomedical Sciences, University of Saskatchewan, Saskatoon, SK, Canada
[d] Department of Zoology, and Center for Integrative Toxicology, Michigan State University, East Lansing, MI, USA
[e] Department of Biology & Chemistry and State Key Laboratory in Marine Pollution, City University of Hong Kong, Kowloon, Hong Kong, SAR, China
[f] School of Biological Sciences, University of Hong Kong, Hong Kong, SAR, China
[g] State Key Laboratory of Pollution Control and Resource Reuse, School of the Environment, Nanjing University, Nanjing, China

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ABSTRACT

There is no clear single factor to date that explains colony loss in bees, but one factor proposed is the wide-spread application of agrochemicals. Concentrations of 14 organophosphorous insecticides (OPs) in honey bees (*Apis mellifera*) and hive matrices (honey and pollen) were measured to assess their hazard to honey bees. Samples were collected during spring and summer of 2013, from 5 provinces in the middle delta of Egypt. LC/MS–MS was used to identify and quantify individual OPs by use of a modified Quick Easy Cheap Effective Rugged Safe (QuEChERS) method. Pesticides were detected more frequently in samples collected during summer. Pollen contained the greatest concentrations of OPs. Profenofos, chlorpyrifos, malation and diazinon were the most frequently detected OPs. In contrast, ethoprop, phorate, coumaphos and chlorpyrifos-oxon were not detected. A toxic units approach, with lethality as the endpoint was used in an additive model to assess the cumulative potential for adverse effects posed by OPs. Hazard quotients (HQs) in honey and pollen ranged from 0.01–0.05 during spring and from 0.02–0.08 during summer, respectively. HQs based on lethality due to direct exposure of adult worker bees to OPs during spring and summer ranged from 0.04 to 0.1 for best and worst case respectively. It is concluded that direct exposure and/or dietary exposure to OPs in honey and pollen pose little threat due to lethality of bees in Egypt.

1. Introduction

Honey bees (*Apis mellifera* L.) fulfill important ecological and economic roles as pollinators of crops and produce honey that can be harvested for consumption. Approximately 35% of arable crops depend directly on pollinators (Klein et al., 2007), accounting for an annual value of 153 billion Euros (Gallai et al., 2009). Egypt has ~1.3 million hives, 7700 are mud hives and approximately 270,000 beekeepers (The first international Forum for the Egyptian Beekeepers, 2009). There is limited statistical information on the beekeeping industry in Egypt, such as its annual revenue and production volumes, but it is estimated to be one of the most influential in the Middle East and Africa (http://www.beekeeping.com/articles/us/arab_countries.htm). Egyptian beekeepers based along the Nile River have reported increased colony losses over winter with no clear cause for this phenomenon (Hassan, 2009).

There is a global concern about the decline of populations of the honey bee (UNEP, 2010; Van Engelsdorp and Meixner, 2010; Fairbrother et al., 2014). The term 'Colony Collapse Disorder' (CCD) has been coined to identify this issue (Cox-Foster et al., 2007; Williams et al., 2010). During the 1990s 15–20% of hives failed this loss was considered manageable and losses were attributed to a range of factors such as disease, pathogens and pesticides, in the mid-2000s losses have risen to >30% in some locations. Although causes of CCD are still unclear, results of some studies suggest that extensive use of insecticides might be responsible or a significant co-factor for increased colony losses. Genome sequencing of the honeybee provides a possible explanation for their sensitivity to pesticides. Relative to other insects, the honeybee genome is deficient in a number of genes encoding detoxification enzymes (Claudianos et al., 2006). A strong association between disease,

* Corresponding author at: Toxicology Center, University of Saskatchewan, Saskatoon, SK S7N 5B3, Canada. Fax: +306 966 4796.
E-mail address: Yehia.elnagar@science.tanta.edu.eg (Y. Al Naggar).

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pathogens and pesticides have been cited as potential causes of CCD (Cox-Foster et al., 2007; Ratnieks and Carreck, 2010). This has led to the theory that the syndrome might be the result of multiple stressors (Van Engelsdorp and Meixner, 2010). The syndrome is most commonly associated with infections of the Varroa mite and the diseases it carries (Fairbrother et al., 2014).

In Egypt several classes of pesticides including organochlorine (OC), organophosphorus (OP), carbamates, ureas, anilides and pyrethroids are used. OPs have become the major compound group used in pest control, while OC use has declined. In 1995 over 80% of all insecticide used in Egypt were OPs (Badawy, 1998 and Mansour, 2004). Contamination of food with OPs and their residues is of concern; the widespread application has been questioned as a potential risk to human health (Pico et al., 1996).

To address potential effects of pesticides on pollinators, several tools have been developed. These tools range from relatively simple hazard assessments, such as evaluating lethality, to more sophisticated assessments of risk (Fairbrother et al., 2014). Assessments of risk integrate probabilities of response based on hazard or potency with probability of exposure (Giesy et al., 2014). If sufficient information is available, it is generally considered to be more relevant for estimation of potential adverse effects than the simpler hazard quotient (HQ). However, since all the information on probabilities of exposure is not yet available for the honey bee, a HQ approach was used as an initial assessment.

Although there have been several studies on concentrations of pesticides in bee matrices and their potential risks to bees (Rissato et al., 2007; Mullin et al., 2010; Wiest et al., 2011; Chauzat et al., 2011; Cutler et al., 2014) there have been no such studies of the potential effects of pesticides on colonies of bees in Egypt. This study of 14 OPs pesticides in honey and bee matrices from 5 different agricultural governances in Egypt and their potential for lethality from direct and dietary exposure represents the first study of its kind in Egypt.

2. Materials and methods

2.1. Study areas

Within Egypt the main region of agriculture is the Nile River Valley, particularly the Nile Delta region. During spring and summer 2013 honey, bees, and pollen stored in the comb were collected from 15 locations (3 apiaries per location) from the 5 primary agricultural governorates in the Nile Delta of Egypt: Kafr El-Sheikh, Al Gharbiya, Al-Menofiya, Al-Beheira and Al-Dakahlia (Fig. 1). In Egypt, there are three main seasons for pollination; 1) Citrus season during the first two weeks in April; 2) Clover season from May until the first week of June and 3) Cotton season during August and September. Egyptian clover, *berseem*, is the major forage crop cultivated in the Nile Valley and Delta and occupies 1.2 million hectares. Egyptian clover is planted between the 1st of September and 1st of June and flowering starts by the 1st of April. European honeybees forage largely on clover from the beginning of spring until the 1st week of June, after which cotton, maize, vegetables and pumpkins represent the predominant sources of nectar and pollen during summer in these locations. Samples were collected during clover and cotton growing seasons of 2013.

2.2. Experimental

2.2.1. Beehive samples

Three hives were selected at random in each apiary. Pollen was collected by cutting a 6 cm^2 piece of comb containing stored pollen using a disposable plastic knife and placed in a 15 mL Falcon tube (Fisher Scientific). Fresh honey was squeezed from the comb into a 50 mL polyethylene Falcon tubes. Worker bees were brushed into disposable polyethylene bags. Worker bees were collected from the honey combs located on the farthest side walls of the hive from the entrance. These were normally older worker bees, which were more likely to have accumulated pesticides, and should represent similar ages. All samples were transported in a cool box with ice packs and frozen at -20 °C in the laboratory until extraction (Al Naggar et al., 2013). The total number of samples collected was 39, 31 and 34 for honey, pollen and bees respectively.

2.2.2. Chemicals and reagents

Standards used for quantification of pesticides were all technical grade (> 98% purity, Accu Standard, New Haven, CT, USA). All solvents (Hexane, MeOH, MeCN, etc.) were of HPLC grade or better and tested for OP contamination prior to use, (VWR supplier). Anhydrous sodium sulfate (NaAc) and Magnesium sulfate (MgSO4) were from (Sigma Aldrich, Ca.). Individual stock standard solutions

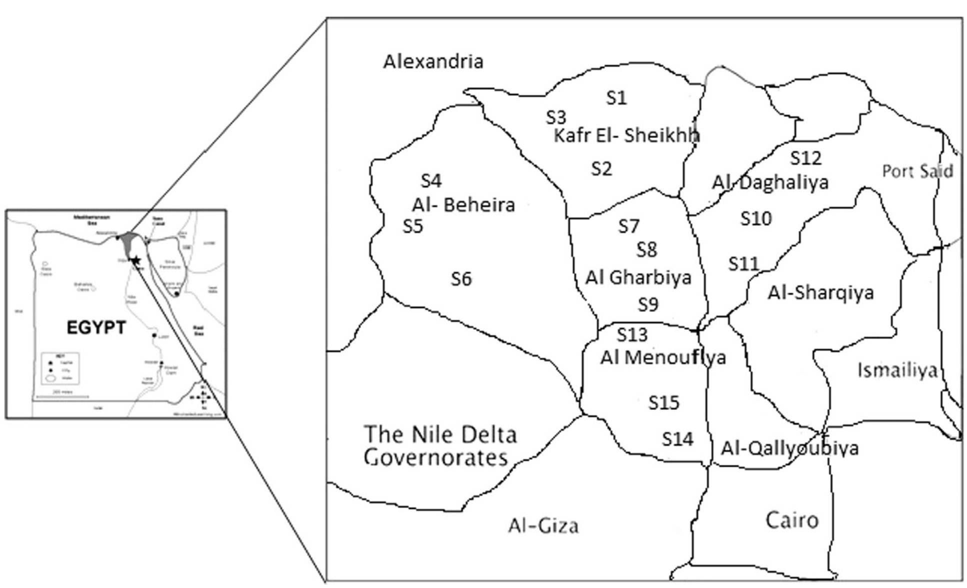

Fig. 1. Map of study sites (S1-15) in the main agricultural governorates in the Nile Delta of Egypt.