短　報（Short communication）

Acute contact toxicity of three insecticides on Asian honeybees *Apis cerana*

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Abstract

The Asian honeybee, *Apis cerana*, is one of the major native honeybee species in Asia. The effects of insecticides on honeybees are currently a major concern worldwide, making it important to evaluate the impact of new insecticides on non-targeted beneficial species. This study determined the acute contact toxicity of ethiprole, acephate, and flubendiamide in Japanese *A. cerana* (*A. c. japonica*). Based on 48-h contact tests, the LD_{50} of ethiprole and acephate on *A. cerana* was 0.0036 and 0.11 μg/bee, respectively, while the LD_{50} of flubendiamide could not be determined from the concentrations tested (*i.e.* up to 100.0 μg/bee). These results provide evidence regarding the susceptibility of *A. cerana* under laboratory conditions to insecticides commonly used.

Key words: *Apis cerana japonica*, pesticide, phenylpyrazole, organophosphorus, diamide

1. Introduction

The Asian honeybee, *Apis cerana*, is one of the major native honeybee species in Asia. It is distributed widely throughout southern, south-eastern, and eastern Asia (Oldroyd and Wongsiri 2006) and provides a range of ecosystem services. *A. cerana* flexibly uses a wide range of plant resources for food (Nagamitsu and Inoue 1999, Taki et al. in press) and plays a significant role as a pollination service provider for wild plants and crop species. In addition, honeybee species are a source of honey, wax, pollen, propolis, and royal jelly (Winston 1991), which, in turn, often have cultural and social roles involving indigenous and local people, and are protected for their economic value in many countries of Asia (Oldroyd and Wongsiri 2006).

Currently, the effects of insecticides on honeybees are a major concern worldwide. Inappropriate use of insecticides can negatively affect non-targeted beneficial species (Stanley et al. 2015, Tan et al. 2015). Several studies have raised concerns over the potential impact of pesticides on wild bee species, particularly systemic insecticides such as neonicotinoids, whose use has increased dramatically within a short period of time (Godfray et al. 2014, Godfray et al. 2015). As insecticides have multiple uses, they have many exposure pathways in natural and human-altered ecosystems, and a species often responds differently to a variety of pesticides (Pisa et al. 2015, Van der Sluijs et al. 2013). Thus, it is important to evaluate the impact of various new insecticides on non-targeted beneficial species (Desneux et al. 2007).

Our study examined, under laboratory conditions, the insecticide susceptibility of *A. cerana* to insecticides commonly used in its natural habitat. Although a previous study of *A. cerana* tested the acute contact toxicity of several insecticides, including neonicotinoids, fipronil, organophosphorus pesticides, and synthetic pyrethroids (Yasuda et al. 2017), more data are required. Here, we examined the acute contact toxicity of three pesticides in *A. cerana*: ethiprole (a phenylpyrazole), acephate (an organophosphorus), and flubendiamide (a diamide). The domestic use of ethiprole, acephate, and flubendiamide in Japan in 2014 amounted to 43,646, 354,223, and 25,431 tons, respectively, and the amounts of ethiprole and flubendiamide have increased in the last 5 years according to data from WebKis-Plus (http://w-chemdb.nies.go.jp/), provided by the National Institute for Environmental Studies (Tsukuba, Japan).

2. Materials and Methods

We studied bees from Japanese populations of *Apis cerana*, which is often classified as the subspecies *Apis cerana japonica*. The worker bees used in this experiment were collected as larvae from a hive at the National Agriculture and Food Research Organisation in Tsukuba, Ibaraki. A hive frame with larvae was stored at 35°C until the larvae emerged as young adult workers. We used workers <2 days old for the experiments (Yasuda et al. 2017).

Pure ethiprole (purity 98%) and acephate (99%) were

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purchased from Wako Pure Chemicals (Osaka, Japan), and flubendiamide (>95%) was obtained from Nihon Nohyaku (Tokyo, Japan). To prepare the test solutions, the insecticides were dissolved in acetone (Wako Pure Chemicals). The doses studied were based on LD_{50} (lethal dose) values reported for the Western honeybee, *Apis mellifera*, using the compiled data (Kimura et al. 2014, Sanchez-Bayo and Goka 2014). The concentrations selected for ethiprole were 0.00016, 0.00033, 0.00065, 0.0013, 0.0026, 0.0052, and 0.0104 μg/bee, those for acephate were 0.0225, 0.045, 0.09, 0.18, 0.36, 0.72, and 1.44 μg/bee, and those for flubendiamide were 1.5625, 3.125, 6.25, 12.5, 25.0, 50.0, and 100.0 μg/bee.

We conducted the acute contact toxicity tests in October 2016. We made a small hole in the centre of a polypropylene cup (180 mL, 81×58×58 mm) (Shingi, Hiroshima, Japan) and the centre of a single Kimwipe (Crecia, Tokyo, Japan) was pushed out through the hole (Iwasa et al. 2004). The cup was placed in a second cup with a reservoir of a 50% aqueous sucrose syrup (Nacalai Tesque, Kyoto, Japan), and the syrup was available to the bees via the Kimwipe. Then, we anesthetised bees with carbon dioxide and immediately transferred them to a cup containing 10 bees/cup. The bees were treated with 1 μL of the appropriate dose of pesticide per bee on the dorsal surface of the abdomen. The control group was treated with 1 μL of pure acetone. After treatment, the polypropylene cup was covered with a nylon mesh sheet fastened with a rubber band and kept in the dark (L:D = 0:24) in a temperature-controlled chamber at 25.4 ± 0.5°C and 45.4 ± 4.5% relative humidity. Mortality was recorded 48 h after the treatment. We did not consider moribund bees that were unable to walk or fly as dead (Laurino et al. 2013). We repeated this procedure three times for each treatment.

All bees from the control group that was treated only with pure acetone were alive after 48 h. We then conducted a probit analysis using PriProbit ver. 1.63 (Sakuma 1998) to obtain acute LD_{50} values for contact toxicity at 48 h for ethiprole, acephate, and flubendiamide. We also used the Finney equivalent method, assuming a normal function distribution, to calculate confidence intervals, assuming a binomial distribution and an all-or-nothing response parameter (Finney 1978).

3. Results

The acute LD_{50} values for contact toxicity at 48 h for ethiprole and acephate on adult workers of *A. cerana* were 0.0036 and 0.11 μg/bee, respectively (Table 1). None of the bees died with the concentrations of flubendiamide tested, so the expected acute LD_{50} value for flubendiamide could not be estimated (>100 μg/bee).

4. Discussion

The previous study examined the acute contact toxicity of 11 insecticides commonly used in Japan: five neonicotinoids (acetamiprid, imidacloprid, clothianidin, dinotefuran, and thiamethoxam), two organophosphates (diazinon and fenitrothion), and one each of a phenylpyrazole (fipronil), anthranilic acid amide (chlorantraniliprole), synthetic pyrethroid (etofenprox), and carbamate (carbaryl) (Yasuda et al. 2017). They found acute LD_{50} values at 48 h ranging from 0.0014 to 0.278 μg/bee. The acute LD_{50} values we determined for the contact toxicity of ethiprole and acephate were within this range, whereas flubendiamide should be much less toxic than these tested insecticides.

Despite our findings, studies will need to determine the chronic effects of realistic field doses of these insecticides on *A. cerana*. For instance, several studies have shown that low doses of some insecticides can have negative effects on other bee species because foraging bees occasionally translocate insecticide residues into the pollen and nectar of plants (Gill and Raine 2014, Rundlöf et al. 2015, Whitehorn et al. 2012). Experiments showed that these effects included memory loss and a decline in navigation skills in *A. mellifera* (Decourtye et al. 2005, Han et al. 2010, Henry et al. 2012). These previous studies of other bee species demonstrate the need for further experiments on *A. cerana* in the future.

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Table 1. Acute (48 h) contact toxicity of pesticides in *Apis cerena*

Class	Chemicals	Slope ± SE	Intercept ± SE	LD_{50} (μg/bee)		Goodness of fit test		
				Estimate	95%CI	d.f.	Likelihood ratio χ^2	p
Phenylpyrazole	Ethiprole	8.006 ± 1.57	19.54 ± 3.79	0.0036	0.0031-0.0042	22	25.3	0.283
Organophosphorus	Acephate	5.27 ± 0.92	5.05 ± 0.87	0.11	0.093-0.129	22	24.58	0.318
Flubendiamide*	Diamide			>100				

*The LD_{50} of flubendiamide could not be determined from the concentrations tested.

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トウヨウミツバチ *Apis cerana* における 3 薬剤による急性経皮毒性試験

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要旨

トウヨウミツバチはアジアで最も主要な野生ミツバチの一種である。近年殺虫剤がミツバチに及ぼす影響について世界的に大きな関心がもたれ、非対象の有益種に対する新しい殺虫剤の影響を評価することが重要となっている。本研究では、日本のトウヨウミツバチ（亜種ニホンミツバチ）において、エチプロール、アセフェートおよびフルベンジアミドの急性接触毒性試験を行った。48 時間の接触試験の結果、トウヨウミツバチにおけるエチプロールおよびアセフェートの LD_{50} 値は、0.0036 および 0.11 μg/bee であったが、フルベンジアミドの LD_{50} 値は、試験した濃度（100.0 μg/bee まで）では明らかにならなかった。これらの結果は、一般的に使用されている殺虫剤における実験室条件下でのトウヨウミツバチの感受性に関する情報の蓄積に寄与する。

キーワード：ニホンミツバチ、農薬、フェニルピラゾール、有機リン、ジアミド

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