

**TOXICITY STUDY OF INSECTICIDE FORMULATIONS ON STINGLESS BEE
Heterotrigona itama (COCKERELL) (APIDAE: MELIPONINI)**

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ABSTRACT

Application of insecticides is a must in crop cultivation to produce high quality product in market. The excess application of insecticides is a major concern as it could harm insect pollinators, which results in broken colonies of pollinator especially stingless bee. This study aimed to determine the toxicity level of selected insecticides toward stingless bee populations. The bioassay on the toxicity level that cause mortality on stingless bee population, *Heterotrigona itama* population was assessed with five common insecticides used in rock melon cultivation, namely deltamethrin, cypermethrin, imidacloprid, abamectin and malathion. Imidacloprid exhibited the highest toxicity causing 50% mortality after 24 hours with a concentration as low as 57.53ppm. The insecticide with the lowest toxicity on *Heterotrigona itama* was malathion, where a high concentration of 500.76 ppm resulted in 50% mortality after 24 hours. Results reaffirmed that the use of highly toxic insecticides are detrimental to populations of pollinators.

Keywords: Bioassay, *Heterotrigona itama*, insecticides, mortality, toxicity level

ABSTRAK

Penggunaan racun serangga adalah kemestian dalam penanaman sayuran dan buahan bagi memastikan produk yang berkualiti tinggi di pasaran. Penggunaan racun serangga secara berlebihan boleh membimbangkan kerana ia boleh membahayakan serangga pendebunga, yang menyebabkan koloni serangga pendebunga terganggu terutamanya kelulut. Kajian ini bertujuan untuk menentukan tahap ketoksikan racun serangga terpilih terhadap populasi kelulut. Ujian bioasei pada *Heterotrigona itama* bertujuan untuk menentukan tahap ketoksikan yang menyebabkan kematian pada populasi kelulut. Populasi *H. itama* dinilai dengan lima insektisida biasa yang digunakan dalam penanaman tembikai batu dan tanaman melon, iaitu deltamethrin, cypermethrin, imidacloprid, abamectin dan malathion. Imidacloprid menunjukkan ketoksikan tertinggi dengan kepekatan serendah 57.53 ppm, mengakibatkan 50% kematian selepas 24 jam. Hasil kajian menunjukkan racun serangga yang mempunyai ketoksikan terendah terhadap *H. itama* adalah malathion, di mana kepekatan tinggi 500.76 ppm mengakibatkan 50% kematian selepas 24 jam. Penggunaan racun serangga yang sangat toksik adalah merugikan kepada populasi serangga pendebunga.

Kata kunci: Bioasei, *Heterotrigona itama*, kematian, racun serangga, ketoksikan

INTRODUCTION

The stingless bee is one of the important pollinators to keep ecological interaction sustainable and is a suitable candidate for the sexual reproduction of most plant species (Franceschinelli et al. 2013). The bee often exhibits behavior of carrying pollen grains during foraging, which serves as a key indicator of frequently visited floral sources. Melittopalynology, a subfield of palynology, focuses on studying pollen collected by bees to determine which flowers they favor most (Ghazi et al. 2018). This field provides valuable insights into bee foraging ecology and the geographical origins of pollen sources (Ponnuchamy et al. 2014). Additionally, it aids in assessing floral availability for bees and helps understand the reduction of botanical sources in certain regions.

Introduction of the stingless bee as a pollinating agent is a winning way to improve pollination of rock melon plants, although keeping stingless bee in a sustainable ecosystem is considered more important. Intensive and injudicious use of insecticides may result in abnormal behaviour, broken colonies, and a reduction in bee products, incurring economics losses (Goldman et al. 2007). The adverse effects that broad uses of insecticides have on non-target beneficial insects are major causes of decline in pollinator populations in cultivated areas (Blacquiere et al. 2012). There is widespread concern about the contribution that insecticides that may cause insect populations to decline (Martelli et al. 2020). Therefore, there is a need to determine the most suitable doses of insecticides that can be applied without harming stingless bees, among the diversity of pollinators. The indicator species should be sufficiently sensitive to detect even small amounts of insecticides and should express the response with increasing concentrations (Chhillar et al. 2007).

The bioassay method used is direct bioassay which is acute toxicity test. Test organisms are exposed to various concentration of the substance, and mortality or severe effects are observed over a short period (usually 24-96 hours). The most common type of toxicity test is the acute mortality test is usually conducted to obtain information about the median lethal concentration (LC₅₀). The median lethal concentration (LC₅₀) is the concentration that has been shown to cause death in 50% of a tested group of animals. It serves as an initial assessment of

toxic manifestations and is one of the first screening experiments typically performed with all compounds (Saganuwan 2011). The most widely used pesticide among organophosphates, which were touted as an eco-friendly alternative to organochlorines, is glyphosate. Other insecticides that are well-known in this class include dimethoate, parathion, malathion, and others with a history of endocrine disruption (Kaur et al. 2024). Therefore, the objective of this study was to measure the immediate toxic effects, LC₅₀ on rock melon cultivation using several insecticide formulations namely Deltamethrin, Cypermethrin, Imidacloprid, Abamectin and Malathion.

MATERIALS AND METHODS

Study Area

The study was conducted at the Entomology Laboratory, Department of Plant Protection, Faculty of Agriculture (2.986365868626896, 101.735318574435) Universiti Putra Malaysia, Serdang, Selangor, under controlled condition at room temperature of 24°C with dark and light ratio of 1:1 (12 hours:12 hours)

Sample Preparation

Individuals of the stingless bees (*Heterotrigona itama*) were collected from hives located at the Integrated Farm (Ladang 10), UPM, Serdang, Selangor. This activity was done at 8 am to 9 am, during this time the stingless bee were active moving flying in and flying out from hive for foraging activities. Only active and healthy foragers was chosen for subsequent treatments. Healthy foragers can be described by healthy colonies, which have crowded and lively colonies. A plastic bag was used to catch the foragers. The entrance or landing pads of the stingless bees were covered with a plastic bag and stingless bees that came out from hives were collected. Stingless bees caught were transferred into plastic cages and acclimatized for one (1) hour at the farm before being taken to the laboratory. In each plastic cage, twenty (20) stingless bees were kept and fed with stingless bee honey. The bees were acclimatized for one (1) day in the laboratory to ensure that stingless bees were healthy when treatments were applied.

Preparation of Insecticides

Before serial dilutions of the insecticides were prepared, preliminary tests were conducted based on concentrations from previous studies. Initially, the common insecticides namely deltamethrin, cypermethrin, imidacloprid, abamectin and malathion were diluted with distilled water to prepare the stock solution. Using the stock solution, a serial dilution was prepared according to the concentrations used (5 different concentrations including control (distilled water was used as control) as stated in Table 1. The dilutions were prepared starting from the highest concentration to the lowest concentration (450ppm to 45ppm) to avoid technical errors during the experiment.

Table 1 Insecticides concentration used as treatments

Insecticide	Concentration Used (ppm)				
Deltamethrin (D)	0	45	90	135	180
Imidacloprid (I)	0	45	55	65	75
Cypermethrin(C)	0	50	100	150	200
Abamectin (A)	0	100	200	300	400
Malathion (M)	0	150	250	350	450

Insecticides Toxicity on Stingless bee

Bioassay using filter paper-dip method was conducted following procedure of Sayyed et al. (2008) with some modification in of concentration of insecticides used. The bioassays were conducted on healthy stingless bees. Five different insecticides with five different concentrations were prepared. Five replicates were prepared for each concentration, each replication 500 stingless bee was used, 100 stingless bees for each insecticide and 20 stingless bees for each cage that consist of five concentrations for each insecticide. A filter paper was dipped into an insecticide solution with a stated concentration and placed on a corrugated aluminium foil to allow air drying for approximately 2 hours. The filter paper was put into the cage and exposed to the stingless bees. Mortality of the stingless bees after 24 hours and 48 hours of exposure to pesticides was recorded.

Data Collection

The numbers of *H. itama* mortality were counted and recorded in series of of LC₅₀ values correspondingly. The data mortality of stingless bees was collected after 24 hours and 48 hours.

Data Analysis

The percentage of mortality and corrected mortality was computed following Abbott (1925).

$$\text{Percentage mortality} = \frac{\text{total number of dead stingless bee}}{\text{total number of stingless bees}} (100)$$

$$\text{Corrected mortality} = \frac{\text{mortality in treatment} - \text{mortality in control}}{100 - \text{mortality in control}} (100)$$

Probit analysis was used to analyze the mortality data of the stingless bee, conducted using POLOPLUS software (LeOra Software 2003) to determine the LC₅₀, 95% fiducial limit (FL) and the slope ($\pm$ SE) of the log-dose probit line.

The different concentration of each treatment was analyzed using Analysis of Variance (ANOVA) and mean comparison using Tukey Post Hoc test at 5% of level significant. The experimental design implemented was complete randomized design (CRD). Further analysis on data were conducted using SAS 9.4 version software.

RESULTS AND DISCUSSIONS

The Effect of Toxicity of Insecticides Against Stingless Bees

There are multiple routes of exposure to insecticides that affect the stingless bees, in direct contact with an insecticide solution the most common ones. It is assumed that its response also depends on intrinsic factors, including ecology, biology, physiology, and morphology, as well as extrinsic factors including climate and flora. Oral LC₅₀ is a measurement that allows one to obtain the lethal concentration of an insecticide in a cage containing stingless bees.

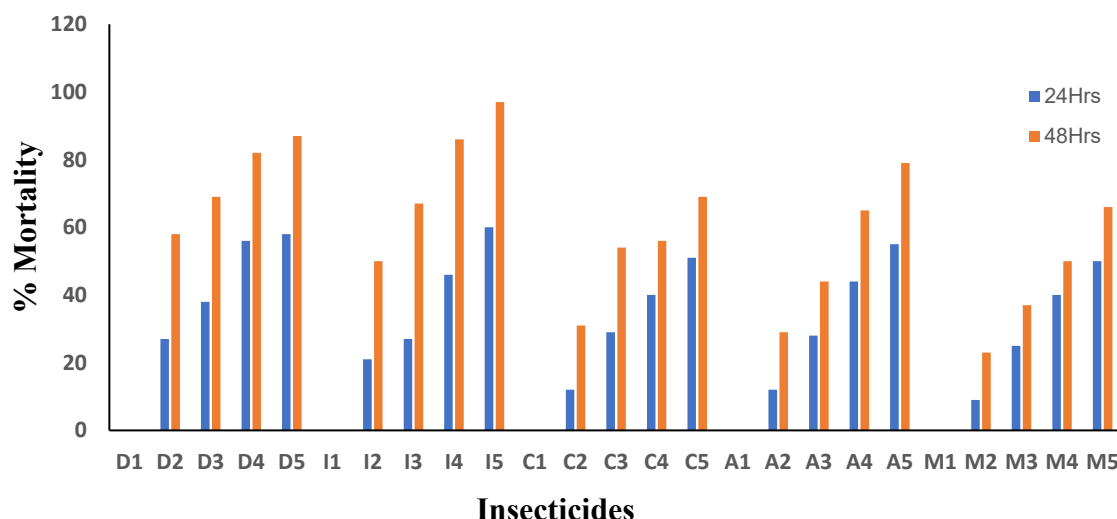


Figure 1 Mortality percentage of stingless bee against insecticides

The mortality percentage of stingless bees after 24 and 48 hours exposed to the insecticides is has shown in Figure 1. Imidacloprid incurred more than 50% of stingless bee mortality after 48 hours of exposure. At the concentration of 65 ppm (I4), 46% of mortality was recorded, while at 75 ppm (I5) mortality was record is 60%. Malathion caused the lowest mortality percentage, which is 50% of mortality at 450 ppm (M5) of concentration, which is the highest concentration applied on stingless bee. Deltamethrin incurred 58% mortality at a concentration 180 ppm (D5). Abamectin showed the result of 55% mortality at a concentration 400 ppm (A5) and cypermethrin showed 51% mortality at a concentration 200 ppm (C5).

At the lowest concentration used of imidacloprid and deltamethrin at 45 ppm, the mortality of the stingless bee in deltamethrin was higher than imidacloprid which was 27% and 21%, respectively. However, higher concentrations were needed to kill the insects compared to imidacloprid. Similarly, malathion, cypermethrin, and abamectin required higher concentrations to cause 12% mortality. Based on observations, the mortality of stingless bees increased with higher concentrations of insecticides used. Higher concentrations of insecticides exposed to stingless bees increased toxicity, making them harmful to the bees.

Among the five insecticides evaluated, imidacloprid proved to be the most toxic, causing the highest level of mortality. Even at low concentrations, imidacloprid resulted in significant mortality of *H. itama*. Imidacloprid, which mimics nicotine, a naturally occurring compound in plants like tobacco, is known for its insecticidal properties. It is available in various formulations such as liquids, granules, and dusts, and is widely used not only in crop production but also for controlling sucking insects, termites, certain soil insects, and fleas on pets.

Oral exposure to residues, or products translocated by the plants present in the pollen or nectar of planted or associated plants within or at the crop border and nearby crops, all the exposed aerial applications, the application of granules to the soil, and seed treatment or the absorption of residues from previous grown crops are considered highly hazardous (Godfray et al. 2014). Imidacloprid disrupts nerve function by preventing normal signaling, effectively causing the nervous system to malfunction. Gervais et al. (2010) highlighted that imidacloprid is particularly toxic to insects and other invertebrates compared to mammals and birds,

primarily because it binds more effectively to receptors in insect nerve cells. As a systemic insecticide, imidacloprid is absorbed by plants through the soil or leaves and then disperses throughout the stems, leaves, fruits, and flowers. This systemic action ensures that insects feeding on these treated plants inevitably ingest the imidacloprid, which damages their nervous systems and leads to their eventual death.

Table 2 Effects of prolonged insecticides exposure in 24 hours based on LC₅₀ and 95% CI values of five insecticides on *H. itama*

Insecticide	LC ₅₀ (ppm)	95%CI (ppm)	Slope±SE	X ² (0.05)
Deltamethrin	92.98	65.63–124.85	1.05±0.56	1.17
Imidachloprid	57.53	55.11–60.75	6.33±0.56	2.54
Cypermethrin	184.99	153.77–248.77	1.82±0.65	1.43
Abamectin	262.59	198.21–464.28	1.77±0.71	0.93
Malathion	500.76	399.07–822.85	1.68±0.94	1.84

Table 2 above shows lethal concentrations (LC₅₀) at 24 hours of insecticide exposure to stingless bees. Deltamethrin LC₅₀ is 92.98 ppm, lower than the previous study by Del Sarto et al. (2014), which is 134 ppm. However, LC₅₀ for imidacloprid was 57.53 ppm, similar to the previous study. Cypermethrin LC₅₀ showed higher concentration compared to Hassan Iqbal et al. (2012) that is 184.99 ppm and 110 ppm, respectively. The LC₅₀ of abamectin was not different from the LC₅₀ from the previous study by Groten et al. (2001), that is, 262.59 ppm compared to 250 ppm. In comparison to the malathion previous study, the LC₅₀ was at 500.76 ppm, which was higher than 300 ppm, as reported by Johnson et al. (2010).

Comparing the results of this study with those obtained by the mentioned authors, it is observed that *H. itama* are more susceptible than species used in previous study. When *A. mellifera* was exposed topically to fipronil, Vidau et al. (2011) obtained an LC₅₀ of 417 ppm, which shows that both species are more susceptible than the domestic bee. In relation to imidacloprid, Valdovinos-Nuñez et al. (2009) obtained a topical LC₅₀ of 440ppm to *Nanotrigona perilampoides*, which reflects a susceptibility, approximately six times greater than *H. itama*. Even so, the topical LC₅₀ reported by Zhu et al. (2015) on *A. mellifera* was equivalent to 410ppm, greater than the obtained value to *H. itama*. Finally, the research carried out by Tomé et al. (2015) showed that Imidacloprid is lethal in doses used in the field for the stingless bee, as has also been observed in the present study, where thiamethoxam and fipronil were lethal to all individuals of the two species, when exposing the bees to the doses used during field application.

This type organophosphate insecticide was widely used in agricultural settings to manage diverse insect pests (Gervais et al. 2010). Available in various forms such as liquids, dusts, wettable powders, and emulsions. Malathion is employed not only to control mosquitoes but also to target insects affecting fruits, vegetables, landscape plants, and shrubs (Bassett et al. 2019). It can also be formulated in products for ant control, as well as tick and flea treatments for pets. Malathion's insecticidal action hinges on disrupting the nervous system. In a healthy insect, nerves communicate through chemical messengers, which are halted by an enzyme to stop the signal. Malathion, however, binds to this enzyme, preventing it from functioning, which leads to an unending transmission of nerve signals (Gallo & Lawryk 2005). This

continuous signaling interferes with the insect's normal movement and breathing, eventually leading to death (Petruzzello et al. 2014).

Malathion is significantly less toxic than other insecticides used. The World Health Organization classifies neonicotinoid insecticides, like imidacloprid, as insecticides with acute toxicity, whereas malathion is categorized as an unclassified insecticide with low acute toxicity. Malathion has been widely applied in public health campaigns to control mosquitoes and other disease vectors, as well as in agriculture to manage crop pests. It is commonly used as a spray with protein hydrolysate or incorporated into yeast bait to target fruit flies (Matthews 2018). In many cases, low doses of organophosphates like malathion have shown reduced hazards to honeybees over time (Suchail et al. 2001). Among five insecticides used, malathion showed the minimal used of insecticide not harmful to the small insect especially pollinator such as stingless bee.

Table 3 Effects of prolonged insecticides exposure in 48 hours based on LC₅₀ and 95% CI values of five insecticides on *H. itama*

Insecticide	LC ₅₀ (ppm)	95%CI (ppm)	Slope±SE	X ² (0.05)
Deltamethrin	46.54	34.24–56.57	2.17±0.56	2.20
Imidacloprid	39.09	32.91–43.01	6.25±1.77	1.62
Cypermethrin	63.87	56.06–73.25	2.71±0.63	2.01
Abamectin	146.26	124.10–185.32	2.23±0.62	2.82
Malathion	141.65	106.91–168.02	2.66±0.97	4.31

Table 3 above indicating the reading of LC₅₀ of insecticides treated on stingless bee after 48 hours. LC₅₀ of Imidacloprid after 48 hours was 39.09 ppm, after long exposure to insecticides with lower concentrations able to cause mortality of *H. itama*. LC₅₀ of deltamethrin after 48 hours was 46.54 ppm, which was slightly higher than imidacloprid. The LC₅₀ of cypermethrin was 63.87ppm, and abamectin at 146.26 ppm and malathion at 141.65 ppm. Imidacloprid showed the highest toxicity towards stingless bee colonies, in contrast to abamectin, imparting the lowest toxicity to stingless bees after 48 hours' exposure.

Imidacloprid is part of the neonicotinoid family, a significant group of neurotoxins that act as antagonists to the nicotinic acetylcholine receptors (nAChR) in insects (Elbert et al. 2008). These types of insecticide application method and route of entry involve initial deposition on leaves and pollen grains, followed by translocation throughout plant tissues, making the plant toxic to insects and potentially other organisms that touch or consume it (Tomlin 2000). In previous study by Laurino et al. (2011) neonicotinoids have been shown to be highly toxic to *Apis mellifera*. While study by Mario et al. (2014) stated that with contact exposure to Imidacloprid proving harmful and toxic to smaller insects. The stingless bee, being significantly smaller than the honeybee, is particularly vulnerable to this insecticide, rendering even brief exposure perilously toxic.

The Toxicity Evaluation Induces by Insecticides on the Stingless Bee

Results showed that there was a highly significant differences among the treatments (F=179.75; df=24,200; $P<0.0001$). The interaction between hours and treatments was highly significant (F=11.77; df=24,200; $P<0.0001$). Table 4 below stated that, imidacloprid with 75ppm at 24 hours was highly toxic than other with mean separation (12.00±1.04^a), followed by

deltamethrin at 180 ppm and 135 ppm (11.60 ± 0.93^{ab} and 11.20 ± 1.06^{abc}). The other three, were abamectin at 400 ppm, cypermethrin at 200 ppm and malathion at 450 ppm was highly toxic than the rest of treatments.

This study indicates that the various insecticides concentrations have different effects on the mortality of *H. itama*. It was obviously also evident that neonicotinoid insecticides affected *H. itama* even when not taken orally. Research by Tomé et al. (2015) revealed that field-level doses of imidacloprid proved lethally potent for another stingless bee species *Scaptotrigona xanthotricha*. Similarly, in the present study, exposure to field-applied doses of thiamethoxam and fipronil resulted in complete mortality across all individuals of both studied species, underscoring the devastating impact of these insecticides on bee populations (Quiroga-Murcia et al. 2017). Insecticide exposure is not limited to compounds directly applied to stingless bees; foraging bees that contact contaminated plant surfaces can transfer these effects to their colonies as well (Gill et al. 2012).

Due to its high-water solubility, imidacloprid poses a unique risk, potentially exposing insects that consume pollen, nectar, and plant exudates, while also impacting aquatic insects in runoff water (DiBartolomeis et al. 2019). Although lower levels of imidacloprid have been detected in pollen, honey, and beebread samples (Nguyen et al. 2009), high insecticide concentrations are known to be fatal to foragers, and even sublethal doses can disrupt essential colony functions (Decourtye et al. 2005). For stingless bees, insecticide exposure can also impair flight and foraging behavior, critically affecting their ecological role.

The toxicity of insecticides for 48 hours showed that imidacloprid 75 ppm (I5) was significantly higher than deltamethrin at 180 ppm (D5) and imidacloprid 65 ppm (I4). The mean separation was 19.40 ± 0.24^a , 18.40 ± 0.24^a and 17.20 ± 0.37^a , respectively. The rest of the concentration was not significantly different compared to the I5, I4 and D5. However, even the toxicity was lower than these three insecticides concentration, the LC_{50} is also major concern in determining the appropriate used of insecticides in rock melon cultivation.

The results reported corroborates with the findings of Carvalho et al. (2009) for the insecticide lufenuron. Acetamiprid and deltamethrin were of medium toxicity to bees when ingested. According to Ramirez-Romero et al. (2005), in addition to causing mortality, deltamethrin may significantly affect the ability of foraging. The authors also verified that deltamethrin caused reduction in the movements of the remaining bees, impairing locomotion and feeding. When sprayed directly on bees acetamiprid, deltamethrin, and cartap exhibited higher toxicity. However, abamectin was extremely toxic to *Apis mellifera* when ingested, as reported by Carvalho et al. (2009). The toxicity of the pyrethroid deltamethrin was also reported by Nica et al. (2004), Rhodes et al. (2006), and Carvalho et al. (2009).

Table 4. Means comparison between the concentration used in each treatment replications

Treatments	Mortality (Mean $\pm$ SE)	
	24 hours	48 hours
D1	0.00 ± 0.00^j	0.00 ± 0.00^i
D2	5.40 ± 0.51^{gh}	11.60 ± 0.81^{cde}
D3	7.60 ± 0.51^{efg}	13.80 ± 0.66^{bc}
D4	11.20 ± 1.06^{abc}	16.40 ± 0.24^{ab}
D5	11.60 ± 0.93^{ab}	18.40 ± 0.24^a

I1	0.00±0.00 ⁱ	0.00±0.00 ⁱ
I2	4.20±0.37 ^{hi}	10.60±0.32 ^{def}
I3	5.40±0.24 ^{gh}	13.40±0.67 ^{bc}
I4	9.20±0.37 ^{bcd}	17.20±0.37 ^a
I5	12.00±1.04 ^a	19.40±0.24 ^a
C1	0.00±0.00 ^j	0.00±0.00 ⁱ
C2	2.40±0.24 ^{ij}	6.20±0.73 ^{gh}
C3	5.80±0.58 ^{fgh}	10.80±0.73 ^{cde}
C4	8.00±0.54 ^{def}	11.20±0.37 ^{cde}
C5	10.20±0.37 ^{abcd}	13.80±1.01 ^{bc}
A1	0.00±0.00 ^j	0.00±0.00 ⁱ
A2	2.40±0.24 ^{ij}	5.80±0.37 ^{gh}
A3	5.60±0.51 ^{fgh}	8.80±0.37 ^{fgh}
A4	8.80±0.37 ^{cde}	13.00±1.00 ^{cd}
A5	11.00±0.44 ^{abc}	13.00±1.00 ^{cd}
M1	0.00±0.00 ^j	0.00±0.00 ⁱ
M2	1.80±0.20 ^{ij}	4.60±0.40 ^h
M3	5.20±0.37 ^{gh}	7.40±0.24 ^{fgh}
M4	8.00±0.31 ^{def}	10.00±0.32 ^{def}
M5	10.00±0.32 ^{abcde}	13.20±0.37 ^c

Note: Means separation with the same letter(s) within the column are not significantly different (Tukey's test $P < 0.05$)

Imidacloprid testing initially utilized a straightforward systemic treatment method involving a filter paper dip in insecticides solution for indicating applying insecticides to leaves, which quickly became a standard for assessing systemic insecticides (Cahill et al. 1996). Typically, bioassay methods for adult mortality involved using clip cages on plants or excised leaves, with insecticide applied by direct drenching indicating systemically through the leaf petiole (Smith & Giurcanu 2013). Bioassays have been conducted using treated leaves in petri dishes or cup containers. Leaf petiole treatments can result in high absorption of systemic insecticides, potentially skewing mortality data upwards compared to field applications. However, the literature does not clearly indicate whether systemic (root or petiole) treatments or leaf dips significantly alter insecticide response values. Our results suggest that both application routes through the root/petiole or directly on the leaf yield similar adult mortality rates.

Excessive insecticide use can result in significant forager mortality and, in severe cases, the collapse of entire colonies. Numerous agricultural chemicals have been detected in pollen, underscoring the need for rigorous assessments of their impact on bees as essential pollinators (Soldà et al. 2013). Bees encounter toxic chemical residues through direct contact when gathering pollen in the field or within the stored pollen in the comb. Exposure also occurs through chronic dietary ingestion of contaminated nectar, honey, and pollen. Foragers, though primarily consuming nectar directly from flowers rather than stored honey, carry nectar in their honey stomachs and process it into honey, often handling more nectar than they actually consume (Sanchez-Bayo & Goka 2014). Certain insecticides further exacerbate this risk; for instance, deltamethrin exposure causes epithelial cells to become contiguous, and abamectin exposure leads to cell merging within the epithelial layer, ultimately jeopardizing colony survival (Aljedani 2017). This cellular damage threatens the health and stability of stingless bee colonies.

CONCLUSIONS

The LC₅₀ of each insecticide commonly used in rock melon cultivation was determined. Imidacloprid showed the most toxicity than other insecticides; even at low concentration, it was able to kill nearly 100% of *H. itama* samples. The LC₅₀ of imidacloprid is 57.53 ppm and 39.09 ppm at 24 hours and 48 hours, respectively. Malathion exhibited the lowest toxicity to *H. itama* amongst the insecticides evaluated, with LC₅₀ 500.76 ppm and 141.65 ppm at 24 hours and 48 hours, respectively. The higher toxicity concentration among the treatments was determined, where imidacloprid at 75 ppm imparted the highest toxicity. The use of imidacloprid as an insecticide should be avoided in rock melon cultivation to maintain sustainability of the stingless bees.

Insecticides are emerging micropollutants in the environment and can have adverse effects on human health. In the recent decade, the production and use of insecticides has escalated due to unscrupulous farmers scrambling to increase crop production. Thus, proper education is necessary for farmers and consumers on the uses of insecticides and their optimal doses to control insects, to avoid overdosage of insecticides. In addition, implementation of existing regulations can contribute to the reduction of the insecticides deleterious effects on beneficial insects and human health.

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Conflict of Interest

The authors declare that they have no conflict of interest.

Ethics Declarations

No ethical issue required for this research.

Data Availability Statement

My manuscript has no associated data.

Authors' Contributions

Siti Asma' Samsudin (SAS) and Nur Azura Adam (NAA) conceived this research, Siti Asma' Samsudin (SAS) and Nur Azura Adam (NAA) designed the experiments; Siti Asma' Samsudin (SAS), Mohamad Syukri Tan Shilan (MSTS) and Wan Nur Asiah Wan Mohd Adnan (WNAWMA) do the research and collect the samples from the field. Siti Asma' Samsudin (SAS), Nur Azura Adam (NAA), Norida Mazlan (NM), and Puteri Edaroyati Megat Wahab (PEMW) participated in the interpretation of the data. Siti Asma' Samsudin (SAS) wrote the paper and participated in the revisions of it. All authors read and approved the final Manuscript.

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