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Research

Foraging Activity and Preference of Pollen Sources by Stingless Bee, Heterotrigona itama in Tropical Lowland Forest of Terengganu

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ABSTRACT

Stingless bees (Hymenoptera: Apidae: Meliponini) are a highly diversified group of eusocial corbiculate bees distributed in tropical and subtropical areas. However, studies on the relation of stingless bee activeness with environmental parameters and pollen source preference are very limited particularly from the lowland forest of East Coast Peninsular Malaysia. Hence, this study aimed to determine the relationship between stingless bee, *Heterotrigona itama* foraging activity with environmental factors (temperature, humidity, and light intensity) and preference of floral sources in a meliponiary of Marang, Terengganu. The foraging activities of five healthy colonies of H. itama were observed from 0700 to 1700 hours on hot sunny days for eight months of study period (August 2020 – September 2021). The foraging activity was enumerated using camera recording for 10 minutes at one-hour intervals (0700-1700 hours). In this study, the peak time for *H. itama* to go forage was observed at 0900 until 1100 hours in the early morning and declined rapidly from 1100 until 1700 hours. October 2020 recorded the highest activeness of stingless bees, followed by August 2021 and September 2021. There was a significant difference in the interaction of stingless bee activeness between time and months [$F67,424 = 3.498$, $p<0.05$]. However, temperature, humidity and light intensity did not significantly influence the activeness of stingless bees. Acacia mangium was the most frequent pollen grain collected by *H. itama*, followed by *Melastoma malabathricum* and other pollen types were considered minor pollen types (<15%). Findings from this study can help beekeepers to understand the foraging behaviour of stingless bee colonies and the preference of the available pollen resources for sustainable meliponiculture management practice.

Key words: Environmental factors, foraging activity, *Heterotrigona itama*, pollen, tropical forest

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INTRODUCTION

Meliponiculture commonly known as stingless bee beekeeping is vigorously developed and getting more interest due to being economically important, particularly in Malaysia (Rattanawannee & Duangphakdee, 2020; Jalil *et al*., 2022). Stingless bees are eusocial insects belonging to the corbiculated bee group within the Meliponini tribes and family of Apidae (Smith-Pardo & Engel, 2011). Stingless bees are distributed in tropical and sub-tropical climates such as South America, Southeast Asia, Africa, and Australia (Hrncir *et al*., 2016). More than 500 species of stingless bees worldwide have been described so far (Souza *et al*., 2021). Sixty species were found in the Indo-Malayan region of Asia (Al-Hatamleh *et al*., 2020) and currently, Peninsular Malaysia holds 35 species of stingless bees (Jaapar *et al*., 2016). Like the other bees, stingless bees are important pollinators in Peninsular Malaysia's dipterocarp forests (Nagamitsu *et al*., 1999) and agricultural ecosystems (Wahizatul *et al*., 2019).

Stingless bees produce honey, bee bread, and propolis which received increasing demand in Malaysia (Rozman *et al*., 2022). Various reports revealed that high antioxidant, anti-diabetic, antimicrobial, and anti-inflammatory are some of the medicinal benefits found in stingless bee honey

(Pimentel *et al*., 2021). Thus, meliponiculture has become a phenomenon in Malaysia due to the high nutrition and medicinal value of their pollen and honey, which contribute to the high demand for honey products in the market (Omar *et al*., 2016). Despite stingless bees producing a smaller quantity of honey, its price is significantly higher compared to honey produced by Apis honey bees. This is attributed to the belief in the stingless bee honey's superior medicinal properties and healing qualities. (Kek *et al*., 2017). Besides honey, stingless bees also produce other bee products, such as bee bread and propolis (Mohd & Zin, 2020). Stingless bee honey is made from the nectar of flowers (Shamsudin *et al*., 2019). Bee bread is formed with a mixture of regurgitated honey, bee salivary enzymes, and pollen from flowers, which undergo fermentation by microbes in the pollen pot (Mohammad *et al*., 2021). Propolis is produced from the resin that is collected by the bees from trees such as Dipterocarpacea, and they use the propolis mainly for nest constructions (Shanahan & Spivak, 2021). The nectar and pollen collected by the worker bees are used to feed the larvae (Fletcher *et al*., 2020). Bee bread is also a part of the bee diet, primarily as a source of protein for larvae and young bees' development (Mohammad *et al*., 2020). Pollen and nectar are stored in the hive to maintain the colony's survival, ensure continuous food supply, and for colony growth and development (Vollet-Neto *et al*., 2017).

According to Bharath *et al*. (2020), the bee that is going out to forage (per unit of time) acts as an indicator for the colony strength. Based on the Agricultural Department of Malaysia (2020), an active colony of stingless bees would have approximately 70-80 bees/min fly in and fly out (FIFO). In addition, the activity of bee workers in foraging the pollen, resin, nectar, and other materials also determines the survival of the colony (Bharath *et al*., 2020). The foraging activity was influenced by abiotic factors such as temperature, humidity, and light intensity (Ramadani *et al*., 2021). Stingless bees preferred to forage at temperatures 29°C to 32°C (Jaapar *et al*., 2018), humidity 62.8% to 67.8% (Benedick *et al*., 2021) and light intensity 46.875 to 91.347 Lux (Salatnaya *et al*., 2020). Stingless bee foraging activity has two peak hr between 0900 -1100 and 1500-1600 (Layek & Karmakar, 2018; Souza-Junior *et al*., 2019). However, the peak time depended on stingless bee species (Basari *et al*., 2018), climatic factors, and resource availability (Aleixo *et al*., 2017). Stingless bees are most likely attracted to flowering trees to collect pollen as their protein sources and to produce honey (Ocaña-Cabrera *et al*., 2022). Cholis *et al*. (2020) reported that stingless bees tend to choose the pollen size they can carry back to their hive. In tropical regions, Gaona *et al*. (2019) found that stingless bees prefer to collect pollen from plants that belong to Fabaceae, Malvaceae, and Boraginaceae. However, the pollen collection varies depending on the seasons and availability of the resources (Aleixo *et al*., 2017). Information on the foraging activity of the stingless bee in tropical climates especially in Malaysia is still limited (Wan Nur Asiah *et al*., 2015) and pollen analysis is crucially important to trace the botanical origin of the honey and floral sources of stingless bees (Zaki & Abdul Razak, 2018). Stingless bees foraging activity depends on the availability of resources. Acacia tree provides pollen (Midgley & Turnbull, 2003; Koutika & Richardson, 2019), nectar (Dukku, 2003), and resin (Hayward, 2004) which is one of the preferred floral sources by the bees. Acacia is a fast-growing tropical tree, widely distributed from Queensland, Papua New Guinea, and Indonesia (Hegde *et al*., 2013). It was first introduced to Sabah in 1966 and to Peninsular Malaysia in 1978. Acacia forest can be found abundantly at Marang, Terengganu, and possibly can be the preferred location for meliponiculture due to the abundance of floral resources for the stingless bees. This study was conducted to determine the relationship between stingless bee (*Heterotrigona itama*) foraging activity about environmental factors (temperature, humidity & light intensity) and to determine the preferred floral and pollen sources collected by *H. itama*.

MATERIALS AND METHODS

Study site

This study was conducted in 2020 (August, September, October) and 2021 (February, March, April, August & September) at a meliponiary located in Marang, Terengganu where the coordinates lie at 5°4'20"N, 103°414'27"E. The meliponiary covers approximately 0.3 hectares. Approximately 300 colonies of stingless bee, *H. itama* are reared in the meliponiary. This area is dominated mainly by the *Acacia mangium*, and other plant species such as coral vines (*Antigonon leptopus*), straits rhododendron (*Melastoma malabathricum*), and shy plant (*Mimosa pudica*).

Observation of stingless bee activeness

Five active colonies of *H. itama* that were actively hovering in and out of approximately 70-80 bees/min FIFO were selected for hive activeness observation from 0700 to 1700 hr. The stingless bee's FIFO from their hive was recorded using Redmi Note 8 mobile phone camera (Model M1908C3JG) for 10 min at one-hr intervals throughout the sampling time (0700-1700 hr) during hot sunny days. The stingless bees' movement in and out of their hive were counted using a tally counter based on the video footage. After that, the numbers of recorded *H. itama* going in and out from their hives were extrapolated to the individual per min (ind/min) to determine the stingless bee activeness.

Data collection on environmental factors

The environmental factors (temperature, relative humidity & light intensity) were measured once every hr (0700-1700). Temperature and relative humidity were measured using a portable wind speed meter (Model LM-8000A), while light intensity was measured using a portable lux meter (Model H197500).

Pollen samples collection and palynological analysis

Foragers of *H. itama* (*n*=5/month) with pollen loads were captured randomly, using entomological nets, in front of their nest entrance and transferred into a microcentrifuge tube (1.5 mL) with contained 1.0 mL of 70% ethanol. The pollen residue in the tube was dyed with 20 µL Safranin O and vortexed for 5 sec, then left overnight (Jayadi & Susandirin, 2020). Pollen loads were mounted in the glass slide and covered with coverslips for microscopy examination. Pollen images were observed under the Leica DM750 microscope with 1000x magnification. The width and length (in µm) of pollen grains were measured. Identification of the botanical species of the observed pollen was based on Kiew & Muid, (1991). The quantitative pollen analysis is based on a method recommended by the International Commission for Bee Botany (Louveaux, 1978). All samples were analyzed in the Apiculture Laboratory of Universiti Malaysia Terengganu (UMT).

Statistical analysis

All dependent variables were tested for normality of distribution (Kolmogorov–Smirnov test) before conducting One-way ANOVA and Two-way ANOVA statistical tests. One-way ANOVA was used to calculate the differences between bee activeness with time and bee activeness with months. Two-way ANOVA was used to calculate the differences between bee activeness with time and months. Regression Analysis was used to determine the relationship between the bee's activeness with temperature, humidity, and light intensity. All analyses were performed using the Statistical Package for the Social Science (SPSS) 23.0 statistical software. The frequencies of pollen grains collected by the stingless bees followed the system introduced by Louveaux *et al*., (1978). The frequency classes used comprised of the following: “Dominant pollen” (more than 45% of the pollen grains counted); “Accessory pollen” (16-45%), “Isolated pollen” (3-15%), and “Occasional pollen” (less than 3%).

RESULTS AND DISCUSSION

Stingless bee activeness between time and months

The activeness of stingless bees, *H. itama* from five different colonies throughout the study period was significantly different between 0700-1700 [$F_{(10,414)}$ = 28.040, $p<0.05$]. Figure 1 shows that the highest peak of stingless bee activeness was observed between 0900 (22.79 ± 2.92 ind/min) and 1100 hr (27.73 ± 2.88 ind/min). The number of bee activeness increased significantly at 1000 hr (32.79 ± 3.60 ind/min) but then reduced rapidly from 1100 (27.73 ± 2.88 ind/min) until 1700 hr (3.29 ± 0.28 ind/min).

The result suggests that the stingless bee activeness was significantly higher in the morning and could be associated with the flower anthesis time, which usually occurred between 8:00 am and 11:00 am for tropical plants (Willmer & Stone, 1997; Baskorowati, 2011; Ghazi *et al*., 2014; Hatfield & Prueger, 2015; López-Sampson & Page, 2019; Alves-de-Lima *et al*., 2023). Wahizatul *et al*., (2015) found that the highest foraging of *H. itama* occurred in the morning due to the need to fulfill their energy requirement. Basari *et al*. (2018) also obtained a similar result on the foraging activity of *H. itama* which increased from 0700 until 1100.

Stingless bee movement in and out was significantly different between months [$F_{(7,417)}$ = 7.473, $p<0.05$]. Stingless bee activeness significantly increased from August until October 2020. However, bee activeness gradually decreased from October 2020 until March 2021, but the trend increased steadily again from March until September 2021. Based on Figure 2, October 2020 showed the highest mean number of stingless bee activeness going in and out from the beehive’s entrances (24.69 ± 2.97 ind/min) followed by August 2021 (19.29 ± 3.21 ind/min) and September 2021 (13.95 ± 1.90 ind/min). However, there was no significant difference in bee activeness in (August & September) 2020, and (February, March, April & September) 2021. The activeness of stingless bees in August 2021 (19.29 ± 3.21 ind/min) was higher compared with August 2020 (12.12 ± 1.41 ind/min).

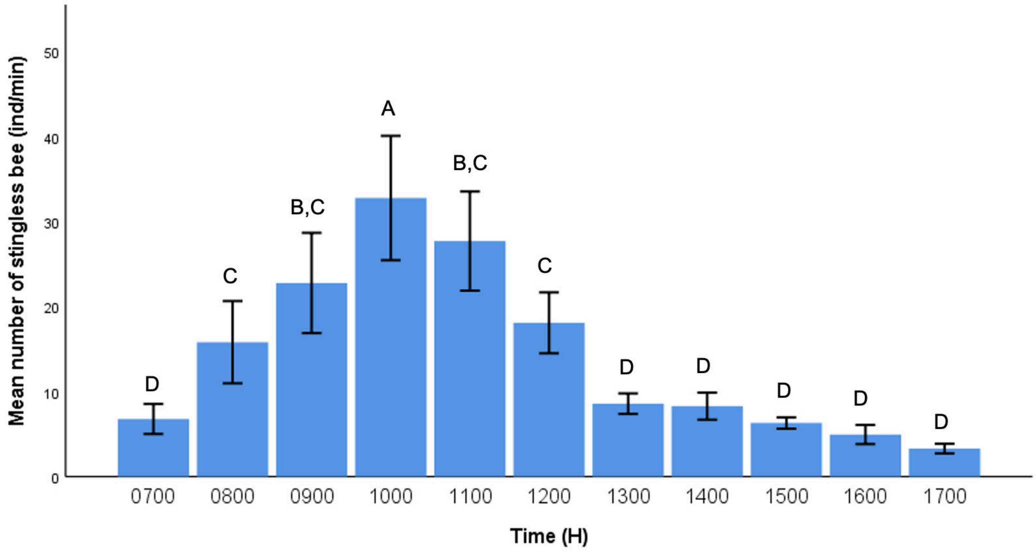

Fig. 1. The mean activeness of *Heterotrigona itama* from 0700 to 1700. Error bars represent the standard error. Different letters in the graph indicate significant differences between time at *p*<0.05, Turkey HSD analysis.

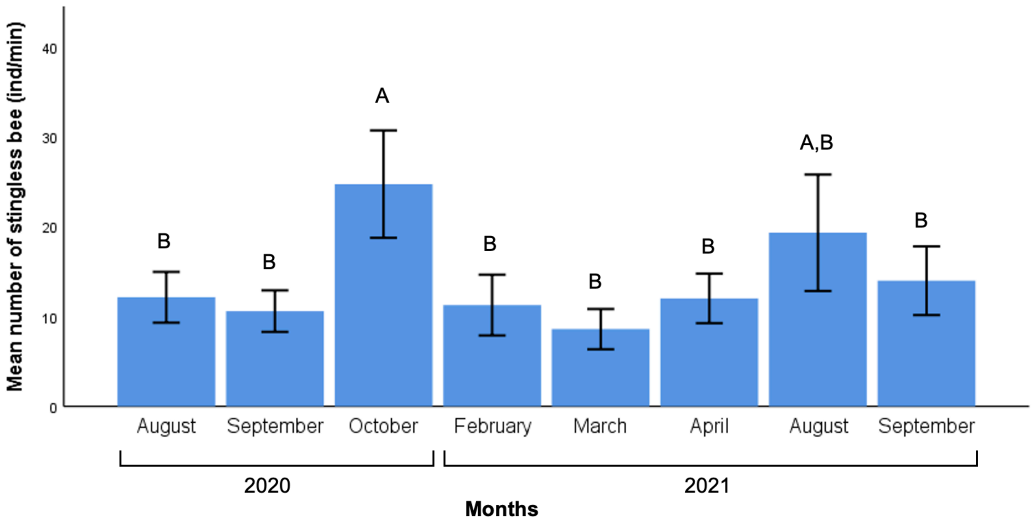

Fig. 2. The mean activeness of *Heterotrigona itama* according to months. Error bars represent the standard error. Different letters in the graph indicate significant differences between months at *p*<0.05, Turkey HSD analysis.

According to Neupane and Thapa (2005), seasons significantly impact the number of foragers. They also found that the highest number of foragers occurs in spring rather than the rainy season. Our findings suggest that *H. itama* also behaves similarly to honey bees as reported by He *et al.* (2016), where they found that honey bees collect more food and display increased activity before the onset of the monsoon season, which occurred in November, December and January in Malaysia (Saad *et al.*, 1999). Based on Figure 2, samplings were conducted during the dry weather season in 2020 (August, September, October) and 2021 (February, March, April, August & September), and the highest bee activeness was recorded in October 2020 and August 2021 which is before the monsoon season.

Results show that there was a significant difference in stingless bee activeness between months for five colonies of *H. itama* [$F_{(7,424)}$ = 17.303, *p*<0.05]. The activeness of stingless bees also significantly differed between times, ranging from 0700 to 1700 hr [$F_{(10,424)}$ = 46.215, *p*<0.05]. Hence, there was a significant difference in the interaction of stingless bee activeness between time and months [$F_{(67,424)}$ = 3.498, *p*<0.05].

The relationship of stingless bee activeness with environmental factors

In this study, the stingless bees started to forage early in the morning at a temperature of 25.04 ± 9.47°C and a relative humidity of 79.67 ± 30.11%. The minimum average light intensity was recorded at 2,791 ± 1.06 Lux (0700-0800 hr), while the maximum average light intensity reached 15,596 ± 5.51 Lux (1400-1500 hr). However, according to Table 1, temperature (R^2=0.000), humidity (R^2=0.022), and light intensity (R^2=0.021) did not significantly influence the activeness of stingless bees, indicating a weak correlation.

Table 1. Regression analysis of stingless bee activeness with temperature, relative humidity, and light intensity

Environmental Factors	Model	R	R Square	Adjusted R Square	Std. The error in the Estimation
Temperature	1	.009[a]	.000	-.002	14.97540
Humidity	1	.148[a]	.022	.019	14.81194
Light Intensity	1	144[a]	.021	.018	14.82104

From the results, this study contradicted Wan Nur Asiah *et al*., (2015), who found that the temperature, relative humidity, light intensity, and weather conditions consistently stimulate the stingless bee foragers and affect their flight activity. In this study, the minimum average light intensity recorded was 2,791 ± 1.06 Lux (0700-0800 hr), while the maximum average light intensity reached 15,596 ± 5.51 Lux (1400-1500 hr). According to previous findings, Wicaksono *et al*. (2020) study found that the peak time occurred at 1200 hr with a temperature of 37°C, humidity of 39.3%, and light intensity of 25.5 Klux. However, the peak time in this study was at 1000 hr with a temperature of 30.8°C, humidity 76.4%, and light intensity of 13.3 Klux. The results differed from those of Wicaksono *et al*. (2020) due to variations in climate and study sites. Wicaksono *et al*. (2020) conducted their research on *Lepidotrigona terminata* in Bogor, Indonesia, while our study focused on *H. itama* in Terengganu, Malaysia. Different surrounding areas of the sampling site may also be the factor that changes the abiotic factors' value and not influence the bee movement. The forest sampling site in this study provided a canopy area that reduces the light intensity, thus making the surrounding area a little dim. The forest canopy provided a shaded area for the colonies which the bees have used as a trail to forage, similar to a study conducted by Wan Nur Asiah *et al*., (2021). Shaded areas reduce the temperature from the sunlight, therefore not influencing the bees to go forage. These variations significantly contribute to the differences observed in the results.

From our results, the stingless bees started their foraging activities at approximately 25.04 ± 9.47°C and the optimal level of relative humidity at 79.67 ± 30.11%. These results were consistent with a study by Hilario *et al*., (2000) which was conducted at São Paulo in Brazil, and Benedick *et al*., (2021) at a residential area in Sabah, Malaysia. However, these results were inconsistent with the previous study by Jaapar *et al*. (2018), which revealed that the ideal temperature for *H. itama* foraging activity was 29°C to 32°C. Our results were inconsistent with Jaapar *et al*. (2018) which might be related to differences in time of foraging observation (1000 hr to 1100 hr) and landscape pattern areas as their study was done in the primary forest of Taman Tropika Tasik Kenyir, Terengganu, whereas we were carried out in Acacia secondary forest.

Dominant pollen collected by *Heterotrigona itama* from acacia forest in Terengganu

Results show that *Acacia mangium* was the most dominant pollen type collected by *Heterotrigona itama* (Figure 3). *Melastoma malabathricum, Commelina diffusa, Antigonon leptopus*, *Cleome rutidosperma*, *Turnera subulate* and *Sporobolus diander* were the important minor pollen types while *Mimosa pudica*, *Ischaemum muticum*, *Melaleuca cajuputi,* and *Capsicum frutescens* were the minor pollen type collected by *H. itama*. All the pollen types collected belonged to nine different families: Cleomaceae, Fabaceae, Melastomataceae, Myrtaceae, Polygonaceae, Solanaceae, Commelinaceae, Poaceae, and Passifloraceae (Table 2).

Acacia mangium belongs to the family Fabaceae, and this tree was planted in the hardwood industry in Malaysia (Hegde *et al*., 2013). Our data shows that this species has been foraged by *H. itama* abundantly as found by Gaona *et al*., (2019). As a preferred tree, this species provided multiple resources for the bees (Warui *et al*., 2018). Other than pollen as their protein source, *Acacia* sp. also produces nectar for stingless bee's carbohydrate source and resin for nest construction. *Acacia* sp. is an evergreen tree that seasonally flowers annually (Hedge *et al*., 2013), which also gives the bees an advantage to visit and collect pollen from *Acacia* sp. every year. As stated by Ghazi *et al*. (2018), family members of Fabaceae produce yellowish-colored flowers that are attracted by *H. itama* and this could be one reason *H. itama* prefers to collect pollen from the *A. mangium* trees.

In this study, no secondary type of pollen was collected by the stingless bees. The least number of pollen collected by the bees (important minor pollen and minor pollen) regarding the size of the pollen also matters to the stingless bees. Stingless bees tend to choose pollen size that they can carry back to their nest (Cholis *et al*., 2020). The bigger the pollen size, the less the number of pollens that they can carry. Moreover, the least number of pollens is also due to the unavailability of resources that can be collected by the foragers (Ramadani *et al*., 2021). Foraging distance is also one of the factors that contribute to the collection of pollen. Stingless bees mainly focus on collecting floral resources near their nest within a range of approximately 500 m (Leonhardt *et al*., 2014). This differs from *Apis mellifera* which can travel more than 2 km from its nest (Abou-Shaara, 2014).

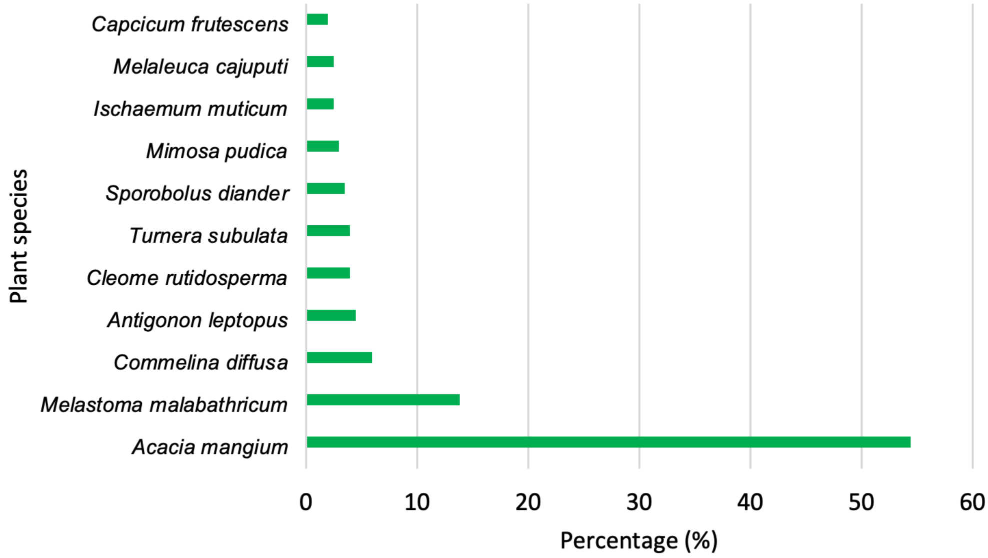

Fig. 3. Frequencies of plant species collected by *H. itama* in 2020 (August, September, October) and 2021 (February, March, April, August & September) in the tropical lowland forest of Terengganu. Total foragers collected (*N*=40).

Table 2. Pollen loads samples of *Heterotrigona itama* from Acacia Forest in Marang. Notes: D: Predominant pollen type >45%, S: Secondary pollen type 16-45%, I: Important minor pollen type 3-15% & M: Minor pollen type <3% (Louveaux *et al*., 1978)

Plant families	Pollen types	Local names	Habits	Pollen frequencies classes
Cleomaceae	*Cleome rutidosperma*	Pokok Maman	Herb	I
Fabaceae	*Acacia mangium*	Akasia Lebar	Tree	D
	Mimosa pudica	Pokok Semalu	Creeper	M
Melastomataceae	*Melastoma malabathricum*	Senduduk	Shrub	I
Myrtaceae	*Melaleuca cajuputi*	Gelam	Tree	M
Polygonaceae	*Antigonan leptopus*	Air Mata Pengantin	Climber	I
Solanaceae	*Capsicum frutescens*	Pokok Cili	Shrub	M
Commelinaceae	*Commelina diffusa*	Rumput Aur	Herb	I
Poaceae	*Sporobolus diander*	Rumput Telor Belalang	Grass	I
	Ishaemum muticum	Rumput Tembaga Jantan	Grass	M
Passifloraceae	*Turnera subulata*	Lidah Kucing	Shrub	I

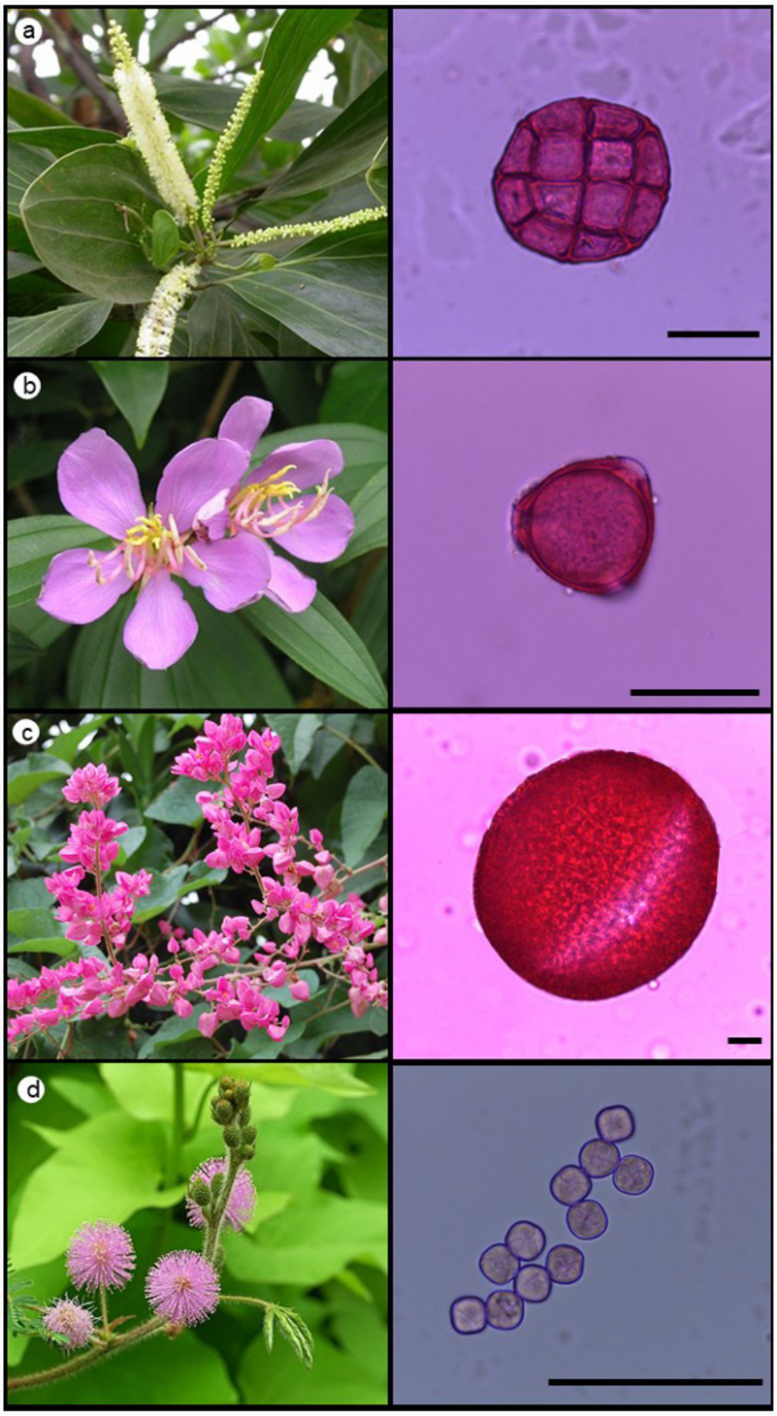

Fig. 4. Images of flowers and photomicrographs of the pollen collected by *Heterotrigona itama:* (A) *Acacia mangium,* (B) *Melastoma malabathricum*, (C) *Antigonon leptopus*, (D) *Mimosa pudica*. Scale bar 10 μm.

CONCLUSION

In conclusion, this study provides valuable information on the preferred time for the foraging activity of *H. itama* and floral sources in Marang, Terengganu. *Heterotrigona itama* were found actively foraging in the morning (0900 hr until 1100 hr) as the flowers become fully open and functional for the pollination process (anthesis) in the morning, thus attracting pollinators such as stingless bees. The highest activeness of stingless bees was recorded in October 2020, followed by August 2021 and September 2021. However, this study showed that the temperature, relative humidity, and light intensity did not significantly influence the activeness of stingless bees. This study also found that *Acacia mangium* is an important food source for *H. itama* as more than 50% of collected pollen belongs to this specific species. *Melastoma malabathricum* was the second-highest pollen type collected by the stingless bees, followed by *Commelina diffusa* and other pollen types. It shows that the stingless bees selected multiple plant habits for pollen collection. This study is crucial to understand the preferred time of foraging activity and pollen sources of *H. itama* which then provides the information to the beekeepers to determine the active colony, thus sustaining and developing the meliponiculture industry in the future.

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ETHICAL STATEMENT

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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