Ecotoxicology and Environmental Safety 147 (2018) 200–205

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Assessment of acute sublethal effects of clothianidin on motor function of honeybee workers using video-tracking analysis

Abdulrahim T. Alkassab*, Wolfgang H. Kirchner

Ruhr University Bochum, Faculty of Biology and Biotechnology, Universitätsstrasse 150, D-44801 Bochum, Germany

ARTICLE INFO

Keywords:
Clothianidin
Sublethal
Locomotor activity
Tracking
Honeybee

ABSTRACT

Sublethal impacts of pesticides on the locomotor activity might occur to different degrees and could escape visual observation. Therefore, our objective is the utilization of video-tracking to quantify how the acute oral exposure to different doses (0.1–2 ng/bee) of the neonicotinoid "clothianidin" influences the locomotor activity of honeybees in a time course experiment. The total distance moved, resting time as well as the duration and frequency of bouts of laying upside down are measured.

Our results show that bees exposed to acute sublethal doses of clothianidin exhibit a significant increase in the total distance moved after 30 and 60 min of the treatment at the highest dose (2 ng/bee). Nevertheless, a reduction of the total distance is observed at this dose 90 min post-treatment compared to the distance of the same group after 30 min, where the treated bees show an arched abdomen and start to lose their postural control. The treated bees with 1 ng clothianidin show a significant increase in total distance moved over the experimental period. Moreover, a reduction in the resting time and increase of the duration and frequency of bouts of laying upside down at these doses are found. Furthermore, significant effects on the tested parameters are observed at the dose (0.5 ng/bee) first at 60 min post-treatment compared to untreated bees. The lowest dose (0.1 ng/bee) has non-significant effects on the motor activity of honeybees compared to untreated bees over the experimental period.

1. Introduction

The bioavailability of neonicotinoids is considered to be at a high level throughout the year depending on the respective pest control profiles in a wide range of agricultural and horticultural plants (Bonmatin et al., 2015). Neonicotinoids exhibit long persistency in soil, e.g. the half-life of clothianidin in soil is between 148 and 6900 days (Rexrode et al., 2003). Moreover, their high ability to diffuse throughout the plants due to their systemic properties allow them to spread through the xylem in growing plants. Thus, the uptake by crops and wild plants as well as the diffusion in several matrices lead to contaminated nectar, pollen (Cutler et al., 2014; Botias et al., 2015) and water (Joachimsmeier et al., 2012; Samson-Robert et al., 2014) which were collected by bee foragers and transported to the nest.

However, under field-realistic conditions, little information is known about the level of oral or contact exposure either via contaminated food (nectar, pollen, and water) or other surfaces and matrices (reviewed by Alkassab and Kirchner, 2017). Therefore, the exposure of non-target organisms, e.g. *Apis* and non-*Apis* bees, to pesticides through the residues at different concentrations is currently a vital issue in the risk assessment process (Spurgeon et al., 2016).

It has been reported that the field-relevant concentrations of these pesticides ranged between 1–10 µg/kg depending on the most frequently detected residues in pollen and nectar in the seed-treated crops (Cresswell, 2011; Botias et al., 2015; Kunz et al., 2015). Recently, Rundlöf et al. (2015) reported that the detected residues of clothianidin in the pollen or nectar of seed-treated canola ranged between 6.7–16 µg/L in nectar and 6.6–23 µg/kg in pollen. Subsequently, the exposure of pollinators to neonicotinoids at sublethal concentrations is not excluded. Nowadays, increasing attention is paid to sublethal effects due to their subsequent impacts on the development of the insect pollinators (Schneider et al., 2012; Arce et al., 2016). Among them, *Apis* and non-*Apis* bees are considered as the most important pollinators worldwide, as they play a key role in the maintenance of biodiversity and food production (Kleijn et al., 2015; Potts et al., 2016).

Moreover, the concerns about adverse effects of neonicotinoids on insect pollinators have led to two-year restrictions on the use of three neonicotinoids (clothianidin, imidacloprid, and thiamethoxam) as seed treatment in bee-attractive crops in the European Union to evaluate their potential environmental impacts (European Commission, 2013).

* Corresponding author.
E-mail addresses: Abdulrahim.Alkassab@rub.de (A.T. Alkassab), Wolfgang.H.Kirchner@rub.de (W.H. Kirchner).

http://dx.doi.org/10.1016/j.ecoenv.2017.08.047
Received 31 January 2017; Received in revised form 12 August 2017; Accepted 18 August 2017
0147-6513/

The neonicotinoids' mode of action is known as acetylcholine mimics acting as agonists of nicotinic acetylcholine receptors (nAChRs), which in turn activate persistently the cholinergic receptors leading to hyperexcitation and eventually death (Jeschke and Nauen, 2008). Moreover, Palmer et al. (2013) reported that the exposure to neonicotinoids causes a depolarization-block of neuronal firing and inhibition in the nicotinic responses.

Although imidacloprid and clothianidin were classified in the same group "N-nitroguanidines," clothianidin acts as a super agonist compared to imidacloprid serving as a partial agonist (Brown et al., 2006). However, imidacloprid-related effects on honeybees have been well investigated by many researchers (e.g. Decourtye et al., 2004; Eiri and Nieh, 2012; Blanken et al., 2015; Wegener et al., 2016), whereas few studies were carried out to assess clothianidin-related effects on honeybees (e.g. Cutler et al., 2014; Rundlöf et al., 2015)

Recently, most ecotoxicological studies have been carried out to test the effects of sublethal exposure to pesticides on various endpoints, especially the behavioral endpoints, due to the sensitivity and effectiveness of these endpoints in the ecological risk assessment (see review Alkassab and Kirchner, 2017). Motion activities play an important role for *Apis* and non-*Apis* bees, since they are involved in different behavioral aspects, e.g. foraging and communication. Some studies looked at the influence of xenobiotics on bees' mobility by investigating the locomotion modifications and foraging activity (Williamson et al., 2014; Schneider et al., 2012). Special attention was given to assessing sublethal effects on foraging behavior, which plays a key role in the development and fitness of the colony (Sherman and Visscher, 2002). In relation to the effects on motor function, imidacloprid was reported to reduce the foraging activity of honeybees as well as bumblebees (Decourtye et al., 2004; Feltham et al., 2014; Arce et al., 2016), delay a forager's return visit to the feeder (Yang et al., 2008; Schneider et al., 2012), impair navigation and homing flights (Schneider et al., 2012; Fischer et al., 2014), and lead to fewer waggle dance circuits (Eiri and Nieh, 2012). Moreover, bees treated with imidacloprid exhibit trembling or may decrease the frequency of waggle dancing upon their return to the nest (Kirchner, 1999).

Few studies have been conducted to investigate the related effects of these pesticides on the motion activities of honeybees because there are sometimes limitations to determining and quantifying the effects using an efficient tool. A preliminary visual observation was performed, showing an increased motor activity of bees contact-treated with imidacloprid at 1.25 ng/bee even after 15 min of the treatment, whereas impairment of the movement was observed at doses ≥ 5 ng/bee (Lambin et al., 2001). On the other hand, bees treated orally with clothianidin exhibited no changes in walking, sitting and flying but spent more time laying on their backs (upside down) after 24 h at the dose 0.34 ng/bee (Williamson et al., 2014).

Nevertheless, sublethal impacts on the locomotor activity might occur at different degrees and could escape visual observation. However, if the foragers are foraging permanently from a monoculture of crops seed-treated with neonicotinoids, they might be exposed to sublethal doses from the contaminated nectar during their foraging trips. Thus, our objective is to use the video-tracking method to quantify how the acute oral exposure to clothianidin influences the motor activity of honeybees in the time course experiment. The total distance, resting time and the period of laying upside down are measured by analysis of the video-recordings. To our knowledge, this study provides the first detailed data about clothianidin-related effects on the locomotor activity of honeybees, where low sublethal doses were also tested to correspond to the realistic field exposure levels.

2. Materials and methods

2.1. Pesticide

Clothianidin was obtained in dry powder (99% purity) from Bayer Crop Science, Germany. The solubility of clothianidin in water is high (327 mg/L), but due to the difficulty in dissolving the crystals in water, a stock solution was pre-dissolved in acetone with a concentration of 200 mg/L. Then, the previous solution was mixed with distilled water, thereby gaining a solution of 1 mg/L. For acute oral treatment, dilution series were implemented to obtain concentrations in a 2 M sucrose solution of 5, 25, 50, 100 µg a.i./kg syrup which are equivalent to dosages per 20 mg syrup of 0.1, 0.5, 1 and 2 ng/bee. Untreated bees were fed a 2 M sucrose solution, in addition to an equal amount of solvent 0.0025–0.05%. Fresh solutions were prepared weekly from frozen aliquots of the stock solution. The sucrose solution was prepared with distilled water and kept in the refrigerator at 2–4 °C.

2.2. Bees

Winter workers of the honeybee *Apis mellifera* were collected from a single healthy colony form an apiary at the Ruhr University, Germany. A treatment against *Varroa destructor* was performed in the late summer with 80% formic acid. The colony comprised about 7000 workers and a fertile 1-year-old queen.

2.3. Experimental protocols

For each dose, 24 individual winter bees were randomly collected from the colony, then placed in the arena (a 9 cm Petri dish with filter paper and four small holes on the side for ventilation), transferred to the laboratory and left to acclimatize for 30 min under red light. Since the bees need only a short time to find the food, we offered the sugar solution (20 mg in the cap of a 1.5 ml centrifuge tube) after 25 min of acclimatization for the three pairs of bees, i.e. six Petri dishes, and observed them; when two bees (one treatment and on control) ate the total amount at the same time (± 1 min) within the 5 min, the caps were removed, and the time of the experiment started. Within the next 30 min, the next three pairs were collected and left to acclimatize. All treatments were recorded at the same time of day, 10:00–15:00 o'clock.

The time course experiment was conducted to analyze the locomotor activity of bees, where pairwise (treated and untreated bees) video recording (Canon camera; Powershot SX500 IS; 30 photo/s) was carried out for 10 min during 30–40, 60–70, 90–100 min post-treatment.

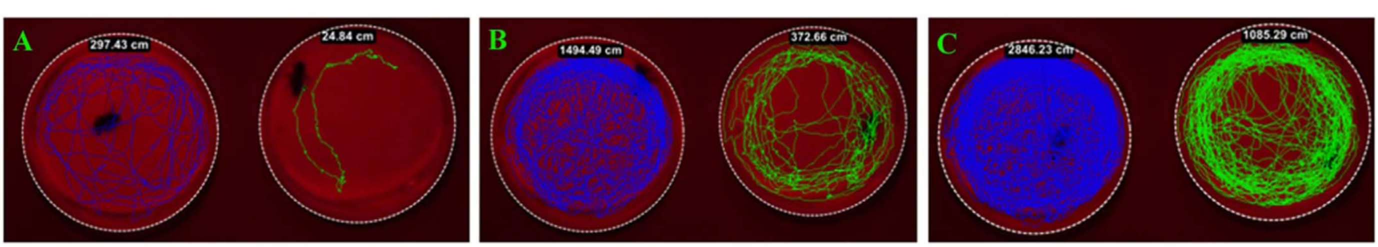

Fig. 1. Example of the tracking process and the distance moved inside the tested arena. Treated honeybee (blue track) with 2 ng after 30 min of the treatment compared with untreated bee (green track) throughout the 10 min of video recording. (A) total distance moved after one minute, (B) total distance moved after five minutes, (C) total distance moved after ten minutes. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)