

Article

# Food reward and distance influence the foraging pattern of stingless bee, *Heterotrigona itama*

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**Abstract:** Stingless bee beekeeping provides new opportunities to improve the incomes of many households in Malaysia through the sale of honey and other bee products. While *Heterotrigona itama* is one of the most commonly cultured species of stingless bees, its behavior is not very well understood. Hence, we conducted this study to investigate the behavior of *H. itama* in exploiting food sources by ascertaining the nectar sugar concentration preferred by the bee. We also aimed to determine the preferred distance of food source from the bee hive. Our results suggest that *H. itama* prefers high sugar concentrations of 35% and above, and they would fly up to 7 m from the hive to collect food. We discuss how nectar concentration and food distance influence the number of bees exploiting food sources and the overall foraging pattern of *H. itama*.

**Keywords:** Foraging activity; food exploitation; sugar nectar concentration; tropical species; meliponiculture; bee farming

## 1. Introduction

The stingless bee is a eusocial insect which belongs to the Family Apidae and the tribe Meliponini [1–3]. The behavior of the stingless bee is dynamic in exploiting plant-based resources [4] such as nectar, pollen, resin, latex, leaves, scents, oil and seed during their foraging flight [5]. Besides plant-based food sources, the stingless bee is also known to collect other sources, mainly for nest construction, such as animal feces, water and clay [5]. There are at least 600 species of stingless bees in the world classified under about 60 genera, as compared with only 11 species of honeybees in a single genus, *Apis* [6]. Because of their high diversity, many species of stingless bees are still under study. Each species may have its own specific behavior and requirements that need to be understood not only to facilitate their culture for honey collection, but also to ensure sustainability of the colonies especially in the farming areas.

Meliponiculture, or stingless bee keeping, has been gaining more attention in Malaysia recently. It provides new opportunities to increase household income, especially of rural folks, through the sale of honey and other bee products such as bee bread and propolis. About 35 species of stingless bees (tribe Meliponini) have been identified in Peninsular Malaysia [7]. Among these, *Heterotrigona itama* and *Geniotrigona thoracica* are the most popular species in meliponiculture [8]. Previous studies have been carried out on the foraging behavior of various stingless bee species, for example *Hypotrigona gribodoi* and *Meliponula ferruginea* [9], *Melipona panamica* [10], and *Tetragonula biroi* [11]. However, the foraging behavior of *H. itama* remains largely unknown and a comprehensive

study in this respect was, therefore, undertaken. With meliponiculture becoming an important economic resource for many households not only in Malaysia but also in other tropical countries [12–13], an intensification of research on stingless bees to understand their foraging behavior is appropriate. This would help maximize honey production and also to ensure that their colonies remain healthy.

Different species of stingless bee behave differently [14]; they have their own specific requirements and preferences in food resources. In general, the concentration of sugar in nectar collected by the bees is between 35% and 65% [11, 14]. Different species may have a preference for nectar of different sugar concentrations. For example, *M. fasciata*, *M. compressipes triplaris*, *M. fuliginosa* and *M. marginata* collect flower nectar with sugar concentrations ranging from 21% to 60% [15]. *Hypotrigona gribodoi* has been recorded to prefer nectar with 14.2% to 67.4% sugar concentration while *M. ferruginea* collects nectar with sugar concentrations ranging between 9.1% and 63.4% [9]. Another study on the sugar preference by *M. fasciata* revealed that this species might exploit flower nectar with 15% sugar concentration but would switch to nectar of a higher sugar concentration (95%) when the latter was available [16].

Distance from food source affects foraging behavior of stingless bees. As the bees recruit foragers to an area rich with food sources [10], some species may show a significant preference towards food located at a certain distance. Jarau et al. [1] found that *M. scutellaris* foraged 30 m from their nests while *M. quadrifasciata* preferred to forage 40 m from their nest. Other species such as *T. biroi* preferred to exploit food source located close to (1 m) their colonies [11].

The stingless bee *Trigona iridipennis* has two peak hours of foraging activity, from 09:00 h until 11:00 h, and from 15:00 h until 16:00 h [3], while the peak foraging hour for *Geotrigona subterranean* is between 13:00 h and 14:00 h [17]. Another stingless bee *Plebeia remota* carries out peak foraging activities at different times according to the season. In its reproductive diapause, nectar foraging centers around noon while it is around 10:00 h during its reproductive phase [5].

Previous studies have shown that different species of stingless bees exhibit their unique behaviors in exploiting food sources. Our study was conducted to examine how sugar concentration preference and distance from food source influenced the foraging pattern of *H. itama*. This study focused to help beekeepers and farmers to better arrange bees colonies and the food sources around the farming areas. Hence, the experimental set-up in this study was designed to be suitable for such areas. We are aware that the bees foraging behaviour may be quite different under their natural environment where the food sources occurred naturally and scattered in the wild. We aimed to answer the following questions: (1) What sugar concentration would the stingless bee *H. itama* prefer? (2) What is the preferred foraging distance? and (3) How do variations in sugar concentration and food distance affect the foraging pattern of *H. itama*?

We hypothesized that (a) the stingless bee *H. itama* would choose higher concentrations of sugar solution (better reward) compared to lower concentrations, (b) *H. itama* would prefer to forage food sources nearer to the nest as compared to those further away, and (c) *H. itama* would begin foraging in the morning from around 08:00 h. with the peak foraging activity between 10:00 h and 12:00 h; foraging activity would cease from the afternoon (13:00 h).

The information gained from this study would be helpful to agriculturists and stingless bee keepers in deciding the position of bee hives in relation to food sources on their farms as well as in

crop fields [11]. This study can also enhance stingless bee keepers' knowledge of the specific behavior of *H. itama*, thus helping them sustain the bee colonies.

## 2. Materials and Methods

### 2.1. Study site

This study was carried out at Universiti Malaysia Terengganu (UMT), located in Kuala Nerus, Terengganu (5.406020N, 103.086918E).

### 2.2. Experimental design

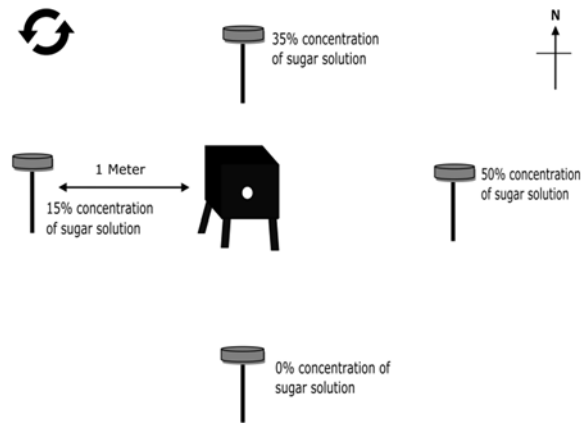
Two experiments were conducted: 1) sugar concentration preference; 2) foraging distance. The experimental set-up for Experiment 1 and Experiment 2 were modified from the previous set-up by Ciar et al. [11]. Ten well-established colonies of *H. itama* were used in the two experiments. A repeated measure design was used in this study. The ten colonies of *H. itama* were used alternately to minimize the number of colonies involved in the experiments. Each colony was used not more than three times throughout the experiments.

#### Experiment 1: Sugar concentration preference

Four feeder stands were placed 1 m around the trial bee colony following Ciar et al. [11] (Figure 1). The four feeders were placed in four cardinal directions to the colony, i.e. north, south, east and west. The height of the feeders was set up according to the height of the bee colony's entrance tube, which was about 1 m from the ground.

The trial bee colony was moved from the rearing site to the experimental area a day before each trial was conducted to familiarize the bees to the experimental environment. During the actual trial day, the trial colony was presented with four different sucrose water concentrations (0%, 15%, 35%, and 50%) (HmbG® Chemicals, Johor Bahru). Four Petri dishes were placed on the four different feeder stands. The four Petri dishes contained 15 ml of saline water (0%) and the other three Petri dishes contained 15 ml sugar (sucrose) solutions of 15%, 35%, and 50% concentration. Each Petri dish was layered with a cotton pad to prevent the stingless bee from drowning in the Petri dish. The solutions in the Petri dishes were refilled every three hours. The placements of Petri dishes were also rotated clockwise every three hours to reduce bias. In total, ten colonies were used in Experiment 1.

A picture of bees visiting each feeder were taken three times every hour (Figure 2) for eleven hours using a digital camera (Canon IXUS 185, Canon) and the number of bees landed on the feeders were counted using ImageJ 1.32j software (Wayne Rasband, National Institute of Health, USA). Physical parameters such as temperature (°C) and relative humidity (% Rh) were recorded during each experiment. The physical parameters were recorded using a 3-in-1 Hygro-Thermo-Anemometer.



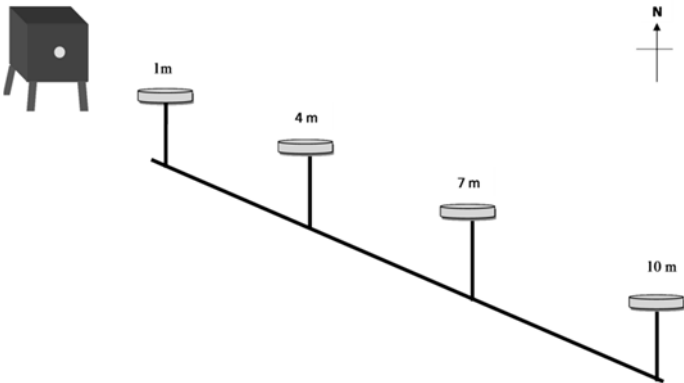
**Figure 1:** The experimental set up for experiment 1. The set-up was a modified version from Ciar et al. [11]. Four Petri dishes contained water (0 %), 15 %, 35 % and 50 % sugar (sucrose) concentration were placed around the colony according to the four cardinal direction; North, South, West, East. The feeder stands were placed 1 m from the trial colony. The Petri dishes were rotated clockwise every three hours. The grey dot on the bee hive represents the colony entrance (faced to the East during the actual experimental trials).



**Figure 2:** Pictures of bees visiting each feeder were taken three times per hour. The red circles show marks left by *H. itama* on the cotton layer soaked in a sucrose solution.

*Experiment 2: Foraging distance*

Experiment 2 began two weeks after the last trial in experiment 1 was conducted to ensure bees did not have memory of the previous experiment. The preferred sugar solution from the result in experiment 1 was used in experiment 2. Fifteen ml of 50% sugar solution in Petri dishes was placed on feeder stands aligned at four different distances. Feeders were placed at a distance of 1 m, 4 m, 7 m, and 10 m from the trial colony. The number of bees visiting feeders was recorded three times per hour for twelve hours.



**Figure 3:** The experimental set up for Experiment 2 was modified from the method used in Ciar et al. [11]. Four Petri dishes contained 50 % sugar (sucrose) concentrations were placed on the feeder stands at four different distances (one meter, four meter, seven meter, and ten meter) in front of the colony entrance. The grey dot on the bee hive represents the entrance of the bee colony. The colony entrance and feeder stands were placed facing East towards the sunrise during the actual experimental trials.

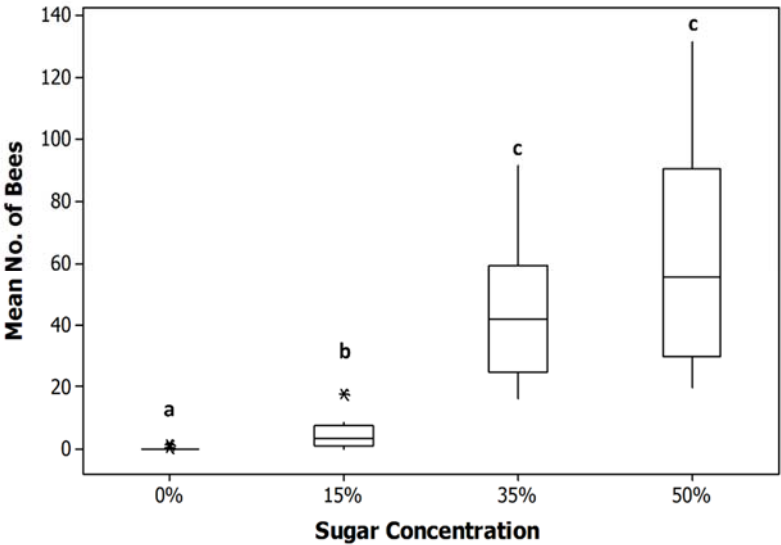
2.3. Statistical analysis

The Shapiro-Wilk normality test showed that our data were not normally distributed even after transformation. Hence, the Kruskal-Wallis test followed by all pairwise multiple comparisons was performed to analyze the difference in the mean number of stingless bee visits in Experiment 1 and 2. We then divided the foraging times into three time bands, i.e. morning (MR, from 07:00 h until 10:40 h; midday (MD, from 11:00 h until 14:40 h) and late afternoon (LA, from 15:00 h until 18:00 h). We conducted Generalized Linear Modeling (GLM) to determine the difference in the number of bees visiting different feeders at different time bands. The final GLM for Experiment 1 included sugar concentration, time bands and interaction between concentration and time bands as predictor variables. For Experiment 2, the full GLM included distance, time bands, and interaction between distance and time bands as predictor variables. For foraging pattern analysis, we pooled the number of bees visiting feeders in each hour and conducted the Kruskal-Wallis test, followed by all pairwise multiple comparisons.

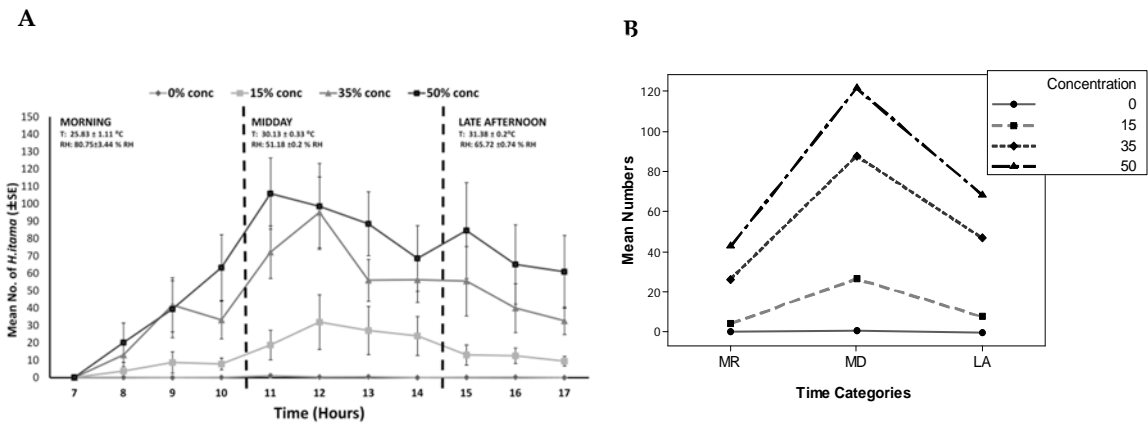
3. Results

3.1 Sugar (sucrose) concentration preference

There was a significant difference in the mean number of stingless bee visiting feeders containing four different sugar concentrations (Kruskal-Wallis test  $H_{(2)}=15.57$ ,  $P<0.01$ ; Figure 4). Further analysis revealed that this significant difference was between 0% versus all concentrations and between 15% versus 35% and 50%. However, the number of bees visiting the 35% and 50% sugar concentrations was not significantly different ( $P>0.05$ ; Figure 4). The number of bees visiting feeders with different concentrations in different time bands (i.e. morning, midday and late afternoon) was also significantly different (GLM=  $F_{2,108}=10.67$ ,  $P<0.001$ ; Figure 5A) but the interaction between time band and sugar concentration was not significant (GLM=  $F_{6,108}=2.04$ ,  $P=0.067$ ; Figure 5B). This indicates that although the number of bees exploiting the 35% and 50% sugar concentration feeders was higher throughout the day (Figure 5B), they seem to be collecting more from the 50% sugar concentration sample during midday (Figure 5A,  $p<0.05$ ).



**Figure 4:** Mean number of *H. itama* visited the four different sucrose concentrations, 0%, 15%, 35% and 50%. The box demonstrates the inter-quartile range (IQR), horizontal line in the box represents the median value of the data. Whiskers extend represent the maximum and minimum value of the data. The asterisks symbolizes the outlier data. Note that the similar letter indicates no significant difference (Kruskal-Wallis test followed by all pairwise multiple comparisons,  $P>0.05$ ,  $N=10$  for all variables).



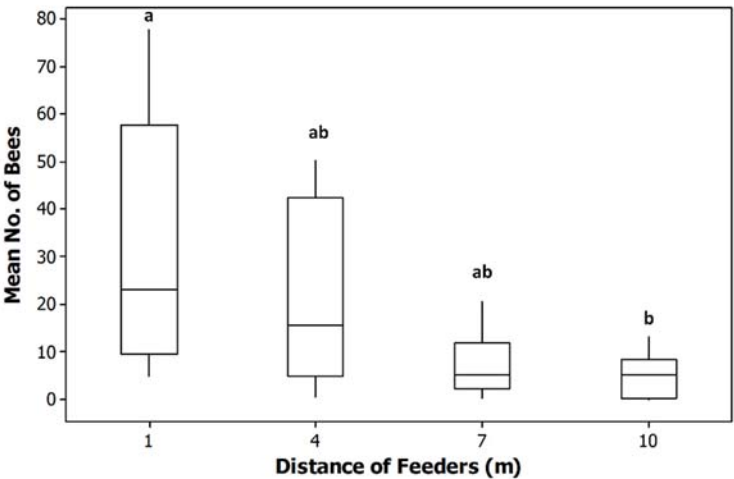
**Figure 5: A)** The mean number of stingless bee, *H. itama* visited the different of sugar concentration (0%, 15%, 35% and 50%) at different time in 11 hours. **B)** The interaction plot of the mean number of bees visiting feeders with different sucrose concentrations across the three time categories. The eleven hours of the experiment were divided into three time categories i.e, morning (MR; 7:00 h - 10:40 h); midday (MD; 11:00 h - 14:40 h) and late afternoon (LA; 15:00 h - 17:40 h). The alphabet, T, in the figure depict the mean temperature; RH depict the mean of relative humidity.

3.2 Food Distance

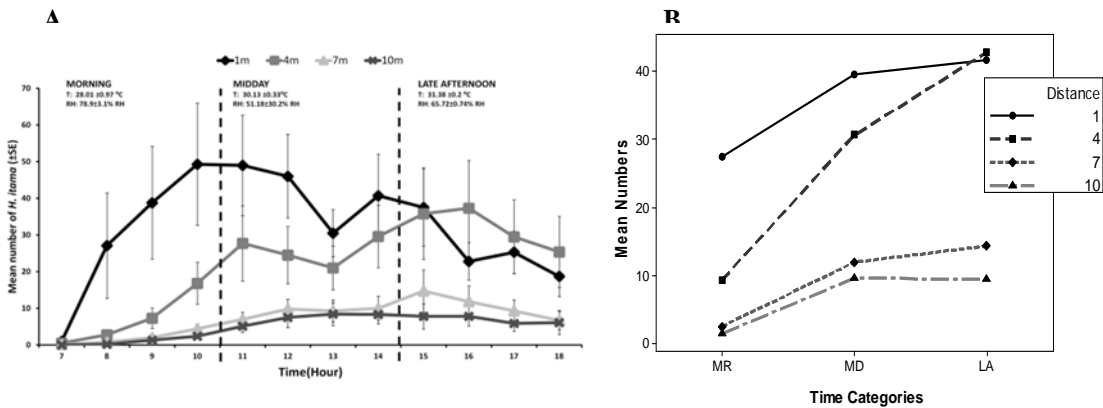
There was a significant difference in the number of bees visiting feeders at different distances (Kruskal-Wallis test;  $H_{(3)}= 12.62$ ,  $P=0.006$ ; Figure 6). Further analysis revealed that only the feeder placed one meter from the bee hive showed a significant difference compared with the feeder placed at 10 m from the bee hive ( $p=0.01$ ). There was also a significant difference in the number of bees



visiting feeders at different distances across the three time bands (i.e. morning vs midday vs late afternoon; GLM=  $F_{3,108}=11.14$ ,  $p<0.01$ ; Figure 7A). Bees visiting the feeder placed one meter from the hive were much higher in number during the midday and late afternoon ( $p<0.05$ ) but there was no effect of interaction between the number of bees visiting feeders at different distances across the three different time bands; GLM=  $F_{6,108}=0.60$ ,  $p=0.731$ ; Figure 7B).



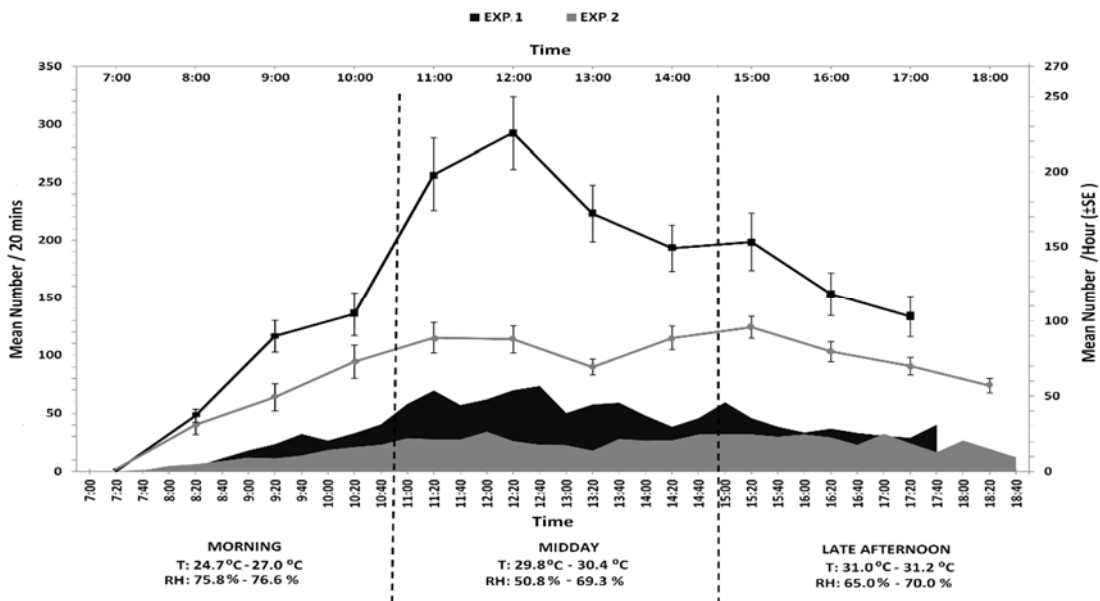
**Figure 6:** Mean number of *H. itama* visiting feeders at different distances; 1m, 4m, 7m and 10m. The boxes show the Inter-Quartile Range (IQR), horizontal line in the box represents the median value of the data. Whiskers are lines running outside of the box to the maximum and minimum value of sample. The asterisks symbolizes the outlier data. The similar letter indicates no significant difference (Kruskal-Wallis test,  $P > 0.05$ ).



**Figure 7:** A) The mean number of *H. itama* visited feeders at different distances (1m, 4m, 7m, and 10m). B) The interaction plot of the mean number of bees visiting feeders at different distances across the three time categories. The time of visitation were categorized into; morning (MR, 7:00 h -10:40 h), midday (MD, 11:00 h -14:40 h) and late afternoon (15:00 h -18:40 h). The alphabet, T, depict the mean temperature  $\pm$  (SE); RH depict mean relative humidity.

3.3 Foraging pattern

Based on the number of foraging bees in the first experiment (Experiment 1: sugar concentration preference), the number of foragers that visited the different sugar concentration feeders (0 %, 15 %, 35 % and 50 %) increased significantly from 07:00 h to reach a peak at noon ( $H_{(10)}=30.40$ ,  $p<0.01$ , Figure 8). In the second experiment to examine the effect of distance of food source from the nest, the same pattern of foraging was observed; the number of bees exploiting the food sources at different distances increased from 07:00 h but in this experiment the foraging activity reached its peaked at 11:00 h ( $H_{(11)} = 39.53$ ,  $p<0.0$ , Figure 8). In both experiments, the number of foragers dropped after midday (from 12:20 h to 13:00 h). The number of foraging bees continued to decline until late afternoon (17:40 h) in Experiment 1. In Experiment 2, the number of bees exploiting the food sources gradually increased again from 14:00 h to reach a second peak at 15:00 h before cessation from 16:00 h until dusk.



**Figure 8:** Foraging pattern of *H. itama* based on the data collected in Experiment 1 (different concentration) and Experiment 2 (equal concentration). The experimental period for Experiment 1 was eleven hours while Experiment 2 was twelve hours at field.

#### 4. Discussion

We looked into the food exploitation of *Heterotrigona itama* by presenting different concentrations of sucrose solutions and by placing the same sugar solutions at different distances from the nest. We then compared the foraging pattern of the stingless bee between the two experimental set-ups.

Food with 50% of sugar concentration was expected to be preferred by *H. itama* as compared with lower sugar concentrations [16]. However, our results showed that the number of *H. itama* foragers exploiting the 35% and 50% of food concentrations were not different, indicating that this species may not differentiate between flowers with nectar concentrations of 35% and above. Bees are known to choose and exploit the most profitable food sources even though both of the food sources are found concurrently [18]. Moreover, for meliponini bees, the higher nectar concentrations ranging



between 20% and 60% are preferred [14]. Preference for high sugar nectar may be determined by nectar-drinking habits. While certain species of bees may drink the nectar via active suction, many other species employ a viscous dipping technique [19]. In nature, nectar with low concentration (35% and lower) is suitable for the active suction drinker (for example the orchid bee), while a higher nectar concentration (50% to 60%) is better suited to the viscous dipping types [19]. Since *H. itama* employs a viscous dipping technique, a much higher nectar concentration would be advantageous. However, bees also have their optimal nectar concentration preference according to their body sizes [14]. Body size plays an important role in foraging the food sources [20-21]. Larger bees prefer higher concentrations of nectar while smaller bees favor lower concentrations of sugar in nectar [20]. For example, *Trigona muzoensis*, with a 6 mm body size, prefers nectar sugar concentration of 30% and its optimal concentration is 60% while the 9 mm *Melipona beechei* has an optimal nectar sugar concentration preference of 65%. In our study on *H. itama*, with its body size of about 5 mm, the preferred sugar concentration ranged from 35% to 50% and the optimal concentration for this species is most likely about 60%. Moreover, our result also showed that highest number of *H. itama* exploited the highest sugar concentration (50%) during the midday as the environmental temperature was rising. This may be a strategic decision made by the bees since dipping into high sugar concentration enables them to collect much of the sugar water in a short time [19, 22], and hence they could save their energy while foraging in the heat.

The body size of the bee may also affect its foraging distance, with stingless bees of different sizes having varying abilities to forage far from their nests. For example, *M. mandacaia*, a small forager, tends to travel only short distances whereas the larger *M. mandacaia* can wander further when foraging [21]. In the case of *H. itama*, body size may be a reason for its foraging range being only about 7 m, with most bees of this species preferring to exploit food sources that are nearer (1 m away) than those from feeders further (10 m) away. Recruitment of nestmates to a food source located a considerable distance away is also problematic because the journey is energy-costly while not always profitable or worth the effort [23]. Nevertheless, under certain circumstances in nature, bees may travel long distances from their hives to seek food [24].

Foraging activities of bees can be influenced by many factors such as temperature, food source availability, and colony condition. Although almost all species of diurnal bees are active from early morning with their foraging activity usually peaking around 11:00 h to 12:00 h [5, 25-26], we found that food reward may change this pattern for *H. itama*. In this study, when food with different sugar concentrations were presented near to the colonies (1 m around the colonies), the foraging activity of the stingless bees increased from morning until about 12:00 h, slowly ceasing after that. High numbers of foragers visited the feeders when the food sources were near the hive. However, when higher concentrations of food sources were presented to the colonies at different distances, the total number of bees visiting all the feeders was smaller, but two peaked foraging hours were recorded in the morning (10:00 h until 12:00 h) and in the afternoon (14:00 h until 16:00 h). Two peaked foraging hours have also been recorded for *Trigona iridipennis* that showed a similar pattern with the first peak foraging hour in the morning (09:00 h to 11:00 h) and the second in the late afternoon (15:00 h -16:00 h) [3]. Our result also showed a sharp decline in the foraging activity during midday from 12:00 until

13:00, probably because of the high temperature (exceeding 30°C). Foraging in high environmental temperature requires more energy and may cause dehydration among the foragers.

Stingless bees are known to leave chemical marks on or near the food sources [11, 27]. We observed *H. itama* leaving obvious marks on the feeder (Figure 2) in both our experiments. We are in agreement with [27] (on *T. hyalinata*) and [11] (on *T. biroï*) that these chemical marks were left by the earlier foragers as a communication strategy used by the stingless bee to recruit other nestmates to exploit the same food source(s). Hence, the number of bees visiting the specific feeder(s) increased over time.

## 5. Conclusions

Our study showed that *H. itama* prefers nectar with 35% to 50% sugar concentration. As the environmental temperature rises, many bees prefer exploiting the highest sugar concentration. While availability of food near the bee hives enables many foragers to exploit the food sources, foraging activity drops as the environmental temperature increases towards mid-day. From the early afternoon, active foraging resumes where food with high sugar concentration (better reward) is abundant. Overall, our results show that nectar concentration and food distance influence the number of bees exploiting food resources and the overall foraging pattern of *H. itama*. The results from this study can help stingless bee beekeepers understand the behavior of stingless bees so that they can make good decisions about the arrangement of hives in relation to distances to food sources, especially in the small crop field.

**Author Contributions:** Conceptualization, N.B; Methodology, N.B; Validation, N.B.; Formal Analysis, N.B. S.N.R. and N.A.S.M.K.; Investigation, S.N.R. and N.A.S.M.K; Writing-Original Draft Preparation, S.N.R; Writing-Review & Editing, N.B; Visualization, N.B and S.N.R; Supervision, N.B.; Project Administration, N.B.; Funding Acquisition, N.B.

**Funding:** This research was funded by the Malaysian Ministry of Higher Education (MOHE) under the Research Acculturation Grant Scheme (RAGS), vote number [57125].

**Acknowledgments:** We would like to thank the Ministry of Higher Education (MOHE) for supporting this research under the Research Acculturation Grant Scheme (RAGS, vote no: 57125) and the School of Marine and Environmental Sciences, Universiti Malaysia Terengganu for laboratory facilities.

**Conflicts of Interest:** The authors declare no conflict of interest.

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