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The Toxicity Test of Synthetic Insecticides on *Tetragonula laeviceps* (Apidae: Meliponini)

Ina Rubiatul Hasanah[1*], Nadzirum Mubin[1], Dewi Sartiami[1], Windra Priawandiputra[2], Dadang[1]

[1]*Department of Plant Protection, Faculty of Agriculture, IPB University Campus Dramaga, Bogor 16680, Indonesia*
[2]*Department of Biology, Faculty of Mathematics and Natural Sciences, IPB University Campus Dramaga, Bogor 16680, Indonesia*

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ABSTRACT

Intense insecticide application is thought to cause a decline in bee colonies worldwide. Bees are effective pollinators in increasing the production of agricultural commodities. The stingless bee *Tetragonula laeviceps* (Apidae: Meliponini) is widely cultivated and found around plantations. The insecticides used in the toxicity test were imidacloprid 200 g/L, fipronil 50 g/L, lambda-cyhalothrin 25 g/L, profenofos 500 g/L, and chlorantraniliprole 50 g/L followed by a semi-field test using imidacloprid, fipronil, and profenofos insecticides on the cucumber plant. The LC_{50} value showed that exposure to imidacloprid, fipronil, and profenofos insecticides caused toxic effects on *T. laeviceps* by contact and orally. Lambda-cyhalothrin was found harmful on contact exposure, in contrast, chlorantraniliprole was harmful through oral. Classification of insecticide toxicity based on LD_{50} contact for imidacloprid, fipronil, lambda-cyhalothrin, and profenofos were very toxic and needed a risk assessment. However, chlorantraniliprole was classified as moderately toxic and low risk. In semi-field test results, imidacloprid and fipronil insecticides caused a significant decrease in the leave-return and bee-visiting activity on cucumber flowers. These insecticides also produced a low average yield of fruit weight. Insecticide application can affect the role of *T. laeviceps* as the pollinator which impacts the production of agricultural commodities.

1. Introduction

Insecticide application is the method of controlling pests commonly used by farmers because it is rated fast, practical, easy, and delivers satisfaction, impacting the farmer's loyalty to using it (Mustikarini *et al.* 2014). Insecticides on the market are prepared ready-to-use, consisting of a mixture of active ingredients and adjuvants, making it easy to apply, maintain user security, and increase effectiveness (Djojosumarto 2020). The intensive application of insecticides on agricultural land was thought to cause colony collapse disorder (CCD) or decrease the number of bee colonies worldwide (Leska *et al.* 2021).

Insecticide exposure for bees could occur either by contact through insecticide droplets or from insecticide-contaminated feed that entered orally (Kuan *et al.* 2018). Insecticide application is generally carried out by high-volume spraying and produces tiny liquid droplets, so it is quickly dispersed by the wind and spread on plants. Insecticides with systemic properties could be absorbed and translocated to whole part plants, including pollen and nectar, the food source for the bee. In addition, insecticide applications can leave residues on plant surfaces, pollen, and bee bodies. (Nai *et al.* 2017; Calatayud-Vernich *et al.* 2018, 2019). The impact of insecticide exposure can cause death directly and changes in the behavior of bee foragers, thereby affecting the foraging process (Sanchez-Bayo and Goka 2016). In addition, insecticide residues on pollen, nectar, or bee bodies can be a source of exposure to colonies in hives (Kumar *et al.* 2020).

Bee has a critical role in pollination, and most bee species are effective pollinators to increase agriculture commodity yields (Khalifa *et al.* 2021). *Tetragonula laeviceps* (Apidae: Meliponini) is a bee widely cultivated and found around agricultural areas. Colonies of *T. laeviceps* can also be found in

* Corresponding Author
E-mail Address: inarubiatulhasanah@apps.ipb.ac.id

nests on trees, bamboo, logs, rocks, and settlements. These bees produce propolis, which has high economic value (Priawandiputra *et al.* 2020). Its propolis contains antioxidant and antibacterial compounds potentially used for medical treatment (Sanpa *et al.* 2015; Popova *et al.* 2022). This bee has a stingless morphological structure that makes it easy and widely cultivated by beekeepers in Indonesia (Buchori *et al.* 2022). This stingless bee actively visits flowers to collect nectar and pollen both in the morning and during the day, has a relatively constant visiting time, and can utilize remaining pollen or nectar on the inside of the flower because of its relatively small body size (Putra *et al.* 2014; Leksikowati *et al.* 2018). *T. laeviceps* bee was known to have the potential to increase the production of several agricultural commodities, such as tomatoes (Indraswari *et al.* 2016), kabocha (Putra *et al.* 2017), chayote (A'yunin *et al.* al. 2019), melons (Bahlis *et al.* 2021), and cucumbers (Zidni *et al.* 2021).

Cucumber (*Cucumis sativus* L.) is a horticultural plant cultivated for direct consumption or as a raw material for skin care and beauty products since it contains antioxidants that benefit body health (Agustin and Gunawan 2019). Therefore, the need for cucumbers continues to increase. However, cucumber production in Indonesia has fluctuated over the last five years and decreased in 2022 (BPS 2023). Fruit formation in cucumber plants occurs through an open pollination process and involves insect pollinators, which are dominated by bees (Hasan *et al.* 2017). The morphology of cucumber flowers, which are bell-shaped, yellow in color, and contain nectar and pollen, cause bees' attraction to them (Agussalim *et al.* 2017). Research by Zidni *et al.* (2021) showed that pollination by bees *T. laeviceps* on cucumber plants increased production yield (fruit set), percentage fruit normality, fruit size and weight, and seed set.

By topical application, *T. laeviceps* was reportedly susceptible to organophosphate, organochlorine, and pyrethroid insecticides (Putra and Badri 2016). Mubin *et al.* (2022) also reported that spinetoram insecticide could attract and cause *T. laeviceps* mortality. In Indonesia, research on the toxicity of insecticides to bees has just been reported in a laboratory using topical and oral methods. However, the three exposure methods (topical, residual, and oral) and confirmation in the field have yet to be widely carried out. Therefore, this study aims to examine the toxicity of insecticides through topical, residue, and oral exposure methods in the laboratory and to confirm the effect of insecticides on the activity of *T. laeviceps* bees at a semi-field scale using cucumber crops.

2. Materials and Methods

2.1. Bee Collection

The bee colonies in this study originated from beekeeping in Banten and were reared in a wooden box hive with an entrance hole on the front side. The hives were placed on open shelves, and the source of bee feed came from plants around the hive area.

2.2. Toxicity Test

The study was conducted at the Insect Physiology and Toxicology Laboratory, IPB University, from March to June 2022. The average relative humidity (RH) and laboratory temperature at the experiment time were 60±5% and 26.5±1°C, respectively.

The tests were carried out using five formulated products with different modes of action of active ingredients based on the Insecticide Resistance Action Committee (IRAC) (Table 1) and control with solvents used to make insecticide test preparations. Formulated products were obtained from agricultural stores in Bogor.

Preliminary tests were carried out using the recommended concentrations listed on the labels (Table 1), then dilutions of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5}. After that, the concentration that caused the death of 10-95% of the bees in the advanced test was used to calculate the lethal concentration of the insecticides.

Table 1. Insecticides and concentrations used for the test

Ingredient active	Formulation	Recommended concentration (ppm a.i)	Group	IRAC code
Imidacloprid	200 SL	200	Neonicotinoids	4A
Fipronil	50 SC	100	Phenyl pyrazole	2B
Lambda-cyhalothrin	25 EC	50	Pyrethroids	3A
Profenofos	500 EC	1,000	Organophosphates	1B
Chlorantraniliprole	50 SC	50	Diamide	28

A total of 10 individual bees were used for each treatment and control with three replications. Bee mortality was calculated 48 hours after treatment (HAT), with the number of mortalities in control maintained at less than 10%.

2.2.1. Acute Topical Toxicity

In testing with the topical method, the concentrations of the insecticide were imidacloprid (0.2; 0.02; 0.002; 0.0002; and 0.00002 ppm a.i), fipronil (1; 0.5; 0.25; 0.125; and 0.0625 ppm a.i), lambda-cyhalothrin (25; 12.50; 6.25; 3.125; and 1.5625 ppm a.i), profenofos (50; 25; 12.5; 6.25; and 3.125 ppm a.i), and chlorantraniliprole (10000; 7500; 5000; 2500; and 500 ppm a.i). The solvent used for the insecticides imidacloprid, lambda-cyhalothrin, and profenofos was acetone, while the insecticides fipronil and chlorantraniliprole used distilled water.

The bees were put into a test tube covered with gauze and anesthetized in the refrigerator at a temperature of -10°C for ±3 minutes to facilitate application. After that, the bees were transferred to a petri dish, and then the 1 µl test solution was dripped directly on the dorsal thorax of the bees with a micro syringe applicator. Then the bees were transferred to a cup or plastic container covered with gauze and fed with a 10% honey solution (Mubin *et al.* 2022).

2.2.2. Acute Residue Toxicity

The insecticide concentrations used in the residue method test were imidacloprid (10; 5; 2.5; 1.25; and 0.0625 ppm), fipronil (5; 2.5; 1.25; 0.625; and 0.3125 ppm), lambda-cyhalothrin (50; 25; 12.5; 6.25; and 3.125 ppm), profenofos (500; 250; 125; 62.5; and 31.25 ppm), and chlorantraniliprole (5000; 1000; 500; 200; and 100 ppm). The solvent used in the residue method was the same as in the topical method test.

The residue toxicity was carried out by taking 500 µl of the test solution using a micropipette, then dripping it evenly on the inner wall of the test tube and coating the entire surface. After the solvent evaporated, the bees were put into the tube and covered with gauze. The bees were left for 5 minutes in the tube, and then the bees were transferred to the cup and fed with a 10% honey solution.

2.2.3. Acute Oral Toxicity

In the oral method, the concentrations of the insecticides used were imidacloprid (0.2; 0.02; 0.002; 0.0002; and 0.00002 ppm), fipronil (0.25; 0.125; 0.0625; 0.03125; and 0 .015625 ppm), lambda-cyhalothrin (500; 250; 125; 62.5; and 31.25 ppm), profenofos (500; 250; 125; 62.5; and 31.25 ppm), and chlorantraniliprole (50; 37.5; 25; 12.5; and 6.25 ppm). The test feed for bees was a 50% honey solution mixed with an insecticide test preparation (Liu *et al.* 2021).

The bees were kept in a cup with a gauze cover and fasted for one hour. Afterward, the bees were fed for 5 minutes using cotton dipped in the test feed. The gauze cover used for treatment was replaced with a new one to avoid insecticide contamination. Bees were fed with 10% honey solution without treatment as additional feed.

2.3. Toxicity Classification

Total mortality of bees is used to obtain LC_{50} and LC_{95} values or concentrations that cause the death of 50–95% of bees. The LC value in the acute topical toxicity test is also used to calculate the lethal dose (LD) value, which is used to classify the LD_{50} value based on the toxicity value of the insecticide in bees according to the US EPA 2014 (Table 2) and the hazard quotient (HQ) according to the European Commission 2002 (Table 3). The HQ formula is as follows:

$$HQ = \frac{\text{Recommended concentration (ml/liter)}}{LD_{50} \text{ in bees (µg a.i/bee)}}$$

2.4. Semi-field Test

The semi-field test was conducted on a smallholder farm in Cikiray Village, Ciampea District, Bogor Regency, West Java (Figure 1) from July to August 2022.

2.4.1. Land Preparation and Planting

A semi-field test to observe the effect of insecticides on the activity of *T. laeviceps* bees was carried out on cucumber (*Cucumis sativus* L.: Cucurbitaceae) Bandana F1 variety in four plots of screen houses for imidacloprid, fipronil, profenofos, and control using

Table 2. Classification of toxicity to bees based on the LD_{50} value

Source	LD_{50} value (µg/bee)	Classification toxicity
US EPA 2014	≥ 11	Slightly toxic
	$2 < LD_{50} < 11$	Moderate toxic
	≤ 2	High toxic

Table 3. Classification of toxicity to bees based on the hazard quotient

Source	HQ value	Classification toxicity
EC 2002	< 50	Low risk
	≥ 50	Need risk assessment

water. The screen house size was 6.5 m × 6.5 m × 2.5 m, made with a bamboo frame, the walls used insect nets, and the roof was covered with UV plastic. The distance between the screen houses was two meters (Figure 2).

The planting plots were prepared by making four beds in each screen house (Figure 3). The bed size was 1 m × 5 m covered with silver plastic mulch, and planting holes were provided with a spacing of 40 cm × 50 cm (20 planting holes per bed). Seeds were sown in seeding boxes and transferred two weeks after planting (WAP). The stakes and ropes are installed when the plants have tendrils.

2.4.2. Placement of Bee Colony Hives and Insecticide Application

The bee colonies were acclimatized around the test site for one week so the bees could adapt to the surrounding environment. The colonies in wooden box hives were placed in each screen house when

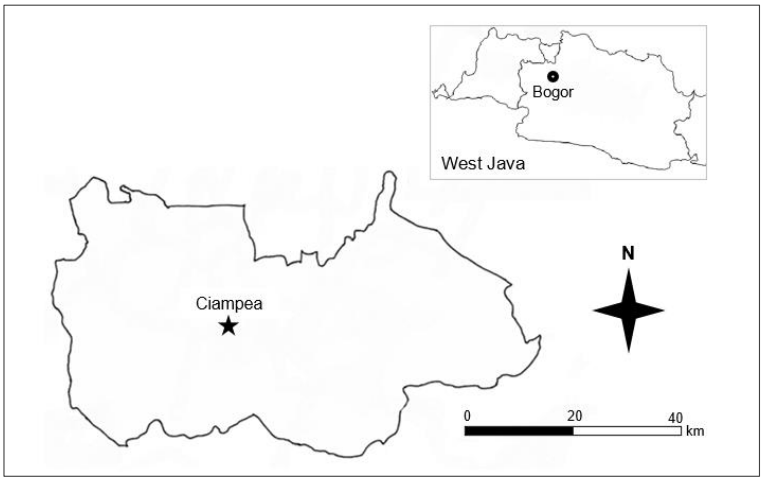

Figure 1. Location of semi-field test: point 1 (6°34'47.7"S 106°42'47.4"E)

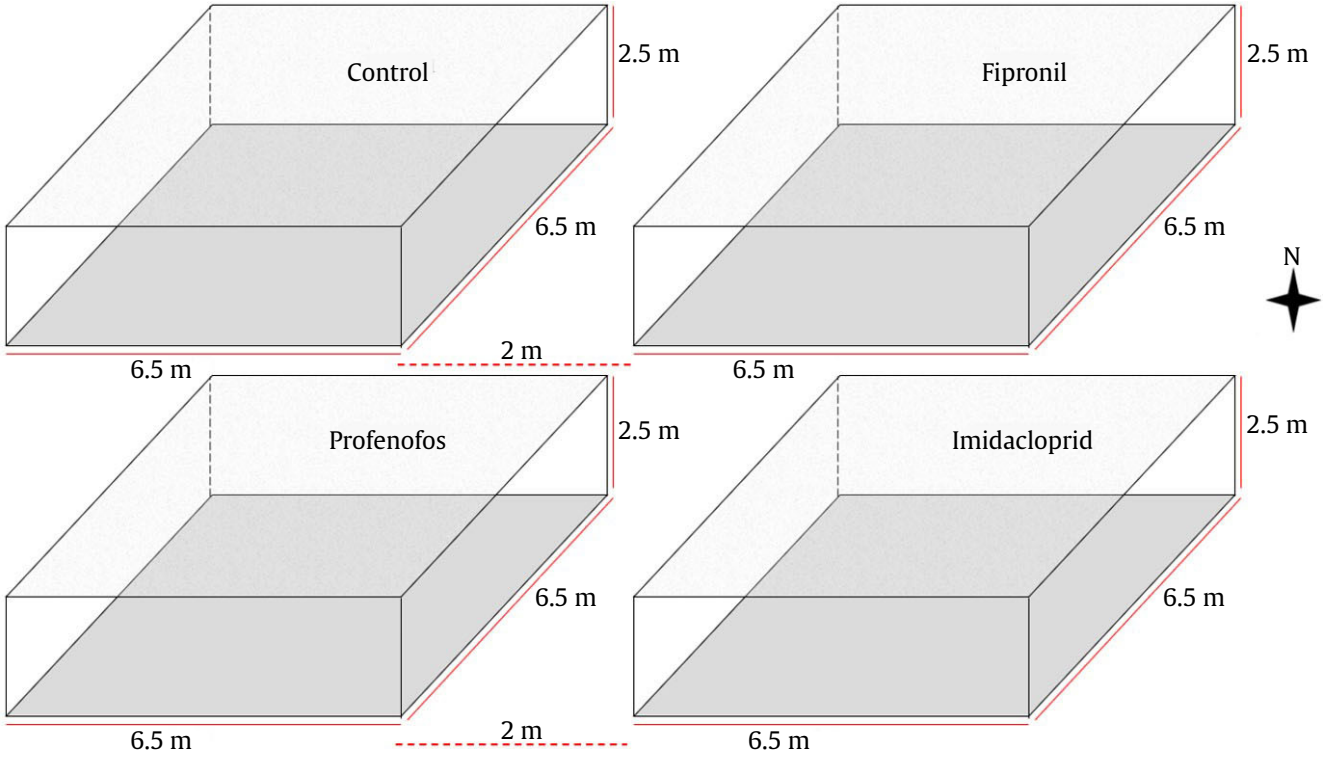

Figure 2. The layout of the treatment screen houses

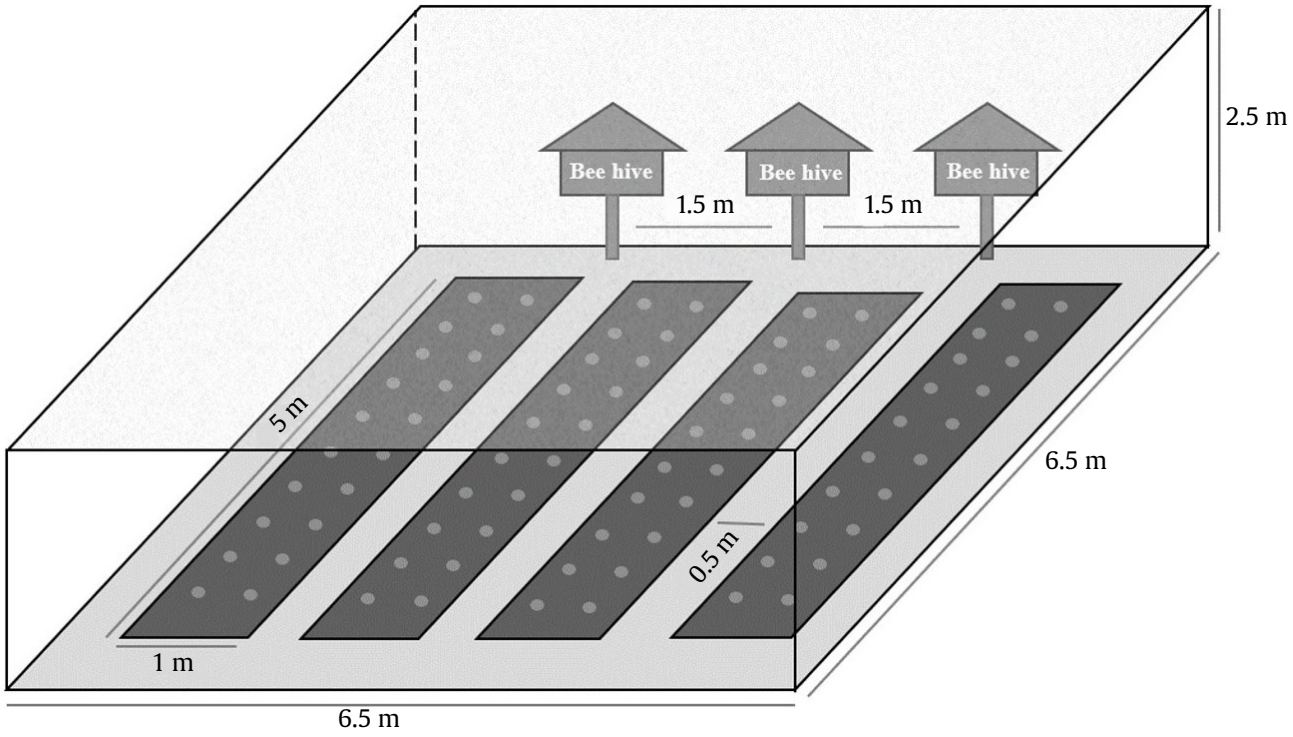

Figure 3. The layout of the planting plot and placing *T. laeviceps* hives in a screen house

the plants started to flower or around 5 WAP, and the distance between the colonies was 1.5 meters (Figure 3). The three colonies were used as a repetition of leave-return activities observation, whereas three individual bees were used as a repetition of bee visiting flowers activities observation.

Insecticide application was carried out once in the second week of the flowering phase (7 WAP). The concentration of the insecticides used followed the usage rules stated on the label, i.e imidacloprid 1 ml/L, fipronil 2 ml/L, and profenofos 2 ml/L water, while the control only used water. Insecticide spraying was carried out using an electric knapsack sprayer with a capacity of 16 liters and followed farmers' application methods in the field by moving the nozzle from the bottom to the top of the plant canopy and from the top of the canopy to the bottom of the plant until the spray liquid hits all parts of the plant.

2.4.3. Bee Activity Observation

Observations of bee activity were conducted one day before to four days after insecticide application at 07.00-09.00, 11.00-13.00, and 15.00-17.00 by the focal sampling method (Klein *et al.* 2008). The leave-return activity observation was counted visually for 5 minutes in each colony using a hand counter. After that, the activity of bees visiting flowers was observed, including the number of flowers visited by each bee (foraging rate) and the duration of visits to each flower (flower handling time) per 5 minutes (Bahlis *et al.* 2021). Counting the number of dead bees was carried out on bees found dead outside the hive and accumulated from the day of insecticide application to four days after application.

2.4.4. Fruit Yields Observation

Yield calculations were carried out on the weight of the cucumbers harvested starting from 7-9 WAP with harvesting intervals every two days. A total of 90 cucumber samples were taken randomly from each plot to be weighed.

2.5. Data Analysis

Bee mortality data was processed using the probit analysis method using the POLO+ and ecotox package in R (Hlina *et al.* 2021) to obtain LC_{50}, LC_{95}, and LD_{50} values. Observational data on semi-field tests to evaluate the effect of insecticide application on leave-return activity and visiting activity bees were carried out by the Kruskal-Wallis test and Dunn's post hoc test at a 5% significance level. In the observational data on bee mortality, the effect of insecticides was examined using the Chi-square test (X2). The data on cucumber fruit weight was processed using the General Linear

Model (GLM) test and Tukey post hoc test at a 5 % significance level. Semi-field test data was processed using Microsoft Office Excel 2019 and R v.4.2.2 (R Core Team 2022).

3. Results

3.1. Insecticide Toxicity to *Tetragonula laeviceps*

Insecticide exposures by contact (topical and residue) and oral to stingless bee *T. laeviceps* caused mortality 48 hours after treatment. No bee mortality was observed in the residue acute toxicity test with chlorantraniliprole insecticide, so the LC value could not be determined. The LC_{50} and LC_{95} values for each exposure method are presented in Table 4. The LC_{50} values of imidacloprid and fipronil insecticides on the topical and oral tests were lower than the residue test. Lambda-cyhalothrin and profenofos insecticides had the lowest LC_{50} values on the topical test method. However, chlorantraniliprole had the lowest LC_{50} on the oral test method.

The imidacloprid, fipronil, and profenofos insecticides in the three test methods had lower LC_{95} values than the recommended concentrations used to control pests. Lambda-cyhalothrin had a lower LC_{95} value than the recommended concentrations only for topical exposure. In contrast to the other four insecticides, the LC_{95} values of chlorantraniliprole were higher than the recommended concentration in both exposure methods.

The comparison of the lethal dose for each insecticide showed that the LD_{50} value obtained on *T. laeviceps* bees is lower than the LD_{50} values of other bee species that have been reported (Table 5). According to the EPA and EC toxicity classification on bees (Table 2 and 3), the LD_{50} and HQ values showed that the insecticides imidacloprid, fipronil, lambda-cyhalothrin, and profenofos were highly toxic and required risk assessment. In contrast, chlorantraniliprole insecticide was classified as moderately toxic with low risk (Table 6).

Table 4. Comparison of lethal concentration values of insecticide treatment on *T. laeviceps* by several exposure methods with recommended concentrations

Insecticide	Recommended concentration (ppm a.i)	Topical method		Residue method		Oral method	
		LC_{50} (ppm)	LC_{95} (ppm)	LC_{50} (ppm)	LC_{95} (ppm)	LC_{50} (ppm)	LC_{95} (ppm)
Imidacloprid	200	0.002	0.099	1.50	6.70	0.004	0.14
Fipronil	100	0.28	0.92	1.11	3.79	0.04	0.09
Lambda-cyhalothrin	50	3.19	11.33	23.55	64.21	151.74	336.81
Profenofos	1,000	11.44	36.22	86.54	273.71	104.10	324.42
Chlorantraniliprole	50	3127.17	12,434	-	-	23.82	93.62

Table 5. Comparison of lethal dose values of insecticide treatment on *T. laeviceps* with other bee species

Insecticide	LD_{50} (µg/bee) on *T. laeviceps*	Acute contact toxicity has been reported		
		LD_{50} (µg/bee)	Bee species	Source
Imidacloprid	0.00000242	0.001	*Leioproctus paahaumaa*	Tai *et al.* (2022)
Fipronil	0.000282	0.00041	*Melipona scutellaris*	Lourenco *et al.* (2012a)
Lambda-cyhalothrin	0.003119	0.53	*Apis mellifera*	Yanfeng dan Huili (2019)
Profenofos	0.015	0.22	*A. ceranaindica*	Gokulakrisnan *et al.* (2022)
Chlorantraniliprole	3.127	95.65	*A. mellifera*	Abbassy *et al.* (2020)

Table 6. Comparison toxicity insecticide to bee *T. laeviceps* based on LD_{50} and value Hazard Quotient

Insecticide	Imidacloprid	Fipronil	Lambda-cyhalothrin	Profenofos	Chlorantraniliprole
LD_{50} (µg/bee)	0.00000242	0.000282	0.003119	0.015	3.127
Classification EPA[a]	Very toxic	Very toxic	Very toxic	Very toxic	Moderate toxic
HQ value	413,223.14	7092.20	641.23	133.33	0.32
Classification EC[b]	Need risk assessment	Need risk assessment	Need risk assessment	Need risk assessment	Low risk

[a]Classification toxicity based on LD_{50} value (EPA 2014): LD_{50} ≤2 highly toxic); LD_{50} = 2.0-10.9 (moderate toxic); and LD_{50} ≥11 (slightly toxic)

[b]Classification mark hazard quotient (HQ) (EC 2002): HQ <50 (low risk) and HQ ≥ 50 (need risk assessment)

3.2. The Effect of Insecticides on the Activity of *Tetragonula laeviceps*

The leave-return bees' activity showed no significant difference in all treatments one day before and during spraying (Figure 4). However, one to four days after spraying, there was a decrease in activity in the plots treated with imidacloprid and fipronil insecticides. The results of observing the activities of bee visits to cucumber flowers showed that one bee could visit 2–6 flowers with visit durations were ranged from 15–62 seconds. In the treatment of imidacloprid and fipronil insecticides, there was a decrease in visiting activity after the insecticide application up to four days after application. Unlike the two insecticides, there was

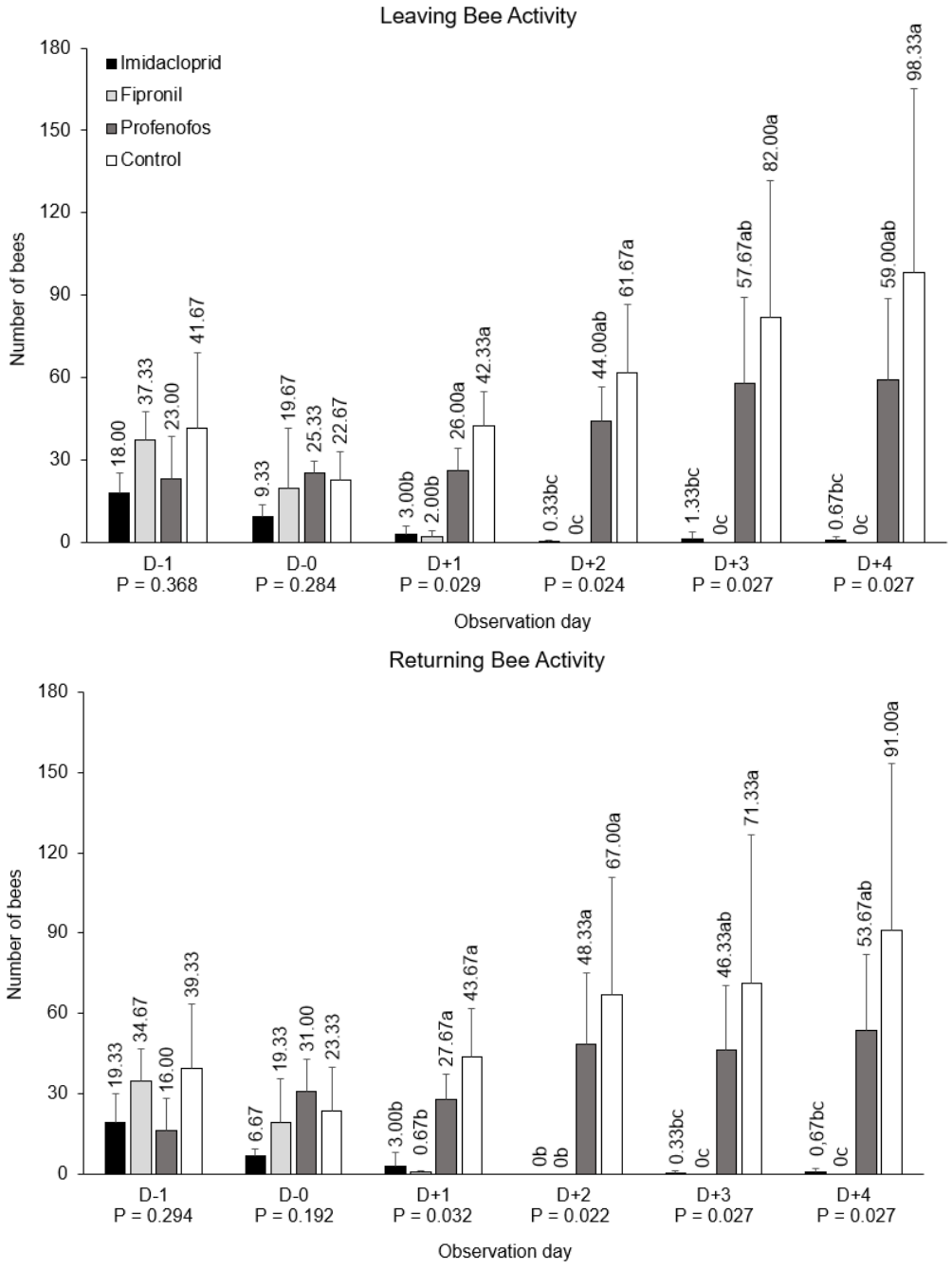

Figure 4. The leave-return activity of *T. laeviceps*. Numbers followed by different letters indicate a significant difference based on Dunn's post hoc test results at a 5% significance level (D-1: one day before spraying, D-0: during spraying, D+1: one day after spraying to D+4: four days after spraying)

no significant difference between the profenofos and control (Figure 5).

Bee mortalities were found in all treatment plots after spraying and increased with observation time. The results of the Chi-square data analysis showed that the insecticide treatment had a significant effect on bee mortality (Table 7). The deaths of bees in all insecticide treatments were significantly different from the control, starting one to four days after application. This result showed that spraying insecticides also caused bee mortality in semi-field conditions.

Based on the yield, insecticide treatment significantly affected cucumber fruit weight. As shown in Figure 6, the average yield weights for the imidacloprid treatment (118.48 grams) and fipronil (114.80 grams) were lower than the profenofos insecticide treatment (124.95 grams) and the control (123.92 grams).

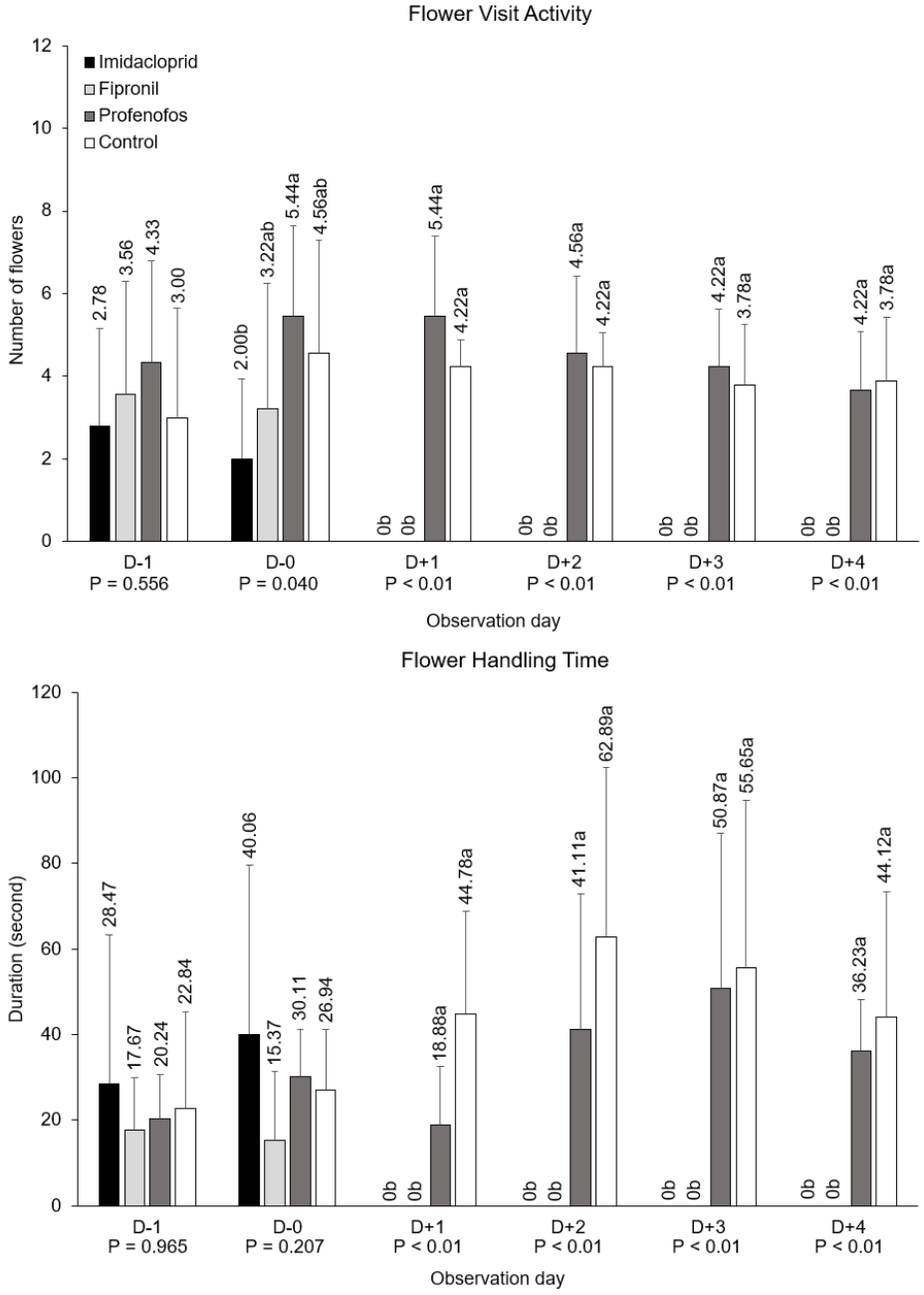

Figure 5. The average number of flowers and the duration of flower visits by *T. laeviceps*. Numbers followed by different letters indicate a significant difference based on Dunn's post hoc test results at a 5% significance level (D-1: one day before spraying, D-0: during spraying, D+1: one day after spraying to D+4: four days after spraying)

Table 7. The mortality of *T. laeviceps* in the semi-field test. (D-1: one day before spraying, D-0: during spraying, D+1: one day after spraying to D+4: four days after spraying)

Insecticide	The total number of mortalities of bees[a]				
	D-0	D+1	D+2	D+3	D+4
Imidacloprid	22**	37**	109**	130**	142**
Fipronil	14*	59**	123**	151**	166**
Profenofos	6	51**	114**	167**	197**
Control	5	13	25	35	48
X^2	10.06	30.50	30.50	86.90	86090
P-values	0.056	0.001	<0.001	<0.001	<0.001

[a]Significant difference to control: (*) P-value <0.05; (**) P-value <0.01

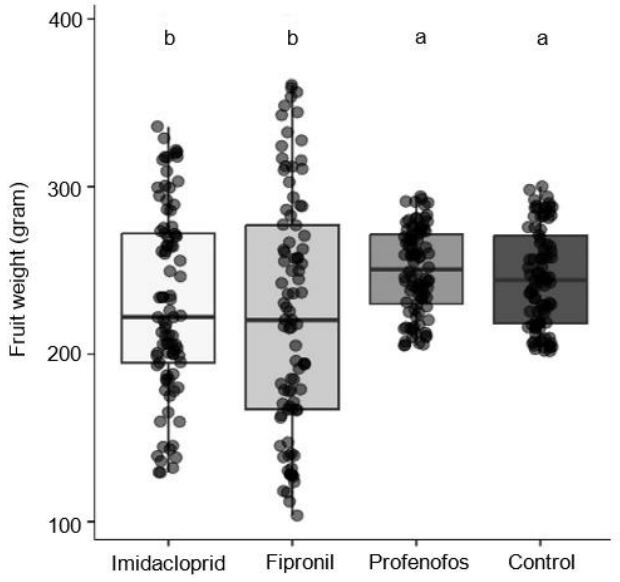

Figure 6. The average weight of cucumber fruit in each treatment. The different letters indicate a significant difference based on Tukey HSD post hoc test results at a 5% significance level

4. Discussion

The lower LC_{50} value indicates that the insecticide is more toxic to bees. In the contact exposure, the LC_{50} value of the acute topical test was lower than the acute residue test for all insecticides because the topical application was carried out by giving insecticide liquid directly to the bee's body parts, and the residue application utilizes the remaining insecticide left on the surface. Insecticide formulations that contain adjuvants, such as stickers, could stick the spray drops to plants so that the insecticide could last quite a long time on the surface (Djojosumarto 2020). Therefore, even though the bees are not exposed to insecticide liquid directly, insecticide residues on the plant's surface can be a source of contact exposure to bee body parts and cause toxic effects (Li 2022). The lower LC_{95} values than the recommended concentration used to control the insect pest indicated that the application of insecticides by farmers could potentially cause 95% of bee population deaths in field conditions through direct contact with the bees' bodies when spraying, as well as due to residues left on plants, and eaten feed contaminated with insecticides (Krupke *et al.* 2012).

The acute topical toxicity test showed that all insecticides caused the higher mortality of *T. laeviceps* bee at 48 HAT except for chlorantraniliprole. Insecticides exposed to bees would be absorbed through the cuticle and enter the body. Then the insecticide continued working according to its mode of action and resulted in the death of the bees. All of the insecticides used in the test have the same way of working, namely acting on the central nervous system. However, the insecticide chlorantraniliprole selectively acts on ryanodine receptors which play a role in muscle contraction. This insecticide binds to specific proteins on the ryanodine receptor in insects (Qi and Casida 2013). Zhou *et al.* (2020) showed that Lepidopteran insects have ryanodine receptor target sites that bind more easily to chlorantraniliprole insecticides than bees, so these insecticides are more toxic to Lepidopteran than bees.

Imidacloprid insecticide could decrease immunity to death in honeybee *Apis mellifera* after topical exposure (Chen *et al.* 2021) and was highly toxic to *Leioproctus paahaumaa* (Tai *et al.* 2022). Fipronil insecticide reported to cause death after 48 hours of exposure on the stingless bee *Melipona scutellaris* (Lourenco *et al.* 2012a) and its residue at 3–24 hours caused the highest death to *A. mellifera ligustica* (Keshlaf *et al.* 2013). Gokulakrishan *et al.* (2022) reported that the profenofos insecticide caused 100% mortality of *A. ceranaindica.* Lambda-cyhalothrin insecticide could cause rapid death (4–16 hours after exposure) when applied directly to *A. mellifera* bees (Tahir *et al.* 2017) and classified as high toxicity based on risk quotient (Yanfeng and Huili 2019). Research on the chlorantraniliprole insecticide was conducted by Abbassy *et al.* (2020), which showed that contact exposure to chlorantraniliprole had a low toxicity to *A. mellifera.*

The acute oral toxicity test result also showed that insecticides caused the mortality of *T. laeviceps*. Imidacloprid and fipronil insecticides have been reported to cause mortality of *M. scutellaris* within 48

hours through ingestion (Costa *et al.* 2015; Lourenco *et al.* 2012b). The mortality also occurred in *A. mellifera jementica*, fed with lambda-cyhalothrin and profenofos insecticides (Yeebyo *et al.* 2020). Likewise, the chlorantraniliprole insecticide caused mortality in *B. terrestris*. Insecticides that enter by ingestion could cause death in bees due to the formation of apoptosis in the midgut and damage to the digestive system, hypopharynx, and brain (Gregorc *et al.* 2018; Castro *et al.* 2020).

The comparison of LD_{50} values of *T. laeviceps* with the other bee species showed that the *T. laeviceps* bee was more sensitive to insecticides compared to *Leioproctus paahaumaa, Melipona scutellaris, Apis mellifera*, and *A. ceranaindica* because *T. laeviceps* had a smaller size than those bee species, which then increased the poisoning risk due to the ratio of body surface area or body weight and the dose of insecticide received is lower than the larger bees (Gradish *et al.* 2018).

The bees' activity is usually influenced by environmental conditions such as temperature (26°C), light intensity (13.000 lux), and humidity (62%) (Salatnaya *et al.* 2020). The average temperature, light intensity, and humidity in the treatment plot at the experiment's time were 37°C, 10,000 lux, and 87% respectively. This study showed that imidacloprid and fipronil treatment could affect the activities of *T. laeviceps* and the average leave-return bees after treatment were below 10 individuals with no visiting activity. The activity of *T. laeviceps* bee starts at 08.00 am to 05.00 pm, and the peak of flying activity occurs at 11.00 am–01.00 pm, with an average number of leave-return bees reaching 60 individuals within 5 minutes (Yustia *et al.* 2017). The bee's activity in the profenofos and control plots continued to increase with increasing observation time and the number of flowers. An increase in bees' activity can also be influenced by the number of flowers as a food source for bees so that worker bees are more active in foraging (Santos *et al.* 2008).

Applying insecticides with high-volume spraying produces droplets that can spread through the air and stick evenly to plants, including pollen and nectar. This process allows bees to be directly exposed to liquid spray from the air, residues on plant surfaces, or consume food contaminated with insecticides. Imidacloprid and fipronil caused a decrease in bee activity and movement due to motor disturbances, thus impacting the opportunities for bees to find foods, their ability to grow and develop, and colony death (Zaluski *et al.* 2015; Tasman *et al.* 2020). Imidacloprid and fipronil have low volatility in the air because they have a high vapour pressure but can still survive in the air during spraying (Bonmatin *et al.* 2015). However, these two insecticides have high persistence and long degradation times (Pisa *et al.* 2015). In contrast, the profenofos insecticide has high volatility in the air, but this insecticide is easily and quickly degraded (FAO 2008), so that in this treatment plot the leave-return and visiting activity of the bees still occurs one day after treatment.

All insecticide applications in cucumber cultivation using the recommended concentration to control pests caused the mortality of *T. laeviceps* bees. This result was similar to Melisie and Damte (2017) study that reported insecticide application on onions caused a decrease in visiting and the mortality of honeybees *A. mellifera*. Bajiya and Abrol (2017) also reported that the direct spray of insecticides in mustard crops caused a high mortality of *A. mellifera* compared to the untreated plot. Monoculture cropping and application of insecticides in agricultural areas caused a decrease in bee abundance and had low colony weight (Buchori *et al.* 2019).

The lower average weight yield of cucumber fruit was consistent with the bee-visiting activity on flowers, which decreased after being treated with imidacloprid and fipronil insecticides. The activity of *T. laeviceps* visiting flowers could help the pollination process that occurs when bees collect pollen or nectar from one to another flower and help in increasing the quantity and quality of fruit yields, such as weight, diameter, length, and perfect fruit formation (Alpionita 2021; Zidni *et al.* 2021).

This study concluded that imidacloprid, fipronil, and profenofos were toxic to stingless bee *T. laeviceps* on topical, residue, and oral exposure. The lambda-cyhalothrin was more toxic on contact exposure, while chlorantraniliprole was on oral exposure. Classification of insecticide toxicity based on the LD_{50} value of imidacloprid, fipronil, lambda-cyhalothrin, and profenofos was very toxic and needed risk assessment. In contrast, chlorantraniliprole insecticide was classified as moderately toxic and low risk. In the semi-field test results, the insecticides imidacloprid and fipronil caused a significant reduction in the leave-return activity and bee-visiting activity, thereby decreasing the average fruit weight yield.

It is necessary to carry out further research on a field scale to determine the impact of insecticide applications on *T. laeviceps* and to evaluate the toxicity of insecticides on other insecticide active ingredients with a different mode of action.

Conflict of Interest

The authors declare no conflict of interest.

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