Bulletin of Insectology **77** *(1): 65-74, 2024*
ISSN 1721-8861 eISSN 2283-0332

Toxicity of synthetic insecticides on *Tetragonula pagdeni* (Hymenoptera Apidae)

Manlika KILASO[1], Chatchawan CHAISUEKUL[2]
[1]*Department of Plant Production Technology, School of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand*
[2]*Integrative Insect Ecology Research Unit, Department of Biology, Faculty of Science, Chulalongkorn University, Bangkok, Thailand*

Abstract

Stingless bees are important pollinators in the ecosystem and crop production in most subtropical and tropical regions of the world. However, the global decline of bee populations is a serious concern. Insecticides are considered crucial factors affecting bee survival. In this study, the acute toxicity of selected synthetic insecticides (imidacloprid, profenofos, and lambda-cyhalothrin) on a stingless bee, *Tetragonula pagdeni* (Schwarz) (Hymenoptera Apidae), was investigated and the LD_{50} and LC_{50} determined through topical, oral, and contact exposure under laboratory conditions. The estimated topical LD_{50} values for imidacloprid, lambda-cyhalothrin, and profenofos were 0.06, 0.53, and 45.39 ng active ingredient (a.i.)/bee, respectively. The corresponding estimated LD_{50} values from oral bioassays were 0.66, 7.52, and 75.04 ng a.i./bee, and LC_{50} values for contact exposure were 48.74, 4,339.83, and 3,062.81 ng a.i./cm^2. Overall, imidacloprid exhibited the highest toxicity among the evaluated insecticides for all routes of exposure. In addition, insecticide toxicity was higher in the topical bioassay than in oral and contact bioassays. These findings demonstrate that this stingless bee is extremely vulnerable to insecticide use. Therefore, selection of insecticides in pest control programs should consider mitigation of the risk to and detrimental effects on stingless bees.

Key words: acute toxicity, pollinator, stingless bee, pesticide, bioassay, mortality, susceptibility.

Introduction

Pollinators are important organisms in the ecosystem that provide essential services to both natural and agricultural ecosystems (Ricketts *et al*., 2008; Ollerton, 2017). In particular, their important roles in pollination services enhance the biodiversity of the world's plant species (Fontaine *et al*., 2005). Potential pollination services from pollinators also assist crop production in the global agricultural system (Garibaldi *et al*., 2013). This is very beneficial to environmental maintenance and humankind. Bee species are remarkable pollinators with high potential of pollinating and are responsible for pollinating most wild plants and agricultural crops (Potts *et al*., 2010). However, there are reports of decline in key pollinator bee populations in many parts of the world, including Europe and North America (vanEngelsdorp and Meixner, 2010). Such declines have been observed in both natural landscapes and domesticated areas (Pettis and Delaplane, 2010; Potts *et al*., 2010). This raises worldwide concerns about declines in pollinator abundance and diversity, plant diversity, natural and agricultural ecosystem sustainability, and agricultural product stability (Garibaldi *et al*., 2011; Thomann *et al*., 2013).

The observed decline in pollinators, especially bees, originates from various factors such as changes in land use, habitat loss, lack of food sources, pathogens, and insecticide exposure (Vanbergen *et al.*, 2013; Patrício-Roberto and Campos, 2014; Goulson *et al.*, 2015); however, insecticides are considered the main contributor to the decline of bee colonies (Sanchez-Bayo and Goka, 2014; Zioga *et al*., 2020) due to the widespread utilization of insecticides for pest control in most agricultural production (Dudley and Alexander, 2017). Bees are considered non-target organisms, but are prone to be affected by non-selective activity of insecticides (Brittain and Potts, 2011; Fairbrother *et al*., 2014). Bee pollinators are at risk of pesticide exposure during their foraging and also in-hive via routes such as direct contact from spray both in and off hive (Botías *et al*., 2017), chemical contamination in collected pollen and nectar (Botías *et al*., 2015; David *et al*., 2016), and chemical residues in wax comb (López *et al*., 2016). Insecticide exposure can bring about lethal or sublethal toxic effects for bee pollinators. High doses of insecticides can exacerbate mortality, resulting in population decline (Tosi and Nieh, 2019). Meanwhile, exposure at sublethal level does not cause death, but can impair foraging activity (Karahan *et al*., 2015), food intake (Azpiazu *et al*., 2019), behaviour (Matsumoto, 2013), memory (Samuelson *et al*., 2016), and learning (Williamson and Wright, 2013). Exposure to sublethal doses was also found to alter biochemical responses and gene expression (Christen *et al*., 2017; Han *et al*., 2019). Therefore, attention needs be paid to the impact of insecticides on bee pollinators according to their toxicological effects.

Studies of insecticide toxicity on bee pollinators have to date mostly focused on honey bees, *Apis mellifera* (L.) (Hymenoptera Apidae), as they are recognized as the main pollinator worldwide with high efficiency of floral visiting in both wild plants and agricultural crops (Fairbrother *et al*., 2014; Hung *et al*., 2018). Insecticide effects on honey bee mortality are well established and typically expressed as the median lethal dose (LD_{50}) or the median lethal concentration (LC_{50}) (Johnson *et al*., 2006; Laurino *et al*., 2013; Shaker *et al*., 2017). However, in addition to honey bees, stingless bees are also key pollinators. They are mainly distributed in subtropical and tropical regions (Free, 1993), and are highly diverse in species with variation in morphology, floral prefer-

ences, foraging behaviour, and nesting (Jacob *et al*., 2019b). Like honey bees, stingless bees are capable of pollinating both wild and cultivated plants (Jacob *et al*., 2019a). They are considered effective pollinators as they can pollinate a variety of plant species and have a behavioural trait referred to as flower constancy (Slaa *et al*., 2006; Rahman *et al*., 2018). However, stingless bees face the same risk of insecticide exposure as honey bees, particularly in the context of agricultural production systems (Brittain and Potts, 2011). Such exposure would result in decreased population of and pollination service from these bee species. Thus, the adverse effect of insecticides on these non-target pollinators is an essential concern that merits study.

While most toxicity studies on pollinators have emphasized honey bees, limited research has been conducted on insecticide toxicity in stingless bees. In particular, a few studies have tested the susceptibility of Neotropical bee species to different insecticides (Jacob *et al*., 2019b; Brito *et al*., 2020; Piovesan *et al*., 2020; Miotelo *et al*., 2021; Viana *et al*., 2021). The common method of assessing the effect of an insecticide on any living organism, such as a non-target pollinator, is to determine the acute toxicity by measuring the LD_{50} and LC_{50} (Stanley and Preetha, 2016). Establishing the LD_{50} and LC_{50} for a bee species provides basic information on its sensitivity to the chemical. This is very useful information and a fundamental step in assessing the insecticide risk posed to pollinators.

Tetragonula pagdeni (Schwarz) (Hymenoptera Apidae) is a common species of stingless bee in Thailand (Sakagami, 1978; Engel *et al*., 2017) that can be generally found in the landscape and in agricultural areas and is one of the most crucial pollinators. To date, the effects of insecticides on this bee species have not been reported. Therefore, the aim of this study is to evaluate the toxicity of representative organophosphate, neonicotinoid, and pyrethroid insecticides commonly used in pest control to *T. pagdeni*, specifically estimating the LD_{50} and LC_{50} through topical, oral, and contact bioassays. The selected insecticides were profenofos, imidacloprid, and lambda-cyhalothrin. Profenofos is an organophosphate insecticide that is widely used in many countries located in North America, South America, Africa, Southeast Asia, East Asia, and South Asia (Nataraj *et al.*, 2017; Abdel Rahman *et al.*, 2020; Sankom *et al.*, 2021; Shi *et al.*, 2021; Verma and Chatterjee, 2021; Manjunath *et al.*, 2023). Imidacloprid is a neonicotinoid insecticide broadly used in several regions situated in North America, South America, Africa, Asia, and Australia (Mengoni Goñalons and Farina, 2015; Xia *et al.*, 2016; Raymann *et al.*, 2018; Sriapha *et al.*, 2020; Kotze *et al.*, 2022; Omongo *et al.*, 2022; Silvanima *et al.*, 2022; Dutta *et al.*, 2024). Finally, lambda-cyhalothrin is a pyrethroid insecticide widely applied in many areas of North America, South America, Africa, the EU, and Asia (Moore *et al.*, 2001; He *et al.*, 2008; Fetoui *et al.*, 2010; Ansari *et al.*, 2012; Li *et al.*, 2014; Rieff *et al.*, 2020; da Silva Sousa *et al.*, 2021; Manjunath *et al.*, 2023). Accordingly, in enabling greater awareness of insecticide effects on non-target insect pollinators, particularly the underrepresented stingless bees, the findings of this work will support improvement of pest management strategies worldwide through selection of the most appropriate insecticide. In addition, this study contributes to minimizing the impact of insecticides on bee pollinators and thereby aids conservation of these pollinators in the environment.

Materials and methods

Insects

The stingless bees (*T. pagdeni*) used in the experiment were obtained from colonies resident at the School of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Thailand in an area free of pesticide use. Forager bees were collected using a net at the hive entrance upon their return after foraging in the morning. The collected bees represented four colonies, and each colony constituted a replicate.

Insecticides

Commercial insecticides were purchased from an agrochemical store in Bangkok, Thailand. The investigated insecticides were profenofos (organophosphate; Karuka 50% EC, Ladda), imidacloprid (neonicotinoid; Bornimida 5E 5% EC, Born Agroscience), and lambda-cyhalothrin (pyrethroid; Karate® 2.5 EC 2.5% EC, Syngenta). The names of these insecticide products are translated from the Thai trademarks on the labels.

Bioassays

The susceptibility of forager *T. pagdeni* to each insecticide was evaluated as LD_{50} and LC_{50} through bioassays representing three exposure routes: topical, oral, and contact exposure. The topical bioassay was adapted from the method of the OECD (1998) and Del Sarto *et al*. (2014); the oral bioassay was modified from Li *et al*. (2017); and the contact bioassay followed Del Sarto *et al*. (2014) with some modification. Each insecticide was diluted in acetone to make a stock solution at a concentration of 10,000 ng active ingredient (a.i.)/µL. This solution was subsequently serially diluted (1:10) in acetone for topical and contact bioassays and in 50% v/v honey solution (1:1 honey:distilled water) for oral bioassays to obtain a range of concentrations. Each bioassay was performed in two steps, a preliminary test and a final test (Piovesan *et al*., 2020). In the preliminary test, a range of log-scale concentrations (0.01 to 1,000 ng a.i./µL for topical and contact and 0.01 to 100 ng a.i./µL for oral exposure) was used for the purpose of range finding, determining the doses that yielded 0% and 100% mortality (data not shown). In the final test, four to seven concentrations of each insecticide were selected based on the preliminary range finding results to determine the LD_{50} and LC_{50}. The honey used in the experiment was 100% honey harvested from longan flowers (*Dimocarpus longan* Lour.) (Sapindales Sapindaceae) purchased in Bangkok (Doi Kham Food Products Co., Ltd., Thailand). It was mixed with distilled water at a ratio of 1:1 to make 50% v/v honey solution, which was used for insecticide dilution in oral bioassays and the preparation of food sources for stingless bees.

Topical bioassays

The forager bees were anaesthetized at −20 °C for 2 minutes prior to topical application. One microlitre of an insecticide solution was applied to the dorsal side of the thoracic region of each insect. The treated bees were transferred to a Petri dish (9 cm diameter) lined with filter paper (Whatman™ no. 1) at the bottom and provided with 50% v/v honey solution (1:1 honey:distilled water). The treatment doses used to determine the LD_{50} were: profenofos, 10, 25, 50, 75, 90, and 100 ng a.i.; imidacloprid, 0.02, 0.04, 0.05, 0.06, 0.07, 0.08, and 0.1 ng a.i.; and lambda-cyhalothrin, 0.2, 0.4, 0.5, 0.6, 0.7, and 0.9 ng a.i. The assay was replicated four times for each dose, with ten bees per replicate and each replicate representing a different colony. Acetone was used as the control treatment (zero dose). The Petri dishes were kept in the laboratory at 29 ± 2 °C and 65 ± 5% relative humidity. Mortality of bees was recorded at 24 hours after treatment. Bees were considered dead if they were immobile when probed with a soft brush.

Oral bioassays

The forager bees were starved for 2 hours prior to testing. One microlitre of insecticide solution was individually fed to each bee using a micropipette, after which the bees were transferred to a Petri dish (9 cm diameter) lined with filter paper (Whatman™ no. 1) at the bottom and provided with 50% v/v honey solution (1:1 honey:distilled water). The treatment doses used to determine the LD_{50} were: profenofos, 20, 40, 60, 80, and 100 ng a.i.; imidacloprid, 0.25, 0.5, 0.75, 1, 2.5, 5, and 10 ng a.i.; and lambda-cyhalothrin, 2, 4, 6, 8, 10, 20, and 40 ng a.i. The assay was replicated four times for each dose, with ten bees per replicate and each replicate representing a different colony. Two separate sets of control bees received only honey solution or honey solution containing 1% acetone. There was no regurgitation observed during feeding. After application, the bees were kept in the Petri dishes and mortality recorded as in the topical bioassays.

Contact bioassays

One millilitre of insecticide solution was dropped on a 9 cm diameter filter paper (Whatman™ no. 1). The treated filter paper was left to dry for 20 minutes and then was placed in a Petri dish (9 cm diameter) provided with 50% v/v honey solution (1:1 honey:distilled water). A set of ten forager bees was then placed in the Petri dish. The treatment concentrations used to determine the LC_{50} were: profenofos, 150, 175, 200, and 225 ng a.i./µL; imidacloprid, 1.5, 1.75, 2, 2.25, 2.5, and 2.75 ng a.i./µL; and lambda-cyhalothrin, 225, 250, 275, 300, and 325 ng a.i./µL. The assay was replicated four times for each concentration, with each replicate representing a different colony. Acetone, as the solvent, was used for the control treatment. After application, the bees were kept in the Petri dishes and mortality recorded as in the topical bioassays.

Statistical analysis

LD_{50} and LC_{50} for each insecticide were determined by Probit analysis via SPSS software version 16.0 (SPSS Inc., Chicago, Illinois, USA). The values were considered significantly different if there was no overlap of their 95% confidence intervals.

Results

In the topical application, the most toxic insecticide to *T. pagdeni* was imidacloprid, followed by lambda-cyhalothrin, with profenofos being the least toxic (table 1). Comparatively, imidacloprid was 8.83-fold more toxic than lambda-cyhalothrin and 756.5-fold more toxic than profenofos, while lambda-cyhalothrin was 85.64-fold more toxic than profenofos. No mortality was observed in the control treatment.

In the oral exposure bioassay, the three insecticides exhibited considerably less toxicity compared to topical application, but a similar order of effect. Imidacloprid was again the most toxic insecticide, followed by lambda-cyhalothrin and then profenofos (table 2). Comparatively, imidacloprid was 11.39-fold more toxic than lambda-cyhalothrin and 113.70-fold more toxic than profenofos, while lambda-cyhalothrin was 9.98-fold more toxic compared to profenofos. No mortality was observed for the control honey solution diet either with or without 1% acetone.

Similarly, the contact exposure bioassay showed imidacloprid to have the highest toxicity; however, profenofos was the second most toxic and lambda-cyhalothrin the least (table 3). In relative terms, imidacloprid was 62.84-fold more toxic than profenofos and 89.04-fold more toxic than lambda-cyhalothrin, while profenofos was 1.42-fold more toxic compared to lambda-cyhalothrin. No mortality was observed in the control treatment.

Overall, the LD_{50} values from topical toxicity were less than those observed for oral toxicity of the same chemical. In particular, the topical LD_{50} values of imidacloprid, lambda-cyhalothrin, and profenofos were 11, 14.19, and 1.65 times lower than the respective oral LD_{50} values. This highlights topical exposure as the more toxic route, compared to oral exposure. No over-

Table 1. Estimated median topical lethal dose (LD_{50}, ng a.i./bee) of insecticides for *T. pagdeni* forager worker bees after 24 hours of exposure.

Insecticide	Number of samples	LD_{50}	95% confidence interval		Slope (± SE)	Intercept (± SE)	χ^2 (df)
			Lower	Upper			
Profenofos	280	45.39	30.71	59.33	0.036 (± 0.002)	−1.617 (± 0.115)	40.993 (5)
Imidacloprid	320	0.06	0.05	0.08	22.146 (± 1.803)	−1.405 (± 0.113)	19.726 (6)
Lambda-cyhalothrin	280	0.53	0.45	0.62	2.725 (± 0.216)	−1.451 (± 0.122)	13.615 (5)

Table 2. Estimated median oral lethal dose (LD_{50}, ng a.i./bee) of insecticides for *T. pagdeni* forager worker bees after 24 hours of exposure.

Insecticide	Number of samples	LD_{50}	95% confidence interval Lower	Upper	Slope (± SE)	Intercept (± SE)	χ^2 (df)
Profenofos	240	75.04	66.26	86.09	0.032 (± 0.002)	−2.375 (± 0.171)	9.537 (4)
Imidacloprid	320	0.66	0.20	1.56	1.685 (± 0.155)	−1.108 (± 0.107)	85.693 (6)
Lambda-cyhalothrin	320	7.52	6.89	8.22	0.398 (± 0.030)	−2.990 (± 0.223)	12.520 (6)

Table 3. Estimated median contact lethal concentration (LC_{50}, ng a.i./cm^2) of insecticides for *T. pagdeni* forager worker bees after 24 hours of exposure.

Insecticide	Number of samples	LC_{50}	95% confidence interval Lower	Upper	Slope (± SE)	Intercept (± SE)	χ^2 (df)
Profenofos	200	3062.81	2776.36	3431.89	0.002 (± 0.000)	−4.882 (± 0.503)	10.265 (3)
Imidacloprid	280	48.74	41.89	69.31	0.046 (± 0.006)	−2.233 (± 0.217)	12.428 (5)
Lambda-cyhalothrin	240	4339.83	3762.74	4981.31	0.001 (± 0.000)	−3.294 (± 0.435)	15.683 (4)

lap of 95% confidence intervals was observed for LD_{50} or LC_{50} values, indicating that this stingless bee species differs significantly in insecticide susceptibility depending on the route of exposure. The low LD_{50} and LC_{50} values obtained for imidacloprid highlight it as being extremely toxic to *T. pagdeni*.

Discussion

There was a marked difference in the susceptibility of *T. pagdeni* to the three tested insecticides. Such variation in sensitivity among bee species has been demonstrated in many reports (Del Sarto *et al*., 2014; Jacob *et al*., 2019a; 2019b; Padilha *et al*., 2020). This study further investigated three routes of exposure, specifically topical, oral, and contact exposure. In the topical bioassay, imidacloprid showed the most toxicity to *T. pagdeni*, followed by lambda-cyhalothrin and then profenofos. This result is similar to the findings of Valdovinos-Núñez *et al*. (2009), which reported another stingless bee species, *Nannotrigona perilampoides* (Cresson) (Hymenoptera Apidae), to be most susceptible to imidacloprid (LD_{50} = 1.1 ng a.i./bee) followed by permethrin (LD_{50} = 14 ng a.i./bee), methomyl (LD_{50} = 120 ng a.i./bee), and diazinon (LD_{50} = 190 ng a.i./bee) at 24 hours after exposure. This study and others agree on the relative toxicity of insecticide classes, with neonicotinoids followed by pyrethroids and then organophosphates. In particular, a study in the stingless bee *Melipona quadrifasciata* (Lepeletier) (Hymenoptera Apidae) and the honey bee *A. mellifera* revealed greater toxicity of a pyrethroid (deltamethrin) than an organophosphate (methamidophos) (Del Sarto *et al*., 2014).

Imidacloprid has been reported to have topical LD_{50} values of 25.2 ng a.i./bee in *Scaptotrigona postica* (Latreille) (Hymenoptera Apidae) (Soares *et al*., 2015) and 2.41 ng a.i./bee in *Melipona scutellaris* (Latreille) (Hymenoptera Apidae) (Costa *et al*., 2015), both of which are also stingless bees. Compared with these results, our study indicates *T. pagdeni* to be more susceptible to topical imidacloprid. Hence, the level of toxicity of a given insecticide can vary depending on the stingless bee species. Meanwhile, various topical LD_{50} values for imidacloprid at 24 hours have also been reported for honey bee species: 24 ng a.i./bee (Suchail *et al*., 2000), 18 ng a.i./bee (Iwasa *et al*., 2004), and 14 ng a.i./bee (Yasuda *et al*., 2017) for *A. mellifera* and 8 ng a.i./bee for *Apis cerana* (F.) (Hymenoptera Apidae) (Yasuda *et al*., 2017). The value obtained in the present study is lower, indicating that *T. pagdeni* is highly susceptible to imidacloprid relative to *Apis* spp.

In oral bioassays, the lowest LD_{50} value was obtained for imidacloprid, which again highlights it as the most toxic to *T. pagdeni* among the insecticides tested. The oral LD_{50} value previously reported for imidacloprid in the stingless bee *M. quadrifasciata* is 23.54 ng a.i./bee (Tomé *et al*., 2015). The lower LD_{50} obtained in the present work indicates *T. pagdeni* to be more susceptible to imidacloprid via oral exposure. The present findings also indicate *T. pagdeni* to have a lower oral LD_{50} than *A. mellifera* (LD_{50} = 8.6 ng a.i./bee) and *A. cerana* (LD_{50} = 2.7 ng a.i./bee) as determined by Li *et al*. (2017); *A. m. mellifera* (LD_{50} = 5 ng a.i./bee) and *A. m. caucasica* (LD_{50} = 5 ng a.i./bee) from the report of Suchail *et al*. (2000); and *A. mellifera* (LD_{50} = 4.5 ng a.i./bee) from the study of Cresswell (2011). Thus, this stingless bee is more susceptible to imidacloprid than honey bee species.

Several prior studies have determined toxicity from oral bioassays in terms of the LC_{50} value. In particular, the oral LC_{50} values reported for imidacloprid in other bee species are: 42.5 ng a.i./µL (Soares *et al*., 2015) or 89.11 ng a.i./µL (Jacob *et al*., 2019a) in *S. postica*, 1.70 ng a.i./µL in *Tetragonisca angustula* (Latreille) (Hymenoptera Apidae) (Jacob *et al*., 2019b), and 22.78 ng a.i./µL in *A. mellifera* (Jacob *et al*., 2019a). Conversion of the present results to oral LC_{50} gave a value of 0.66 ng a.i./µL for *T. pagdeni*, which is again lower in comparison to other reports.

Notably, the ingestion bioassay revealed a lower LD_{50} value for lambda-cyhalothrin than for profenofos, indicating pyrethroid to be more toxic than organophosphate insecticides in *T. pagdeni*. This differs from a pri-

or report in the stingless bee *M. quadrifasciata* that revealed slightly lower toxicity of a pyrethroid (deltamethrin) than an organophosphate (methamidophos), but is similar to the result obtained in the honey bee *A. mellifera* (Del Sarto *et al*., 2014).

The final route of exposure investigated in the present study was contact. Imidacloprid was again the most toxic among tested insecticides; however, the relative ranking of profenofos and lambda-cyhalothrin differed from the report by Del Sarto *et al*. (2014) for *M. quadrifasciata* and *A. mellifera*, in which contact with deltamethrin (a pyrethroid insecticide) was more toxic than methamidophos (an organophosphate insecticide).

The differing susceptibility of *T. pagdeni* to different insecticides may stem from numerous factors, such as particular characteristics of the insecticides or the bee species (Piovesan *et al*., 2020). Similarly, species-level differences in life cycle, life history, body weight, detoxification capacity, foraging behaviour, and nesting activity are considered to potentially affect between-species differences in sensitivity to insecticides (Hardstone and Scott, 2010; Brittain and Potts, 2011; Decourtye *et al*., 2013; Arena and Sgolastra, 2014). Thus, the higher toxicity of imidacloprid in stingless bees compared to *Apis* honey bee species may result from variations in the different bees' morphological, physiological, or behavioural characteristics, including their life history and life story traits (Del Sarto *et al*., 2014). It has been observed that insecticide intake in larger bees is proportionally lower due to the greater increase of body volume relative to body surface (Valdovinos-Núñez *et al*., 2009), and bees of larger size tend to be more tolerant to insecticides than smaller ones (Thompson, 2016). From a previous review of available data in the literature, in general, honey bees are no more sensitive to insecticides than other insect species (Hardstone and Scott, 2010). Meanwhile, a prior report comparing the sensitivity of *A. mellifera* and other bee species to pesticides found most examined stingless bee species to be more sensitive than *A. mellifera* (Arena and Sgolastra, 2014).

Route of exposure is an important factor affecting the susceptibility of stingless bees to insecticides and should be considered when evaluating toxicity. The results of the present study show insecticide toxicity to vary by the route of exposure, with greater effect on *T. pagdeni* from topical exposure than oral exposure. This finding is similar to many reports that insecticides are more toxic to bee species when applied topically (Suchail *et al*., 2000; Costa *et al*., 2015; Soares *et al*., 2015; Dorneles *et al*., 2017). In addition, toxicity of imidacloprid in *Podisus maculiventris* (Say) (Hemiptera Pentatomidae) has been shown to decrease by route in the order of topical exposure, ingestion, and contact, and topical exposure to organophosphate and pyrethroid insecticides has been shown to be more toxic than oral exposure in *A. mellifera* (Suchail *et al*., 2000), which trends are similar to the present findings. Notably, in the contact bioassay, profenofos presented as more toxic than lambda-cyhalothrin. Not all insecticides exhibit significant difference of toxicity according to exposure route (Dorneles *et al*., 2017). For compounds observed to be more toxic in topical exposure, this may be due to the insecticide readily penetrating the integument of the insect; meanwhile, in oral exposure, the insecticide encounters various enzymes involved in metabolism of insecticides in the midgut and requires time for activation (Soares *et al*., 2015), which may lead to toxicity being reduced in the oral route. Finally, in contact exposure, the insect does not contact the insecticide immediately and directly but rather its residue; thus, activation of the insecticide may be reduced and the insect may receive a reduced dose, which may lead to less toxic effect.

The topical bioassay is an efficient and typical toxicity assessment method that simulates exposure of a forager bee to an insecticide while they are foraging nectar and pollen in the field (Nauen *et al*., 2001; Padilha *et al*., 2020). When an insecticide chemical is applied by spraying in the air, there is a risk that the droplets could come into direct contact with bees (Thompson, 2001; Sgolastra *et al*., 2019); this risk is very high for forager bees, and may cause foragers to die before or after returning to the hive, ultimately resulting in loss of the forager bee population and the occurrence of colony collapse (Sanchez-Bayo and Goka, 2016). When preparing the tested insecticides for application, the manufacturer-recommended dilutions are 40 mL/20 L water for imidacloprid, 25 mL/20 L water for lambda-cyhalothrin, and 40 mL/20 L water for profenofos. In terms of the active ingredients, the prepared insecticides exceed the observed topical LD_{50} (determined using 1 µL solution per bee) by 1,663.33 times (99.80 ng a.i./µL vs. 0.06 ng a.i./bee), 58.89 times (31.21 ng a.i./µL vs. 0.53 ng a.i./bee), and 21.99 times (998 ng a.i./µL vs. 45.39 ng a.i./bee), respectively. Thus, the application concentrations of these insecticides represent high levels of toxicity, particularly for imidacloprid, and the stingless bee is at risk from exposure in the course of their normal use.

The oral bioassay is another important toxicity assessment method as it simulates the consumption of contaminated pollen and nectar following insecticide treatment in agricultural areas (Dively and Kamel, 2012; Piovesan *et al*., 2020). Such direct ingestion of an insecticide may result in significant effects, both lethal and sublethal (Azpiazu *et al*., 2019; Brito *et al*., 2020; Miotelo *et al*., 2021). Furthermore, not only forager bees but also nurse bees and the brood in the hive could suffer negative effects from intaking collected dietary product with residue present (Zioga *et al*., 2020). Several compounds have been detected in pollen and nectar at maximum concentrations exceeding the corresponding estimated LD_{50} values for various bee species (Zioga *et al*., 2020). This raises concern about the risk of oral exposure across bee species; however, risk assessments for bee pollinators will be challenging to perform. As with topical exposure, the recommended application concentrations of the investigated insecticides considerably exceed the corresponding oral LD_{50} values (determined using 1 µL solution per bee), with imidacloprid, lambda-cyhalothrin, and profenofos active ingredient application concentrations being 151.21 times (99.80 ng a.i./µL vs. 0.66 ng a.i./bee), 4.15 times (31.21 ng a.i./µL vs. 7.52 ng a.i./bee), and 13.30 times (998 ng a.i./µL vs. 75.04 ng

a.i./bee) higher, respectively. Hence, the normal use of these insecticides, especially imidacloprid, also poses danger for the stingless bee through oral exposure.

The residue bioassay is an alternative assay method that imitates the scenario in which insecticides are applied and some residue remains on plant parts such as leaves and flowers, particularly pollen and nectar (Dively and Kamel, 2012; Stoner and Eitzer, 2012; Goulson, 2013). When forager bees contact a contaminated plant part or collect contaminated pollen and nectar, they tend to experience insecticide exposure (Stoner and Eitzer, 2012; Goulson, 2013; Sanchez-Bayo and Goka, 2014). Moreover, if foragers carry contaminated pollen and nectar to the hive, this can create an accumulation of chemical contamination in stored pollen and nectar; the members of the colony are at risk of exposure from both the forager bee and the carried product (Poquet *et al*., 2016; Boyle *et al*., 2019). On a per-hectare basis, the manufacturer-recommended application rates of the tested insecticides are 500 mL for imidacloprid, 312.50 mL for lambda-cyhalothrin, and 500 mL for profenofos. Considered in terms of the active ingredients, these correspond to concentrations of 25 g a.i./ha, 7.81 g a.i./ha, and 250 g a.i./ha. Thus, for every square centimeter of treated surface area a bee comes in contact with, it could receive on average 5.13 times (250 ng a.i./cm^2 vs. 48.74 ng a.i./cm^2), 0.02 times (78.1 ng a.i./cm^2 vs. 4,339.83 ng a.i./cm^2), and 0.82 times (2,500 ng a.i./cm^2 vs. 3,062.81 ng a.i./cm^2) the LC_{50} dose of imidacloprid, lambda-cyhalothrin, and profenofos, respectively. Hence, imidacloprid applied at recommended rate can also present high level of toxicity for the stingless bee through contact exposure.

All told, the evaluated insecticides present different levels of toxicity to the stingless bee. Imidacloprid is a neonicotinoid, a type of insecticide that is widely used for pest control in crop production due to the advantages of highly effective systemic activity and long residual activity (Jeschke *et al*., 2011). Neonicotinoids act through mimicking the neurotransmitter acetylcholine, interacting with the insect nicotinic acetylcholine receptor (nAChR) to cause hyperexcitation, paralysis, and death (Jeschke and Nauen, 2008; Johnson, 2015). The toxicity of this insecticide has been tested in many bee species, including both honey bees and stingless bees (Costa *et al*., 2015; Zhu *et al*., 2017; Jacob *et al*., 2019a; 2019b), with results indicating that in addition to their high effectiveness in pest control, neonicotinoids can adversely affect bee pollinators in various ways. There are reports of neonicotinoids having less toxicity than imidacloprid, such as thiamethoxam, acetamiprid, and thiacloprid (Valdovinos-Núñez *et al*., 2009; Jacob *et al*., 2019a), but these insecticides are demonstrated to have sublethal effects as well (Christen *et al*., 2016; Tison *et al*., 2016; Jacob *et al*., 2019a; Miotelo *et al*., 2021). Profenofos is an organophosphate insecticide; these molecules act as inhibitors of acetylcholinesterase, resulting in hyperactivation of cholinergic neurons (Fukuto, 1990; Chambers *et al*., 2010), and have likewise been tested for toxicity on many bee species (Stanley *et al*., 2015; Dorneles *et al*., 2017; Yasuda *et al*., 2017; Yao *et al*., 2018). Lambda-cyhalothrin is a pyrethroid insecticide, which compounds act as neurotoxicants that prolong the open phase of the sodium channel, increasing the sodium permeability of insect nerve membranes (Belzunces *et al*., 2012; Christen and Fent, 2017). This group of insecticides has also been tested for toxicity in many bee species (Wang *et al*., 2020a, 2020b; Chibee *et al*., 2021).

The effect of insecticides on mortality of the stingless bee *T. pagdeni* observed in this study indicates that this bee is potentially threatened during insecticide application. Insecticide users should be aware of the adverse effect of insecticides on non-target organisms, particularly beneficial insects like bee species. Furthermore, the present findings support that not only *A. mellifera* should be used as a reference in risk assessments, but also other bee species. Moreover, the results demonstrate that different insecticides present different levels of toxicity to stingless bees, which is useful knowledge for selecting insecticides to mitigate adverse effects on stingless bees. Agrochemical applications should be conducted with care to avoid negative effects, and proper pest management in agricultural production must consider this issue. Rotation of insecticides and use of reduced-risk insecticides could be included in such control programs to help safeguard the stingless bee, conserve its presence in the agricultural landscape, and provide for the sustainability of pollination by this bee.

Acknowledgements

This work was supported by King Mongkut's Institute of Technology Ladkrabang (grant no. KREF186213). The authors wish to thank Yuvarin Boontop, senior professional level, Department of Agriculture, Bangkok, Thailand for identifying the stingless bee species.

Study conception and design, methodology, all experiments, data collection and analysis, and discussion and manuscript writing were performed by Manlika Kilaso. Study design, data analysis, and suggestions were provided by Chatchawan Chaisuekul. All authors read and approved the final manuscript.

This study has been reviewed and approved by the Animal Care and Use Committee, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand (Approval no. ACUC-KMITL-RES/2020/005).

The authors declare no competing interests.

References

ABDEL RAHMAN A. N., MOHAMED A. A.-R., MOHAMMED H. H., ELSEDDAWY N. M., SALEM G. A., EL-GHAREEB W. R., 2020.- The ameliorative role of geranium (*Pelargonium graveolens*) essential oil against hepato-renal toxicity, immunosuppression, and oxidative stress of profenofos in common carp, *Cyprinus carpio* (L.).- *Aquaculture*, 517: 734777.

ANSARI R. W., SHUKLA R. K., YADAV R. S., SETH K., PANT A. B., SINGH D., AGRAWAL A. K., ISLAM F., KHANNA V. K., 2012.- Involvement of dopaminergic and serotonergic systems in the neurobehavioral toxicity of lambda-cyhalothrin in developing rats.- *Toxicology Letters*, 211: 1-9.

ARENA M., SGOLASTRA F., 2014.- A meta-analysis comparing the sensitivity of bees to pesticides.- *Ecotoxicology*, 23: 324-334.

AZPIAZU C., BOSCH J., VIÑUELA E., MEDRZYCKI P., TEPER D., SGOLASTRA F., 2019.- Chronic oral exposure to field-realistic pesticide combinations via pollen and nectar: effects on feeding and thermal performance in a solitary bee.- *Scientific Reports*, 9: 13770.

BELZUNCES L. P., TCHAMITCHIAN S., BRUNET J.-L., 2012.- Neural effects of insecticides in the honey bee.- *Apidologie*, 43: 348-370.

BOTÍAS C., DAVID A., HORWOOD J., ABDUL-SADA A., NICHOLLS E., HILL E., GOULSON D., 2015.- Neonicotinoid residues in wildflowers, a potential route of chronic exposure for bees.- *Environmental Science & Technology*, 49: 12731-12740.

BOTÍAS C., DAVID A., HILL E. M., GOULSON D., 2017.- Quantifying exposure of wild bumblebees to mixtures of agrochemicals in agricultural and urban landscapes.- *Environmental Pollution*, 222: 73-82.

BOYLE N. K., PITTS-SINGER T. L., ABBOTT J., ALIX A., COX-FOSTER D. L., HINAREJOS S., LEHMANN D. M., MORANDIN L., O'NEILL B., RAINE N. E., 2019.- Workshop on pesticide exposure assessment paradigm for non-*Apis* bees: foundation and summaries.- *Environmental Entomology*, 48: 4-11.

BRITO P., ELIAS M., SILVA-NETO C., SUJII E., SILVA D., GONCALVES B., FRANCESCHINELLI E., 2020.- The effects of field-realistic doses of imidacloprid on *Melipona quadrifasciata* (Apidae: Meliponini) workers.- *Environmental Science and Pollution Research*, 27: 38654-38661.

BRITTAIN C., POTTS S. G., 2011.- The potential impacts of insecticides on the life-history traits of bees and the consequences for pollination.- *Basic and Applied Ecology*, 12: 321-331.

CHAMBERS J. E., MEEK E. C., CHAMBERS H. W., 2010.- The metabolism of organophosphorus insecticides, pp. 1399-1407. In: *Hayes' handbook of pesticide toxicology* (KRIEGER R., Eds), 3rd edition.- Academic Press, London, UK.

CHIBEE G. U., OJELABI O. M., FAJANA H. O., AKINPELU B. A., KEHINDE T. O., AWODIRAN O. M., OBUOTOR E. M., OWOJORI O. J., 2021.- Effects of cypermethrin as a model chemical on life cycle and biochemical responses of the tropical stingless bee *Meliponula bocandei* Spinola, 1853.- *Environmental Advances*, 5: 100074.

CHRISTEN V., FENT K., 2017.- Exposure of honey bees (*Apis mellifera*) to different classes of insecticides exhibit distinct molecular effect patterns at concentrations that mimic environmental contamination.- *Environmental Pollution*, 226: 48-59.

CHRISTEN V., MITTNER F., FENT K., 2016.- Molecular effects of neonicotinoids in honey bees (*Apis mellifera*).- *Environmental Science & Technology*, 50: 4071-4081.

CHRISTEN V., BACHOFER S., FENT K., 2017.- Binary mixtures of neonicotinoids show different transcriptional changes than single neonicotinoids in honeybees (*Apis mellifera*).- *Environmental Pollution*, 220: 1264-1270.

COSTA L. M., GRELLA T. C., BARBOSA R. A., MALASPINA O., NOCELLI R. C. F., 2015.- Determination of acute lethal doses (LD_{50} and LC_{50}) of imidacloprid for the native bee *Melipona scutellaris* Latreille, 1811 (Hymenoptera: Apidae).- *Sociobiology*, 62: 578-582.

CRESSWELL J. E., 2011.- A meta-analysis of experiments testing the effects of a neonicotinoid insecticide (imidacloprid) on honey bees.- *Ecotoxicology*, 20: 149-157.

DA SILVA SOUSA J., DO NASCIMENTO H. O., DE OLIVEIRA GOMES H., DO NASCIMENTO R. F., 2021.- Pesticide residues in groundwater and surface water: recent advances in solid-phase extraction and solid-phase microextraction sample preparation methods for multiclass analysis by gas chromatography-mass spectrometry.- *Microchemical Journal*, 168: 106359.

DAVID A., BOTÍAS C., ABDUL-SADA A., NICHOLLS E., ROTHERAY E. L., HILL E. M., GOULSON D., 2016.- Widespread contamination of wildflower and bee-collected pollen with complex mixtures of neonicotinoids and fungicides commonly applied to crops.- *Environment International*, 88: 169-178.

DECOURTYE A., HENRY M., DESNEUX N., 2013.- Overhaul pesticide testing on bees.- *Nature*, 497: 188-188.

DEL SARTO M. C. L., OLIVEIRA E. E., GUEDES R. N. C., CAMPOS L. A. O., 2014.- Differential insecticide susceptibility of the Neotropical stingless bee *Melipona quadrifasciata* and the honey bee *Apis mellifera*.- *Apidologie*, 45: 626-636.

DIVELY G. P., KAMEL A., 2012.- Insecticide residues in pollen and nectar of a cucurbit crop and their potential exposure to pollinators.- *Journal of Agricultural and Food Chemistry*, 60: 4449-4456.

DORNELES A. L., DE SOUZA ROSA A., BLOCHTEIN B., 2017.- Toxicity of organophosphorus pesticides to the stingless bees *Scaptotrigona bipunctata* and *Tetragonisca fiebrigi*.- *Apidologie*, 48: 612-620.

DUDLEY N., ALEXANDER S., 2017.- Agriculture and biodiversity: a review.- *Biodiversity*, 18: 45-49.

DUTTA D., RAY A., GHOSH B., BAHADUR M., 2024.- Assessment of imidacloprid induced genotoxicity in *Pethia conchonius* (Rosy barb), a common freshwater fish of India.- *Drug and Chemical Toxicology*, 47 (1): 101-114.

ENGEL M. S., MICHENER C. D., BOONTOP Y., 2017.- Notes on Southeast Asian stingless bees of the genus *Tetragonula* (Hymenoptera: Apidae), with the description of a new species from Thailand.- *American Museum Novitates*, 3886: 1-20.

FAIRBROTHER A., PURDY J., ANDERSON T., FELL R., 2014.- Risks of neonicotinoid insecticides to honeybees.- *Environmental Toxicology and Chemistry*, 33: 719-731.

FETOUI H., MAKNI M., MOULDI GAROUI E., ZEGHAL N., 2010.- Toxic effects of lambda-cyhalothrin, a synthetic pyrethroid pesticide, on the rat kidney: Involvement of oxidative stress and protective role of ascorbic acid.- *Experimental and Toxicologic Pathology*, 62: 593-599.

FONTAINE C., DAJOZ I., MERIGUET J., LOREAU M., 2005.- Functional diversity of plant-pollinator interaction webs enhances the persistence of plant communities.- *PLoS Biology*, 4: e1.

FREE J. B., 1993.- *Insect pollination of crops*.- Academic press, London, UK.

FUKUTO T. R., 1990.- Mechanism of action of organophosphorus and carbamate insecticides.- *Environmental Health Perspectives*, 87: 245-254.

GARIBALDI L. A., AIZEN M. A., KLEIN A. M., CUNNINGHAM S. A., HARDER L. D., 2011.- Global growth and stability of agricultural yield decrease with pollinator dependence.- *Proceedings of the National Academy of Sciences*, 108: 5909-5914.

GARIBALDI L. A., STEFFAN-DEWENTER I., WINFREE R., AIZEN M. A., BOMMARCO R., CUNNINGHAM S. A., KREMEN C., CARVALHEIRO L. G., HARDER L. D., AFIK O., 2013.- Wild pollinators enhance fruit set of crops regardless of honey bee abundance.- *Science*, 339: 1608-1611.

GOULSON D., 2013.- An overview of the environmental risks posed by neonicotinoid insecticides.- *Journal of Applied Ecology*, 50: 977-987.

GOULSON D., NICHOLLS E., BOTÍAS C., ROTHERAY E. L., 2015.- Bee declines driven by combined stress from parasites, pesticides, and lack of flowers.- *Science*, 347: 1255957.

HAN W., YANG Y., GAO J., ZHAO D., REN C., WANG S., ZHAO S., ZHONG Y., 2019.- Chronic toxicity and biochemical response of *Apis cerana cerana* (Hymenoptera: Apidae) exposed to acetamiprid and propiconazole alone or combined.- *Ecotoxicology*, 28: 399-411.

HARDSTONE M. C., SCOTT J. G., 2010.- Is *Apis mellifera* more sensitive to insecticides than other insects?- *Pest Management Science*, 66: 1171-1180.

HE L.-M., TROIANO J., WANG A., GOH K., 2008.- Environmental chemistry, ecotoxicity, and fate of lambda-cyhalothrin, pp. 71-91. In: *Reviews of environmental contamination and toxicology* (WHITACRE D. M., Eds).- Springer, New York, USA.

HUNG K.-L. J., KINGSTON J. M., ALBRECHT M., HOLWAY D. A., KOHN J. R., 2018.- The worldwide importance of honey bees as pollinators in natural habitats.- *Proceedings of the Royal Society B: Biological Sciences*, 285: 20172140.

IWASA T., MOTOYAMA N., AMBROSE J. T., ROE R. M., 2004.- Mechanism for the differential toxicity of neonicotinoid insecticides in the honey bee, *Apis mellifera*.- *Crop Protection*, 23: 371-378.

JACOB C. R. O., MALAQUIAS J. B., ZANARDI O. Z., SILVA C. A. S., JACOB J. F., YAMAMOTO P. T., 2019a.- Oral acute toxicity and impact of neonicotinoids on *Apis mellifera* L. and *Scaptotrigona postica* Latreille (Hymenoptera: Apidae).- *Ecotoxicology*, 28: 744-753.

JACOB C. R. O., ZANARDI O. Z., MALAQUIAS J. B., SILVA C. A. S., YAMAMOTO P. T., 2019b.- The impact of four widely used neonicotinoid insecticides on *Tetragonisca angustula* (Latreille) (Hymenoptera: Apidae).- *Chemosphere*, 224: 65-70.

JESCHKE P., NAUEN R., 2008.- Neonicotinoids-from zero to hero in insecticide chemistry.- *Pest Management Science*, 64: 1084-1098.

JESCHKE P., NAUEN R., SCHINDLER M., ELBERT A., 2011.- Overview of the status and global strategy for neonicotinoids.- *Journal of Agricultural and Food Chemistry*, 59: 2897-2908.

JOHNSON R. M., 2015.- Honey bee toxicology.- *Annual Review of Entomology*, 60: 415-434.

JOHNSON R. M., WEN Z., SCHULER M. A., BERENBAUM M. R., 2006.- Mediation of pyrethroid insecticide toxicity to honey bees (Hymenoptera: Apidae) by cytochrome P450 monooxygenases.- *Journal of Economic Entomology*, 99: 1046-1050.

KARAHAN A., ÇAKMAK I., HRANITZ J. M., KARACA I., WELLS H., 2015.- Sublethal imidacloprid effects on honey bee flower choices when foraging.- *Ecotoxicology*, 24: 2017-2025.

KOTZE A. C., BAGNALL N. H., RUFFELL A. P., GEORGE S. D., ROLLS N. M., 2022.- Resistance to dicyclanil and imidacloprid in the sheep blowfly, *Lucilia cuprina*, in Australia.- *Pest Management Science*, 78: 4195-4206.

LAURINO D., MANINO A., PATETTA A., PORPORATO M., 2013.- Toxicity of neonicotinoid insecticides on different honey bee genotypes.- *Bulletin of Insectology*, 66: 119-126.

LI M., WANG J., LU Z., WEI D., YANG M., KONG L., 2014.- NMR-based metabolomics approach to study the toxicity of lambda-cyhalothrin to goldfish (*Carassius auratus*).- *Aquatic Toxicology*, 146: 82-92.

LI Z., LI M., HE J., ZHAO X., CHAIMANEE V., HUANG W.-F., NIE H., ZHAO Y., SU S., 2017.- Differential physiological effects of neonicotinoid insecticides on honey bees: a comparison between *Apis mellifera* and *Apis cerana*.- *Pesticide Biochemistry and Physiology*, 140: 1-8.

LÓPEZ S. H., LOZANO A., SOSA A., HERNANDO M. D., FERNÁNDEZ-ALBA A. R., 2016.- Screening of pesticide residues in honeybee wax comb by LC-ESI-MS/MS. A pilot study.- *Chemosphere*, 163: 44-53.

MANJUNATH R., HEMAVATHI, REDDY N. T., CHAITHRA C., MISHRA P., 2023.- Pesticides and its toxicity, 419-428. In: *Encyclopedia of toxicology* (WEXLER P., Ed.).- Academic Press, London, UK.

MATSUMOTO T., 2013.- Reduction in homing flights in the honey bee *Apis mellifera* after a sublethal dose of neonicotinoid insecticides.- *Bulletin of Insectology*, 66: 1-9.

MENGONI GOÑALONS C., FARINA W. M., 2015.- Effects of sublethal doses of imidacloprid on young adult honeybee behaviour.- *PLoS ONE*, 10: e0140814.

MIOTELO L., DOS REIS A. L. M., MALAQUIAS J. B., MALASPINA O., ROAT T. C., 2021.- *Apis mellifera* and *Melipona scutellaris* exhibit differential sensitivity to thiamethoxam.- *Environmental Pollution*, 268: 115770.

MOORE M. T., BENNETT E. R., COOPER C. M., SMITH S., SHIELDS F. D., MILAM C. D., FARRIS J. L., 2001.- Transport and fate of atrazine and lambda-cyhalothrin in an agricultural drainage ditch in the Mississippi Delta, USA.- *Agriculture, Ecosystems and Environment*, 87: 309-314.

NATARAJ B., HEMALATHA D., RANGASAMY B., MAHARAJAN K., RAMESH M., 2017.- Hepatic oxidative stress, genotoxicity and histopathological alteration in fresh water fish *Labeo rohita* exposed to organophosphorus pesticide profenofos.- *Biocatalysis and Agricultural Biotechnology*, 12: 185-190.

NAUEN R., EBBINGHAUS-KINTSCHER U., SCHMUCK R., 2001.- Toxicity and nicotinic acetylcholine receptor interaction of imidacloprid and its metabolites in *Apis mellifera* (Hymenoptera: Apidae).- *Pest Management Science*, 57: 577-586.

OECD, 1998.- *Test No. 214: honeybees, acute contact toxicity test*, OECD guidelines for the testing of chemicals, section 2.- OECD Publishing, Paris, France.

OLLERTON J., 2017.- Pollinator diversity: distribution, ecological function, and conservation.- *Annual Review of Ecology, Evolution, and Systematics*, 48: 353-376.

OMONGO C. A., OPIO S. M., BAYIYANA I., OTIM M. H., OMARA T., WAMANI S., OCITTI P., BUA A., MACFADYEN S., COLVIN J., 2022.- African cassava whitefly and viral disease management through timed application of imidacloprid.- *Crop Protection*, 158: 106015.

PADILHA A., PIOVESAN B., MORAIS M., PAZINI J. D. B., ZOTTI M., BOTTON M., GRÜTZMACHER A., 2020.- Toxicity of insecticides on Neotropical stingless bees *Plebeia emerina* (Friese) and *Tetragonisca fiebrigi* (Schwarz) (Hymenoptera: Apidae: Meliponini).- *Ecotoxicology*, 29: 119-128.

PATRÍCIO-ROBERTO G. B., CAMPOS M. J., 2014.- Aspects of landscape and pollinators-what is important to bee conservation?- *Diversity*, 6: 158-175.

PETTIS J. S., DELAPLANE K. S., 2010.- Coordinated responses to honey bee decline in the USA.- *Apidologie*, 41: 256-263.

PIOVESAN B., PADILHA A. C., MORAIS M. C., BOTTON M., GRÜTZMACHER A. D., ZOTTI M. J., 2020.- Effects of insecticides used in strawberries on stingless bees *Melipona quadrifasciata* and *Tetragonisca fiebrigi* (Hymenoptera: Apidae).- *Environmental Science and Pollution Research*, 27: 42472-42480.

POQUET Y., VIDAU C., ALAUX C., 2016.- Modulation of pesticide response in honeybees.- *Apidologie*, 47: 412-426.

POTTS S. G., BIESMEIJER J. C., KREMEN C., NEUMANN P., SCHWEIGER O., KUNIN W. E., 2010.- Global pollinator declines: trends, impacts and drivers.- *Trends in Ecology & Evolution*, 25: 345-353.

RAHMAN A., DAS P., RAJKUMARI P., SAIKIA J., SHARMAH D., 2018.- Stingless bees (Hymenoptera: Apidae: Meliponini): diversity and distribution in India.- *Apidologie*, 39: 102-118.

RAYMANN K., MOTTA E. V., GIRARD C., RIDDINGTON I. M., DINSER J. A., MORAN N. A., 2018.- Imidacloprid decreases honey bee survival rates but does not affect the gut microbiome.- *Applied and Environmental Microbiology*, 84: e00545-18.

RICKETTS T. H., REGETZ J., STEFFAN-DEWENTER I., CUNNINGHAM S. A., KREMEN C., BOGDANSKI A., GEMMILL-HERREN B., GREENLEAF S. S., KLEIN A. M., MAYFIELD M. M., 2008.- Landscape effects on crop pollination services: are there general patterns?- *Ecology Letters*, 11: 499-515.

RIEFF G. G., NATAL-DA-LUZ T., RENAUD M., AZEVEDO-PEREIRA H. M. V. S., CHICHORRO F., SCHMELZ R. M., SÁ E. L. S. D., SOUSA J. P., 2020.- Impact of no-tillage versus conventional maize plantation on soil mesofauna with and without the use of a lambda-cyhalothrin based insecticide: a terrestrial model ecosystem experiment.- *Applied Soil Ecology*, 147: 103381.

SAKAGAMI S. F., 1978.- *Tetragonula* stingless bees of the continental Asia and Sri Lanka (Hymenoptera, Apidae).- *Journal of the Faculty of Science, Hokkaido University, Series* 6*, Zoology*, 21: 165-247.

SAMUELSON E. E., CHEN-WISHART Z. P., GILL R. J., LEADBEATER E., 2016.- Effect of acute pesticide exposure on bee spatial working memory using an analogue of the radial-arm maze.- *Scientific Reports*, 6: 38957.

SANCHEZ-BAYO F., GOKA K., 2014.- Pesticide residues and bees-a risk assessment.- *PLoS ONE*, 9: e94482.

SANCHEZ-BAYO F., GOKA K., 2016.- Impacts of pesticides on honey bees, pp. 77-97. In: *Beekeeping and bee conservation: advances in research* (CHAMBO E. D., Ed.).- Intech Open, London, UK.

SANKOM A., MAHAKARNCHANAKUL W., RITTIRON R., SAJJAANANTAKUL T., THONGKET T., 2021.- Detection of profenofos in Chinese kale, cabbage, and chili spur pepper using fourier transform near-infrared and fourier transform mid-infrared spectroscopies.- *ACS Omega*, 6: 26404-26415.

SGOLASTRA F., HINAREJOS S., PITTS-SINGER T. L., BOYLE N. K., JOSEPH T., LÜCKMANN J., RAINE N. E., SINGH R., WILLIAMS N. M., BOSCH J., 2019.- Pesticide exposure assessment paradigm for solitary bees.- *Environmental Entomology*, 48: 22-35.

SHAKER N., MESBAH H., KORDY A., ALY G. F., ZAKY S. T., 2017.- Selective toxicity of neonicotinoids compounds against *Apis mellifera* workers.- *Journal of Plant Protection and Pathology*, 8: 261-263.

SHI X., LIU H., ZHANG M., YANG F., LI J., GUO Y., SUN X., 2021.- Ultrasensitive electrochemiluminescence aptasensor based on AuNPs@MWCNTs and Au@AgNPs for detection of profenofos residues.- *Sensors and Actuators B: Chemical*, 348: 130663.

SILVANIMA J., SUNDERMAN-BARNES S., COPELAND R., WOEBER A., MILLER E., 2022.- Regional extent, environmental relevance, and spatiotemporal variability of neonicotinoid insecticides detected in Florida's ambient flowing waters.- *Environmental Monitoring and Assessment*, 194: 416.

SLAA E. J., CHAVES L. A. S., MALAGODI-BRAGA K. S., HOFSTEDE F. E., 2006.- Stingless bees in applied pollination: practice and perspectives.- *Apidologie*, 37: 293-315.

SOARES H. M., JACOB C. R. O., CARVALHO S. M., NOCELLI R. C. F., MALASPINA O., 2015.- Toxicity of imidacloprid to the stingless bee *Scaptotrigona postica* Latreille, 1807 (Hymenoptera: Apidae).- *Bulletin of Environmental Contamination and Toxicology*, 94: 675-680.

SRIAPHA C., TRAKULSRICHAI S., TONGPOO A., PRADOO A., RITTILERT P., WANANUKUL W., 2020.- Acute imidacloprid poisoning in Thailand.- *Therapeutics and Clinical Risk Management*, 16: 1081-1088.

STANLEY J., PREETHA G., 2016.- *Pesticide toxicity to non-target organisms: exposure, toxicity and risk assessment methodologies*.- Springer, Dordrecht, The Netherlands.

STANLEY J., SAH K., JAIN S., BHATT J., SUSHIL S., 2015.- Evaluation of pesticide toxicity at their field recommended doses to honeybees, *Apis cerana* and *A. mellifera* through laboratory, semi-field and field studies.- *Chemosphere*, 119: 668-674.

STONER K. A., EITZER B. D., 2012.- Movement of soil-applied imidacloprid and thiamethoxam into nectar and pollen of squash (*Cucurbita pepo*).- *PLoS ONE*, 7: e39114.

SUCHAIL S., GUEZ D., BELZUNCES L. P., 2000.- Characteristics of imidacloprid toxicity in two *Apis mellifera* subspecies.- *Environmental Toxicology and Chemistry*, 19: 1901-1905.

THOMANN M., IMBERT E., DEVAUX C., CHEPTOU P.-O., 2013.- Flowering plants under global pollinator decline.- *Trends in Plant Science*, 18: 353-359.

THOMPSON H. M., 2001.- Assessing the exposure and toxicity of pesticides to bumblebees (*Bombus* sp.).- *Apidologie*, 32: 305-321.

THOMPSON H., 2016.- Extrapolation of acute toxicity across bee species.- *Integrated Environmental Assessment and Management*, 12: 622-626.

TISON L., HAHN M.-L., HOLTZ S., RÖßNER A., GREGGERS U., BISCHOFF G., MENZEL R., 2016.- Honey bees' behavior is impaired by chronic exposure to the neonicotinoid thiacloprid in the field.- *Environmental Science & Technology*, 50: 7218-7227.

TOMÉ H. V. V., BARBOSA W. F., MARTINS G. F., GUEDES R. N. C., 2015.- Spinosad in the native stingless bee *Melipona quadrifasciata*: regrettable non-target toxicity of a bioinsecticide.- *Chemosphere*, 124: 103-109.

TOSI S., NIEH J., 2019.- Lethal and sublethal synergistic effects of a new systemic pesticide, flupyradifurone (Sivanto®), on honeybees.- *Proceedings of the Royal Society B: Biological Sciences*, 286: 20190433.

VALDOVINOS-NÚÑEZ G. R., QUEZADA-EUÁN J. J. G., ANCONA-XIU P., MOO-VALLE H., CARMONA A., RUIZ SÁNCHEZ E., 2009.- Comparative toxicity of pesticides to stingless bees (Hymenoptera: Apidae: Meliponini).- *Journal of Economic Entomology*, 102: 1737-1742.

VANBERGEN A. J., GARRATT M. P., BAUDE M., BIESMEIJER J. C., BRITTON N. F., BROWN M. J. F., BROWN M., BRYDEN J., BUDGE G. E., BULL J. C., CARVELL C., CHALLINOR A. J., CONNOLLY C. N., EVANS D. J., FEIL E. J., GARRATT M. P., GRECO M. K., HEARD M. S., JANSEN V. A. A., KEELING M. J., KUNIS W. E., MARRIS G. C., MEMMOTT J., MURRAY J. T., NICOLSON S. W., OSBORNE J. L., PAXTON R. J., PIRK C. W. W., POLCE C., POTTS S. G., PRIEST N. K., RAINE N. E., ROBERTS S., RYABOV E. V., SHAFIR S., SHIRLEY M. D. F., SIMPSON S. J., STEVENSON P. C., STONE G. N., TERMANSEN M., WRIGHT G. A., 2013.- Threats to an ecosystem service: pressures on pollinators.- *Frontiers in Ecology and the Environment*, 11: 251-259.

VANENGELSDORP D., MEIXNER M. D., 2010.- A historical review of managed honey bee populations in Europe and the United States and the factors that may affect them.- *Journal of Invertebrate Pathology*, 103: S80-S95.

VERMA S., CHATTERJEE S., 2021.- Biodegradation of profenofos, an acetylcholine esterase inhibitor by a psychrotolerant strain *Rahnella* sp. PFF2 and degradation pathway analysis.- *International Biodeterioration and Biodegradation*, 158: 105169.

VIANA T. A., BARBOSA W. F., LOURENÇO A. P., SANTANA W. C., CAMPOS L. O., MARTINS G. F., 2021.- Changes in innate immune response and detoxification in *Melipona quadrifasciata* (Apinae: Meliponini) on oral exposure to azadirachtin and spinosad.- *Apidologie*, 52: 252-261.

WANG Y., ZHU Y. C., LI W., 2020a.- Comparative examination on synergistic toxicities of chlorpyrifos, acephate, or tetraconazole mixed with pyrethroid insecticides to honey bees (*Apis mellifera* L.).- *Environmental Science and Pollution Research*, 27: 6971-6980.

WANG Y., ZHU Y. C., LI W., 2020b.- Interaction patterns and combined toxic effects of acetamiprid in combination with seven pesticides on honey bee (*Apis mellifera* L.).- *Ecotoxicology and Environmental Safety*, 190: 110100.

WILLIAMSON S. M., WRIGHT G. A., 2013.- Exposure to multiple cholinergic pesticides impairs olfactory learning and memory in honeybees.- *Journal of Experimental Biology*, 216: 1799-1807.

XIA X., XIA X., HUO W., DONG H., ZHANG L., CHANG Z., 2016.- Toxic effects of imidacloprid on adult loach (*Misgurnus anguillicaudatus*).- *Environmental Toxicology and Pharmacology*, 45: 132-139.

YAO J., ZHU Y. C., ADAMCZYK J., LUTTRELL R., 2018.- Influences of acephate and mixtures with other commonly used pesticides on honey bee (*Apis mellifera*) survival and detoxification enzyme activities.- *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, 209: 9-17.

YASUDA M., SAKAMOTO Y., GOKA K., NAGAMITSU T., TAKI H., 2017.- Insecticide susceptibility in Asian honey bees (*Apis cerana* (Hymenoptera: Apidae)) and implications for wild honey bees in Asia.- *Journal of Economic Entomology*, 110: 447-452.

ZHU Y. C., YAO J., ADAMCZYK J., LUTTRELL R., 2017.- Feeding toxicity and impact of imidacloprid formulation and mixtures with six representative pesticides at residue concentrations on honey bee physiology (*Apis mellifera*).- *PLoS ONE*, 12: e0178421.

ZIOGA E., KELLY R., WHITE B., STOUT J. C., 2020.- Plant protection product residues in plant pollen and nectar: a review of current knowledge.- *Environmental Research*, 189: 109873.

Authors' addresses: Manlika KILASO (corresponding author: manlika.ki@kmitl.ac.th), Department of Plant Production Technology, School of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok 10520, Thailand; Chatchawan CHAISUEKUL, Integrative Insect Ecology Research Unit, Department of Biology, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand.

Received April 24, 2023. Accepted February 1, 2024.