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ATTRACTIVENESS AND TOXICITY OF IMIDACLOPRID AND DELTAMETHRIN INSECTICIDES AGAINST *Tetragonula laeviceps* SMITH (Hymenoptera: Apidae: Meliponini)

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ABSTRACT

Tetragonula laeviceps is one of the bees that plays an essential role in pollinating plants. Currently, the existence of bee populations is in danger of decreasing. One of the reasons was the inappropriate use of pesticides. The pesticide groups that influence bee behavior and mortality are neonicotinoid and pyrethroid. This study sought to determine the attractiveness and toxic effect of imidacloprid (neonicotinoid) and deltamethrin (pyrethroid) insecticides on *T. laeviceps*. The experiments were conducted on May to July 2022 at Insect Physiology and Toxicology Laboratory, Department of Plant Protection, Faculty of Agriculture, Bogor Agricultural University. The olfactory response of *T. laeviceps* was carried out using a Y-tube olfactometer with odor sources including imidacloprid (200 ppm), deltamethrin (12.5 ppm), mineral water and honey. Toxicity bioassays of *T. laeviceps* were carried out by topical, residual, and oral applications at five insecticide concentrations. Bees were more attracted to honey and water than imidacloprid 200 ppm or deltamethrin 12.5 ppm, while the attractiveness between imidacloprid 200 ppm and deltamethrin 12.5 ppm did not show a significant difference. The toxic effect of imidacloprid on *T. laeviceps* was greater than deltamethrin for the three different bioassays of insecticides. These two active ingredients were classified as highly toxic (LD_{50} <2 μg/bee) based on EPA classification. The implication of these results is the farmer should not spray the insecticide particularly imidacloprid and deltamethrin on their chili plantation during flowering stages.

Key words: mortality, olfactometer, oral application, residual application, topical application

INTRODUCTION

Most plant species are pollinated by insects (Michener 2007). Roughly one-third of the food is directly or indirectly dependent upon pollination by bees (Peters 2013). One of the native bees in Indonesia is *Tetragonula laeviceps* Smith (Apidae: Meliponini). *T. laeviceps,* known as stingless bee is domesticated by beekeepers as friendly bee because it has no sting. *T. laeviceps* is used to produce honey, pollen and propolis. In addition, *T. laeviceps* can also be used as a plant pollinating agent (Putra et al. 2014). *T. laeviceps* bees actively collect nectar and pollen from various types of plants (Cholis et al. 2019). Cross-pollination by bees significantly increases the quantity and quality of yield in plants (Stein et al. 2017). Plants pollinated by bees have an increased number of seeds and fruit size. Some types of plants, such as cucumber, chili, and tomato, increase fruit size and the number of seeds when pollinated with the help of bees (Zidni et al. 2021; Mubin et al. 2022a).

Pollination by bees contributes to more than half of the US agriculture industry's value of $29 billion per year (EPA 2018). Despite bees' importance, bee colonies' populations have declined in

Europe and North America. Scientists have identified many factors influencing this decline, such as diseases and bee pests, poor bee nutrition, lack of genetic diversity, bee management practices, pesticide use (EPA 2018) as well as colony collapse disorder (CCD) phenomenon (Lu et al. 2012). Some insecticides that are thought to reduce the presence of bee colonies are neonicotinoid such as imidacloprid and pyrethroid such as deltamethrin. Exposure to imidacloprid results in bees experiencing disorientation and lose direction to returning to the hive or their food source (Yang et al. 2008). In addition, bees' learning and memory abilities will generally be impaired in terms of the ability to find and navigate due to exposure to imidacloprid (Zhang and Nieh 2015). Deltamethrin can affect foraging activity and the development of bee colonies. Honey bee (*A. mellifera*) communication through waggle dance was disrupted by deltamethrin-sublethal doses (Zhang et al. 2019). Exposure to the imidacloprid and deltamethrin insecticides disrupted bee activity in foraging for food (Tan et al. 2014; Zhang et al. 2019). The loss of bees will cause a massive problem for the agricultural ecosystems such as chili, tomato, cucumber, melon, citrus, coffee and others. Therefore, this study sought to determine the attractiveness and toxic effect of imidacloprid (neonicotinoid) and deltamethrin (pyrethroid) insecticides on *T. laeviceps*.

MATERIAL AND METHODS

Study site. This research was carried out at the Insect Physiology and Toxicology Laboratory, Department of Plant Protection, Faculty of Agriculture, Bogor Agricultural University from May to July 2022.

Experimental materials. The *T. laeviceps* worker bees used were obtained from beekeepers in Sumedang, West Java-Indonesia. Olfactory and toxicity bioassays were carried out using commercial insecticide products of imidacloprid (5%) and deltamethrin (25 g/l).

Olfactory bioassay. Olfactory bioassay was used to evaluate the attractiveness of bees to insecticides, water and honey. The bioassays can be done in several ways, such as testing with live plants, flowers, prey, or other aroma. In this experiment, the attractiveness of insecticide aroma was evaluated. The single concentration of insecticides was used in olfactory bioassays based on the recommendation rate as shown on the pesticide labels. The concentrations used were 200 ppm and 12.5 ppm for imidacloprid and deltamethrin, respectively (Table 1). Each treatment used 15 bees (as replication) which were acclimatized for 2 hours in a gauze cage (30 cm x 30 cm x 30 cm) before treatment.

Table 1. Sources of aroma treatments for olfactory bioassay.

Sub-test	Code	Treatments
P1	IMD vs HON	Imidacloprid 200 ppm *vs* 10% aqueous honey
	DEL vs HON	Deltamethrin 12.5 ppm *vs* 10% aqueous honey
	IMD-HON vs HON	Imidacloprid 200 ppm in 10% honey solution *vs* 10% aqueous honey
	DEL-HON vs HON	Deltamethrin 12.5 ppm in 10% honey solution *vs* 10% aqueous honey
P2	IMD vs WTR	Imidacloprid 200 ppm *vs* mineral water
	DEL vs WTR	Deltamethrin 12.5 ppm *vs* mineral water
	IMD-HON vs WTR	Imidacloprid 200 ppm in 10% honey solution *vs* mineral water
	DEL-HON vs WTR	Deltamethrin 12.5 ppm in 10% honey solution *vs* mineral water
P3	IMD-HON vs DEL-HON	Imidacloprid 200 ppm in 10% honey solution *vs* Deltamethrin 12.5 ppm in 10% aqueous honey solution
	IMD vs DEL	Imidacloprid 200 ppm *vs* Deltamethrin 12.5 ppm

Description codes: IMD: Imidacloprid 200 ppm, DEL: Deltamethrin 12.5 ppm, HON: 10% aqueous honey, WTR: mineral water, IMD-HON: Imidacloprid 200 ppm in 10% honey solution, DEL-HON: Deltamethrin 12.5 ppm in 10% honey solution

Olfactory bioassays were carried out using a Y-tube olfactometer (Li et al. 2014). Each test solution was pipetted (200 µl) on a piece of foam (2 cm x 2 cm x 2 cm) which was placed inside a small tube (5 cm diameter; 10 cm height) connected to the arm end of the Y-tube olfactometer. The top of the glass tube was covered with gauze and the arm of the Y-tube olfactometer was attached to the mini vacuum pump. Every 20 minutes, the test solution was applied again to reduce loss from evaporation. A flowmeter was installed between the olfactometer and the pump to maintain an air flow rate of 40 ml/min. One *T. laeviceps* bee was placed at the end of the olfactometer arm, and observed for 10 minutes. The olfactometer was used only once, after which it was cleaned and dried for further use.

Toxicity test. Toxicity tests were carried out by topical, residual, and oral applications.

***Topical application*.** The test concentrations of imidacloprid (a.i.) were 200, 20, 2, 0.2, 0.02 ppm, while the concentrations of deltamethrin (a.i.) were 187.5, 125, 12.5, 1.25, 0.125, 0.0125, 0.00125 ppm. Imidacloprid and deltamethrin insecticide formulations were diluted with acetone. Acetone was served as a control.

T. laeviceps worker bees were acclimatized before treatment by placing inside a test tube (30 mm in diameter; 200 mm in height) for one hour and anaesthetized in a refrigerator at -18°C for 3 minutes. Each concentration of insecticide (1 µl) was applied to the dorso-thorax of the bees using a micro syringe applicator (EPA 2014). Each treatment was replicated three times with 10 bees per replication. After treatment, the test bees were placed in a glass tube (10 cm in diameter, 15 cm in height) and fed with 10% honey solution. Mortality of bees was observed at 48 hours after treatment (HAT).

***Residual application*.** The concentrations of imidacloprid tested were similar to topical application test, while the concentrations of deltamethrin as follow 375, 312.5, 250, 187.5, 125, 12.5, 1.25, 0.125, 0.0125, 0.00125 ppm. Acetone was used as solvent. The bioassay method used referred to Syahputra (2001). The test solution (500 µl) was poured inside a glass tube (30 mm in diameter; 200 mm in height), then the inner surface of the glass tube was coated evenly with the test solution by rolling the glass tube thoroughly. After that, the tube was dried until the solvent evaporated. A total of 10 bees were exposed to each treated glass tube for 5 minutes, transferred into a fresh rearing tube and fed with 10% aqueous honey solution absorbed in a cotton swab. Each treatment was replicated three times. Bee mortality was observed at 48 HAT.

***Oral application*.** The concentrations of imidacloprid tested were similar to topical application test, while the concentrations of deltamethrin as follow 1250, 375, 312.5, 250, 187.5, 125, 12.5, 1.25, 0.125, 0.0125, 0.00125 ppm. The insecticides were diluted with 50% aqueous honey solution. Ten *T. laeviceps* were fasted for 1 hour before treatment in tube (10 cm in diameter, 15cm in height). Then, the bees were treated with each concentration of treatment by placing cotton swab containing each concentration of insecticides at the top of the tube. 50% aqueous honey solution was served as control. The bees were let to fed for 5 minutes. After that, all the cotton swabs containing insecticides were removed and then changed to untreated cotton swabs. *T. laeviceps* bees that had been treated were reared and fed with 10% honey solution absorbed in cotton. Each treatment was replicated three times. Mortality observations were made at 48 HAT.

Data analysis. The attractiveness data were analyzed by chi-square test using the Rstudio program. The bee mortality data were analyzed by probit analysis using the PoloPlus program to determine LD_{50} or LC_{50} values. The LD_{50} value was used for the classification of the toxicity value of the test substance on bees according to the US EPA (2014) (Table 2).

Table 2. Toxicity classification to bees based on LD_{50}

LD_{50} (µg/bee)	Toxicity classification
≥ 11	Non-toxic
11 > LD_{50} > 2	Moderately
≤ 2	Highly toxic

Source: US EPA (2014)

RESULTS AND DISCUSSION

Attractiveness of imidacloprid and deltamethrin. Bees rely heavily on their sense of smell (olfactory) for various aspects of their lives, one of which is when bees are looking for food (Paoli and Galizia 2021). *T. laeviceps* spent significantly more time in the arm connected to the honey source over imidacloprid 200 ppm and deltamethrin 12.5 ppm (Fig. 1). There was significant difference in the attraction of *T. laeviceps* for honey compared to imidacloprid 200 ppm and deltamethrin 12.5 ppm (p-value <0.01). This implies that imidacloprid 200 ppm and deltamethrin 12.5 ppm did not disturb the bees visiting behavior for honey (nectar).

Olfactometric response of *T. laeviceps* to mineral water (P2) was higher than response to either imidacloprid 200 ppm, deltamethrin 12.5 ppm or deltamethrin 12.5 ppm in 10% honey solution (p-value <0.05). However, imidacloprid 200 ppm in 10% honey solution did not show significant difference with the mineral water treatment (Fig. 1).

All P3 treatments showed that the attractiveness of imidacloprid 200 ppm and deltamethrin 12.5 ppm was not significantly different with or without honey mixtures (Fig. 1). The average percent attraction of *T. laeviceps* to the scent of imidacloprid 200 ppm was 53.3% compared to 46.7% for deltamethrin 12.5 ppm (P3).

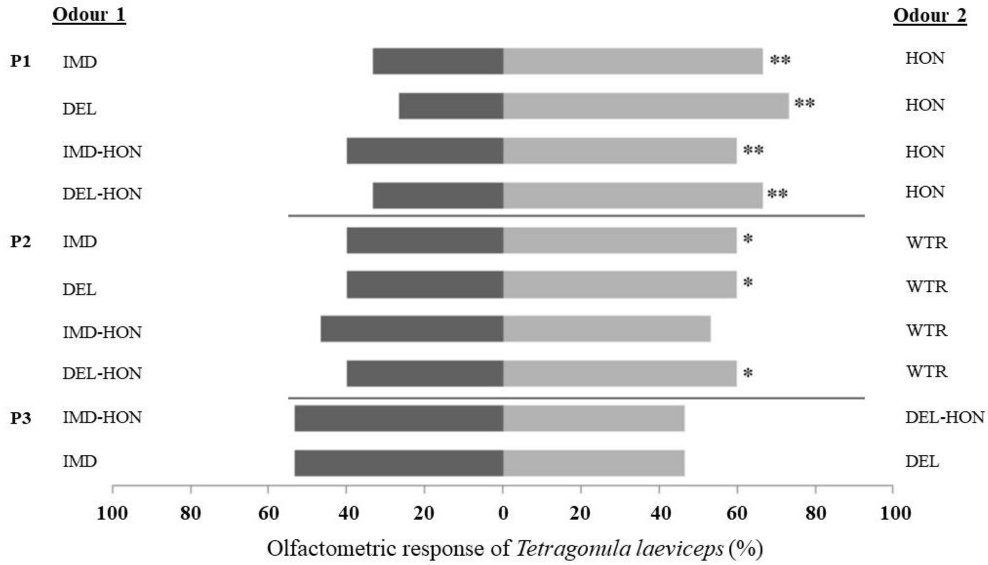

Fig. 1. The olfactometric response of *Tetragonula laeviceps* in the test using a Y-tube olfactometer with two aroma sources. (P1) insecticide -*vs*- honey, (P2) insecticide -*vs*- mineral water, (P3) imidacloprid -*vs*- deltamethrin.**= p-value < 0.01, *= p-value < 0.05 X^2 test. Description: IMD: Imidacloprid 200 ppm, DEL: Deltamethrin 12.5 ppm, HON: 10% aqueous honey, WTR: mineral water, IMD-HON: Imidacloprid 200 ppm in 10% honey solution, DEL-HON: Deltamethrin 12.5 ppm in 10% honey solution

The mixture of insecticide preparations with honey illustrates the field conditions when bees can still visit plants or flowers that have been exposed to insecticides. *Apis mellifera* and *Bombus terrestris* had a higher preference for sucrose solution containing imidacloprid and thiamethoxam than 0.5 M sucrose solution alone under constant temperature and 60% RH (bumblebees: 28 °C; honeybees: 34 °C) (Kessler et al. 2015). Honey and water are necessary for the life of *T. laeviceps* bees. Bees need honey as food and water to regulate the temperature in the hive. Bees consume processed nectar (honey) which provides energy for flight, colony maintenance, and general daily activities (Ellis et al. 2020), while water is used by bees to obtain mineral content and to regulate temperature conditions (thermoregulation) inside the hive when conditions are hot outside the hive (Stabentheiner et al. 2021).

Mortality of *T. laeviceps*. Bees can still visit plants or flowers that have been sprayed with pesticides to obtain food sources such as nectar and pollen to meet the colony's needs. As a result, bees can be exposed to pesticides through contact and oral exposures. Toxicity of imidacloprid against *T. laeviceps* in the three toxicity test methods showed a linear relationship between the concentration of the active ingredient and bee mortality (Fig. 2). 100% mortality was reached at 2 ppm for topical application, while oral and residual applications need higher concentration (20 ppm). These concentrations were far below the recommended spraying concentration (200 ppm).

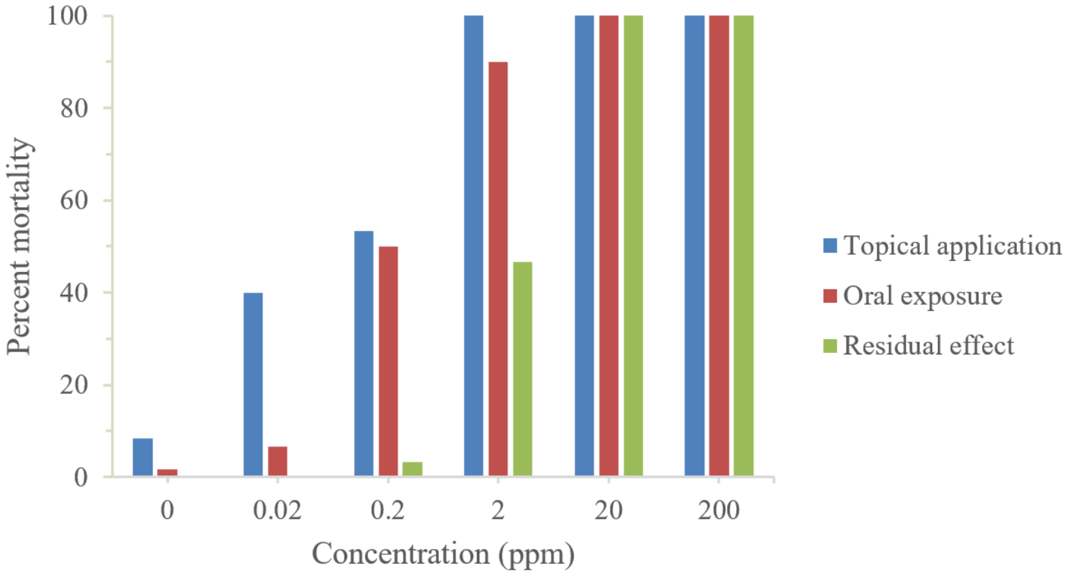

Fig. 2. Toxicity of imidacloprid to *Tetragonula laeviceps*

The exposure of *T. laeviceps* to deltamethrin by the topical application also showed a linear relationship between the concentration of the active ingredient and bee mortality, from 0.00125 to 187.5 ppm deltamethrin (Fig. 3). However, in oral application method, percent of bee mortality increased until 312.5 ppm and then decreased at higher concentrations (375 ppm). The bees stayed away from the test feed containing the insecticide deltamethrin at 375 ppm. The avoidance indicated there was a repellent effect, so bee mortality was lower when the concentration of deltamethrin insecticide reached 375 ppm (Fig. 3).

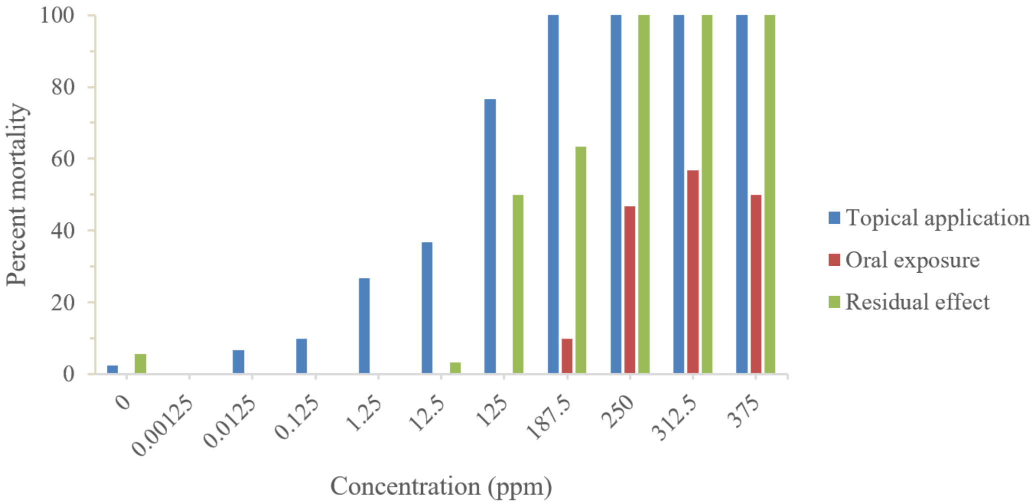

Fig. 3. Toxicity of deltamethrin to *Tetragonula laeviceps*

The mortality tests also proved the disinterest of *T. laeviceps* to deltamethrin aroma at high concentrations (375 ppm) compared to the recommended concentration (12.5 ppm). Based on the results of these tests, *T. laeviceps* was more attracted to the aroma of deltamethrin at 12.5 ppm (p-value <0.05) in five minutes (Fig. 4). It seems that high concentration of deltamethrin (375 ppm) in oral test repelled *T. laeviceps*.

Deltamethrin at concentration 0.52% has a repellent effect on *Aedes aegypti* mosquitoes, so visiting decreased (Bowman et al. 2018). A decrease in foraging of *Apis mellifera* was observed for permethrin at 8 µg/cm^2 and 4.45 µg/cm^2 (filter paper) in flight cage bioassays of bees (Rieth and Levin 1988; Ingram 2013). Deltamethrin and permethrin are an insecticide of the pyrethroid class that acts as contact and stomach poisons (NPIC 2010).

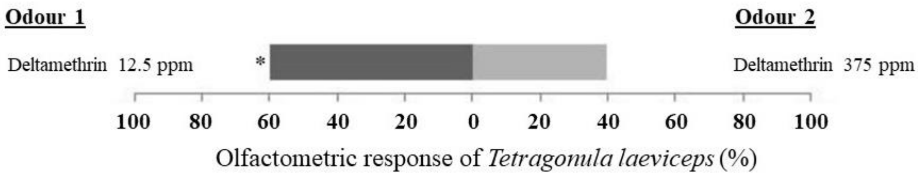

Fig. 4. The olfactometric response of *Tetragonula laeviceps* in the test using a Y-tube olfactometer between deltamethrin 12.5 ppm and deltamethrin 375 ppm aroma sources. *= p-value < 0.05 X^2 test.

Deltamethrin at low dosage (5 g/ha) was used on pollen beetle of oilseed rape, but deltamethrin had a repellent effect on honey bees (Bos and Mason 1983). Although deltamethrin has a repellent effect on bees, it does not mean that it is safe for bees. The repellent effect of pyrethroid compounds did not protect bees visiting plants after application (Rieth and Levin 1988). The repellent effect of pyrethroid compounds is estimated to last about 24 hours, so it is not long enough to prevent bees from residual contact toxicity (Rieth and Levin 1988). After contact with the pyrethroid insecticide, the bees returned to the colony and were inactive for a period ranging from less than 1 hour to a maximum of 24 hours. After recovery, normal foraging resumed and the bees continued foraging at the same site to which they were previously conditioned (Rieth and Levin 1988). At that time, the bees came in contact with the insecticide residues, demonstrating its high toxicity to bees in these topical and residual exposure.

Bees were generally more intolerant to contact (topical) application than oral or residual application. Imidacloprid (0.02 ppm) produced 40% mortality to bees by topical application, while deltamethrin (0.0125 ppm) produced 6.7% mortality (Fig. 2 and 3). The contact toxicity of imidacloprid was generally highly toxic to the *Bombus impatiens*, *Osmia lignaria*, and *Megachile rotundra*; while deltamethrin was less toxic (Scott-Dupree et al. 2009). Treatment of imidacloprid at 0.125 ppm by oral application caused high mortality (more than 45%) to *A. mellifera* (Sanchez-Bayo et al. 2017), while in this study, imidacloprid caused 50% mortality at 0.2 ppm.

Bioassays using the topical application method simulates exposure of bees to insecticides in the field (Fig. 5a). The bees treated by topical application will be exposed to insecticides much more than oral and residual applications at the same concentration so that the mortality of *T. laeviceps* treated by topical application was higher than two other methods.

Honey bee (*A. mellifera*) and stingless bee (*T. laeviceps*) are pollinators, foraging for food to feed the colony. Food source (nectar) that are exposed to insecticides will have a negative impact on bees.

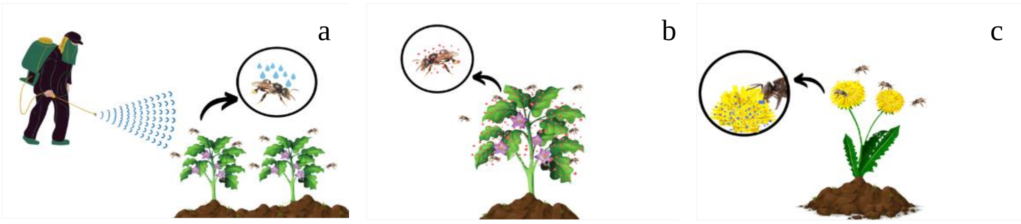

Fig. 5. A simple conceptual model for pesticide exposure in the field based on mortality testing with topical (a), residual (b), and oral application (c).

Bees exposed to imidacloprid dermally (topical and residual) or orally will die. However, other possible impacts of insecticides can harm the bee colony. When bees bring nectar or pollen contaminated with slow-acting insecticides to their hives, the entire colony will be exposed to insecticides (Traynor et al. 2021; Mubin et al. 2022b). The recommended dose of insecticides used in this study could kill the test bees. In addition, sublethal doses of imidacloprid and deltamethrin will reduce foraging activities and other activities at the hive entrance of *Apis mellifera ligustica* in field tests (Decourtye et al. 2004). The exposure of the whole bee colony is caused by trophallactic behavior or the feeding behavior of other colony members (breeding nests) (Gernat et al. 2018).

Toxicity of imidacloprid and deltamethrin to *T. laeviceps*. The toxicity of imidacloprid and deltamethrin insecticides against *T. laeviceps* varied for each type of exposure (Table 3). In general, imidacloprid was more toxic than deltamethrin as shown by the lower LC_{50} values.

Table 3. LC_{50} of imidacloprid and deltamethrin insecticides against *Tetragonula laeviceps*.

Active ingredient	LC_{50} (ppm)		
	Topical application	Residual application	Oral application
Imidacloprid	0.08	1.92	0.23
Deltamethrin	19.08	141.76	279.32

The insecticide imidacloprid acts as a competitive modulator of the nicotinic acetylcholine receptor (nAChR). Imidacloprid binds to nicotinic acetylcholine receptors causing continuous stimulation while deltamethrin works as a sodium channel modulator (IRAC 2022). Deltamethrin will slow down sodium channel closure. Both insecticides will cause the exposed bees to experience convulsions, paralysis, and eventually die.

Although deltamethrin was less toxic than imidacloprid, both insecticides were classified as harmful insecticides to bees (Decourtye et al. 2004). Imidacloprid and deltamethrin could cause a decrease in the activity of *A. mellifera* in foraging (Telangre et al. 2018; Dworzanska et al. 2020). *A. mellifera* and *B. terrestris* sometimes had less active bees than the unsprayed control in deltamethrin plots sprayed, but thiacloprid did not have any tendency to be repellent (Havstad et al. 2019). Deltamethrin interferes with the nervous system of bees, such as dance behavior. Bee workers use social learning when following the waggle dance to learn resource location and quality. Referential communication codes information and the dancer encodes the polar coordinates of a resource relative to the nest (Dong et al. 2023). Bee dance behavior (waggle dance) on bees that consume sucrose syrup with a mixture of deltamethrin insecticide were less precise than those that consumed normal sucrose syrup. The information about the direction and distance to the advertised source conveyed becomes inaccurate compared to bees that consume sucrose alone (Zhang et al. 2019). This causes bees returning to the nest to confuse other nestmates in food recruitment. Forager bees will have difficulty finding food sources from the information provided by previous workers so the pollination process will decrease due to the absence of pollinating bees on the plants.

Based on the EPA classification (2014), both LD_{50} values of contact insecticides, imidacloprid and deltamethrin, were classified as highly toxic insecticides to bees (Table 4). The most apparent effect when bees are exposed to pesticides was the death of worker bees after direct exposure (Gradis et al. 2010). However, pesticides can also cause sub-lethal effects for bees. Exposure to pesticides at sub-lethal concentrations for bees could lead to a shorter life span, behavioral changes, decreased immunity, decreased fecundity, and abnormal development. Bee production and vitality can also decrease when contaminated pollen is collected and fed (Gradis et al. 2010; Chmiel et al. 2020).

Table 4. Contact toxicity of imidacloprid and deltamethrin to *Tetragonula laeviceps* based on EPA classification.

	Imidacloprid	**Deltamethrin**
LD_{50} (µg/bee)	0.0098	0.038
EPA Classification*	Highly toxic	Highly toxic

* Toxicity based on US EPA (2014): LD_{50}< 2 (highly toxic); LD_{50} = 2-11 (moderately); LD_{50}> 11 (non toxic)

CONCLUSION

Water and honey (as food source) are more attractive than imidacloprid 200 ppm and deltamethrin 12.5 ppm for bees to visit the food sources of nectar and pollen. Thus, when farmers spray these two insecticides in chili plantations, the bees are exposed as these are exploring and looking for food sources. However, imidacloprid and deltamethrin are highly toxic to bees, both orally and dermally. Contact of bees to contaminated nectar will lead to bee mortalities, decrease in bee population and less production of cultivated plants. This information is crucial for beekeepers and chili farmers, in particular, so spraying of both insecticides should be avoided during the flowering stage of chili.

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