EFFECT OF RESIDUAL TOXICITY OF SELECTED INSECTICIDES TO THE MALAYSIAN STINGLESS BEE *HETEROTRIGONA ITAMA*

KESAN SISA TOKSIK RACUN SERANGGA PEROSAK KEPADA LEBAH KELULUT MALAYSIA *HETEROTRIGONA ITAMA*

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ABSTRACT

Heterotrigona itama is a Malaysian stingless bee species that actively reared for meliponiculture. This stingless bee is cultivated in a commercial scale for its honey production, propolis and among the greatest commercial potential as crop pollinators. However, this species has been potentially exposed to agronomic practices, among which the use of synthetic insecticides against pests. The indirect toxicity effect of the post-insecticide had affected the mortalities of *H. itama* especially, to the foragers. Due to that, a study has been conducted to determine the lethal concentration of 50% (LC50) and 95% (LC95) of the selected insecticides against stingless bee forager workers through residual exposure. The bioassay test was conducted to the local stingless bee *H. itama* at Agricultural Research Station, Tenom. Four commonly used insecticides in crop protection; Deltamethrin, Chlorpyrifos, Cypermethrin and Malathion were tested at five concentrations that diluted with 500 ml of distilled water in three replications for each insecticide. Lethal concentrations (LC50 and LC95) were obtained from probit analysis after 1-hour dry residues exposure and 24-hour mortality observation. The result shows that; all four tested insecticides were harmful to *H. itama* through dry residue. Deltamethrin shows the higher value of LC50 (1.256 ml) and LC95 (3.582ml) that make it less toxic to the *H. itama* than cypermethrin, malathion, and chlorpyrifos, however, as the concentration gets higher it becomes more toxic.

Keywords: Heterotrigona itama, Insecticides, Dry residues, Lethal concentration, Toxicity

ABSTRAK[2]

Heterotrigona itama merupakan spesis lebah kelulut yang aktif diternak (meliponikultur) di Malaysia. Kelulut ini diternak secara komersil untuk madu, propolis, dan merupakan calon terbaik ejen pendebungaan tanaman. Namun, spesis ini berpotensi besar terdedah dengan penggunaan racun serangga kimia yang disembur pada permukaan tanaman. Kesan sisa toksik selepas pengunaan racun serangga telah memberi kesan terhadap mortaliti *H.itama* khususnya kepada pekerja lebah kelulut. Oleh itu, kajian bioassay telah dilakukan untuk mengenal pasti sisa kering 50% kepekatan kematian (LC50) dan 95% kepekatan kematian (LC95) racun serangga terhadap pekerja lebah kelulut di Stesen Penyelidikan Pertanian, Tenom. Empat racun serangga yang sering digunakan: Deltamethrin, Chlorpyrifos, Cypermethrin dan Malathion, diuji pada lima kepekatan yang berbeza yang dilarutkan bersama dengan 500 ml air suling serta direplikasi sebanyak tiga kali bagi setiap jenis racun. Kepekatan (LC50) dan (LC95) dianalisa mengunakan analisa probit selepas satu jam pendedahan sisa racun serangga terhadap lebah kelulut dan 24 jam pemerhatian mortaliti. Hasil dapatan menunjukan, kesemua racun serangga yang dipilih adalah berbahaya kepada lebah kelulut melalui pendedahan sisa toksik kering. Deltametrin menunjukkan nilai LC50 (1.256 ml) dan LC95 (3.582 ml) yang tinggi, menjadikan racun serangga tersebut kurang berbahaya berbanding racun serangga

yang lain. Walaubagai manapun, semakin tinggi konsentrasi racun maka semakin tinggi kadar toksik dan kematian terhadap pekerja *H. itama.*

Kata kunci: Heterotrigona itama, *racun serangga, sisa toksik, kepekatan maut, Toksik*

1. Introduction

The native Malaysian stingless bee (*Heterotrigona itama*) belong to the order of Hymenoptera (Apidae: Meliponini) is one of the actively reared bee species and most utilised in meliponiculture practice in Malaysia since 2012 until now (Mohd Fahimee, *et al*., 2016). At present, the stingless bee is enthusiastically cultivated for its commercial scale of honey production, propolis and among the greatest commercial potential as crop pollinators. The ability of this stingless bee to pollinate varieties of crops (Slaa *et al*., 2006; Heard, 1994) such as fruit trees and vegetable, that make it suitable to be cultivated in orchard and garden.

However, the foraging activity of pollinator on desired agricultural crop after the insecticide treatment, is the main cause that expose them to agricultural insecticides (Cauich *et al*., 2004). Insecticides are mainly used to control and eradicates the population insect pest existed in the planting area, but, the post-insecticide effect also affecting the non-targeted insect (Aktar *et al*., 2009) which in this case the cultivated stingless bee. According to Renzi (2013), the residual toxicity can occur when the bee foraging at the post-treated area. It is because the insecticides may persist on the plant surface and can completely maintain their toxic characteristics after dried.

Previously, research and studies had been published on the toxicity effect of insecticides to the non-targeted organisms which use *Apis melifera* (honey bee) as an indicator species of ecotoxicity (DiBartolomeis *et al*., 2019). Because *A. melifera* is responsible for the pollination of several important crops and honey production. Unfortunately, the result of insecticide toxicity tested for this species only cannot represent for the other beneficial insects because of the different behavior response, defence mechanism and traits of different insect species. Honey bee and stingless bee shared the same family, however, both come from different tribes which can affect the toxicity result even from the same bioassay procedure and insecticide tested (Dorigo *et al*., 2019). Meanwhile, stingless bee also played a similar role as honey bees that make them vital in the agricultural environment.

Although stingless bee is not the target of insecticide compounds, they may have foraged in the contaminated area and highly vulnerable to the contamination (Tome' *et al*., 2015). This study provide informations to guide and help the farmers in their selection of insecticides. Thus, the aim of this study was to identify the (lethal concentration) LC50 and LC95 of selected insecticides through residual exposure to the *H. itama*.

2. Material and Method

2.1. *Insect*

This study was conducted at the Agriculture Research Station (ARS) Laboratory, Tenom with a controlled room temperature of 27±2°C and 70% relative humidity. The stingless bee used in this study was obtained from *H. itama* colony kept in ARS bee breeding yard before being examined in the laboratory. Only adult forager workers were chosen for the insecticide treatments that caught during morning hour (8.00 am – 11.00 a.m) by using the sweeping net. The collected stingless bees were directly transported to the laboratory and anaesthetised by chilling for 50 seconds at 3 °C to facilitate ease of handling. The chilling method was used with slight modifications as recommended by Thomas and Phadke (2017). During the inactive period of the stingless bee, only healthy, uninjured and uniform sizes were selected for the further experiment.

Figure 1: *Heterorigona itama* forager collecting pollen on *Antigonon leptopus*

2.2. *Insecticide*

Four formulated insecticides were tested in this experiment. The selection of insecticide is based on the common insecticide used by the farmer in controlling agricultural pests. Therefore, four formulated insecticides were tested in this experiment that were Deltamethrin, Chlorpyrifos, Cypermethrin and Malathion as summarised in Table 1. These insecticides were previously tested on serious orchard pest *Bactrocera dorsalis* fruit fly (Wang *et al.*, 2013). Based in Table 1 showed, all four selected insecticides shared a similar mode of action through contact and stomach of contaminated insects.

Table 1: Information of test insecticides

Active ingredient	Class	Mode of action	Group of insecticide
Deltamethrin 1.4% w/w	III	Contact and stomach	Pyrethriod
Chloropyrifos 21.2% w/w	II	Contact, stomach and respiratory action	Organophosphate
Cypermethrin 5.5% w/w	III	Contact and stomach	Pyrethriod
Malathion 84.0% w/w	III	Contact and stomach	Organophosphate

The selected insecticides were examined at five dose concentrations which prepared according to the commercial formulated products guidelines. One of the five concentration was prepared based on the recommended dosages used in the field. The insecticide was diluted with distilled water as a solvent to obtain mortality in the range of 50-100% (Table 2). The control treatment was served with distilled water alone. Replications were made three times with ten stingless bees for each treatment with to obtain precise mortality data.

Table 2: Five different concentrations of insecticides tested against *Heterotrigona itama*

Insecticide	Recommended concentration (mL/10L of water)	Concentration (mL/500 mL of water)				
		C1	C2	C3	C4	C5
Deltamethrin	22.0	1.65	1.40	*1.1	0.83	0.56
Chloropyrifos	6.0	0.60	*0.30	0.15	0.08	0.04
Cypermethrin	10.0	2.00	1.00	*0.5	0.25	0.125
Malathion	22.0	2.20	*1.10	0.55	0.275	0.137

*Note: *Recommended rate in unit of ml/L of water.*

2.3. *Laboratory Residual toxicity bioassay*

Laboratory bioassay is defined as the test where living organisms were exposed to various concentrations of a toxicant or to effluent dilutions under laboratory conditions. Residual toxicity was determined by using dry-contaminated glass jar with insecticide. In this method, five desired concentrations of insecticide were diluted with distilled water. The dilution was coated inside the glass jar by rotating it several times to ensure the diluted insecticide was fully coated and left to dry at room temperature for at least 2 hours. After the jar was completely dried, 10 selected stingless bees from the anaesthetised procedure were introduced in the jar for 1 hour. As for the control jar, it was coated with distilled water and allowed to dry before introducing the stingless bees. Five concentration of diluted insecticide were tested with three replications each. After 1 hour, the stingless bees were transferred into the post cage (9x9x9 inches), where they were fed with 50% sucrose solution diet. Mortality was observed for 24 hours and data were analysed using Probit analysis (SPSS Statistic 21) to obtain the value of LC50 and LC95 for each tested insecticide

2.4. *Statistical analysis*

Mean ANOVA was used in the determination of mortality mean based on the three replications of the experiment. Mortality data was assessed after 24 hours and analysed using Probit Analysis (SPSS Statistic 21) to calculate the LC50 and LC95 values for each tested insecticide.

3. Result and Discussion

Table 3 indicates the mean mortality of *H. itama* foragers after being treated with test insecticides at a recommended concentration based on the product guidelines. Tested concentrations were prepared at the small amount to represent the real concentration diluted

with water. Results from the studies indicated that *H. itama* was vulnerable to all tested insecticides.

Table 3: Mean mortality rate for foragers of *Heterotrigona itama*, treated with test insecticides at recommended concentration

Insecticide	Mean Mortality ± S.E	Recommended insecticide concentration (mL/10L)	Tested concentration (mL/500mL)
Deltamethrin 25.0% w/w	3.00±0	22	1.1
Chlorpyrifos 21.2% w/w	6.33 ± 0.88	6	0.3
Cypermethrin 5.5% w/w	7.00±0.58	10	0.5
Malathion 84.0% w/w	10± 0	22	1.1

Figure 1 depicted the mean mortality of *H. itama* against the tested insecticides. Deltamethrin shows the lowest mortality rate of the foragers by killing at least 30% of the tested bees at recommended concentration by the contact of dry residue. Chlorpyrifos and cypermethrin are two times toxic that deltamethrin which display the mortality rate between 6 to 7 bees. Based on four tested insecticides, malathion was observed to be the most toxic to *H. itama* by killing 100% of the tested organisms. As for the control group, the distilled water was completely safe and has not affected *H. itama* foragers by maintaining 100% mean survival rate.

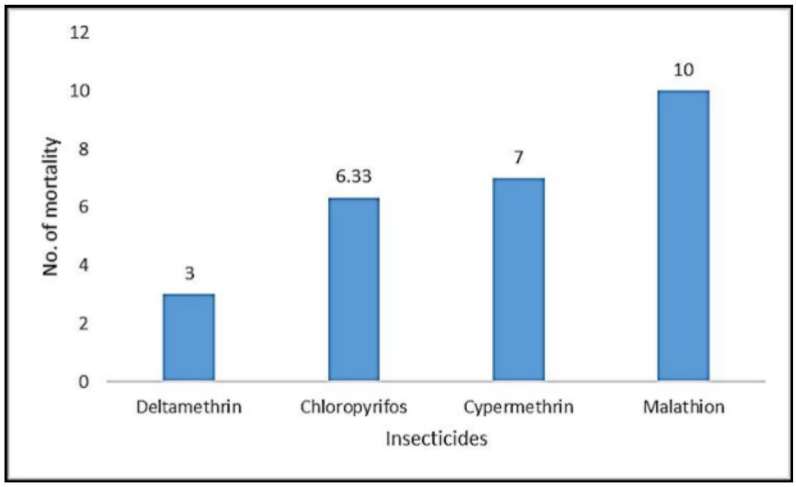

Figure 2: Bar chart of mean mortality of *H. itama* at recommended concentration

Table 4, shows the values of LC50 and LC95 with their 95% fiducial limits (FL) after 3 replications on each tested treatment and 24 hours' mortalities observation. LC50 was used to estimate the values of insecticide that can cause 50% killed of the tested organism. In this studies, LC50 values of the foragers were compared among the test insecticides, it was found that deltamethrin gave the highest value of LC50 compared to the other selected insecticides. It shows that deltamethrin was 1.256 ml (0.978-2.022 ml) 3.99 times less toxic than chlorpyrifos for dry residue to the *H. itama* foragers. cypermethrin 0.315 ml (0.189-0.568 ml) was slightly more toxic, followed by malathion 0.225 ml (0.164-0.304 ml) and chlorpyrifos

0.09 ml (0.049-0.138 ml) that is the most toxic. As for the LC95 value were, deltamethrin 3.582 ml (2.147-41.094), cypermethrin 3.362 ml (0.884-10.873 ml), chlorpyrifos 0.468 ml (0.258-2.469 ml), and malathion 0.447 ml (0.324-1.261 ml). LC95 was a values of insecticide that toxic to the tested organism which killed almost 100% of the tested organism.

Table 4: LC50 and LC95 values of deltamethrin, chloropyrifos, cypermethrin and malathion.

Insecticide	No. of sample per replication	Time (hour)	LC50	LC95	X^2
Deltamethrin	10	24	1.256 ml (0.978 – 2.022)	3.582 ml (2.147-41.094)	1.441
Chlorpyrifos	10	24	0.09 ml (0.049-0.138)	0.468 ml (0.258-2.469)	0.555
Cypermethrin	10	24	0.315 ml (0.189-0.568)	3.362 ml (0.884-10.873)	3.455
Malathion	10	24	0.225 ml (0.164-0.304)	0.447 ml (0.324-1.261)	0.325

In present study, residual exposure was used to evaluate the toxicity responses to deltamethrin, cypermethrin, chlorpyrifos and malathion against *H. itama*. As predicted, it is found that the mortality results for contact residual of insecticides on pollinator *H. itama* was varied among the tested insecticides. In addition, the different insecticide group also affected the mortalities values, in this experiment the tested insecticides were from organophosphate and pyrethroid, which belong in class II and III. In this experiment, the insecticide classes with its active ingredients varying in their sensitivity to different environmental conditions (Tomlin 2009); therefore, differences results obtained in residual bioassay test were expected. *H. itama* was susceptible to the toxicity of insecticides treatment on residual contact.

Observed susceptibility of the tested organism to insecticides can vary based on bioassay methods (Paramasivam & Selvi, 2017). In this study, only residual bioassay has been conducted against *H. Itama*, to compare the insecticides toxic effects on foragers. The pollinator has the behavioural traits of walking on plant surfaces on considerable amount of time of searching for pollen, latex, and water (Cutler *et al.,* 2014), due to that, residual exposure bioassay method is representative of the main insecticide exposure path for this insect. Insecticides can also have life-stage specific effects, but in this research, only adults' foragers were included due to the higher potential of getting contamination to the insecticide during foraging (Xavier *et al.,* 2015). Contact exposure on dry residues of selected insecticides (deltamethrin, chlorpyrifos, cypermethrin and malathion) were considered lethal to *H. itama*. The result revealed, deltamethrin was less toxic than the other tested insecticides, however, the higher the insecticide concentration applied would increase the rate of mortality (Tosi and Nieh, 2019) to the affected pollinator. In this study, chloropyrifos was very toxic to *H. itama* because it showed the lowest LC50 (0.09 ml) and LC95 (0.468 ml) values. Dorneles *et al*., (2017) also got the same result that revealed chlorpyrifos to be the most toxic among the two insecticides (chloropyrifos and phosmet) to both stingless bee (*Scaptotrigona bipunctata* and *Tetragonisca fiebrigi*) species.

However, the toxicity results of controlled environment and in real environment against tested organism were different. A study by Araujo *et al*., (2017) confirm that the toxic effect of residual post-insecticide will degraded overtime because of the positive correlation between the residual period and abiotic factors (wind, rain and temperature). Meanwhile, in the laboratory experiment all of the abiotic factors were remain constant.

The results in this study indicated that chloropyrifos, cypermethrin, malathion were hazardous to *H. itama* population; therefore, there are severals propose measure highlighted

to reduce the insecticide impact on beneficial insect. Indentifying the characteristic and using insecticide with lower toxicity, applying intergrated pest management (IPM) practice by minimizing the usage of insecticide as pest control, avoiding application during foraging and crop blooming time, and alert the presence of bee's colonies existed naturally or managed colony (Rocha, 2012; Dornelesetal *et al*., 2017).

4. Conclusion

The present study, showed that *H. itama* is susceptible to the application of insecticides (deltamethrin, chlorpyrifos, cypermethrin and malathion) for dry residues exposure. Thus, we suggest that further analyses of the toxicity effect of other insecticides including botanical insecticide be carried out on stingless bee species. This research work is important in providing information to the agricultural producer, educators, students and public awareness about the hazardous of insecticide usage, especially to the non-targeted organism.

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