

POLLEN CALENDAR OF SIX STINGLESS BEE SPECIES AT TAMAN PERTANIAN SEKAYU, TERENGGANU

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Abstract. A stingless bee is a eusocial insect from the Meliponine family living in a perennial colony that comprises a queen, workers and drones. They forage nectar, pollen and resin for their provision. This study aims to identify flower preferences among six stingless bee species (*Geniotrigona thoracica*, *Heterotrigona itama*, *Lepidotrigona terminata*, *Tetragonula fuscobalteata*, *Tetragonula minor*, and *Tetragonula testaceitarsis*) and records a pollen calendar for the full 12 months of observation. Stingless bees' pollen samples were collected from the corbícula of returning foragers once a month. The pollens were dispersed in distilled water and observed under a compound microscope to record pollen morphology. Microscopy observation showed variations in pollen size and shapes, indicating stingless bee preferences for a variety of flowers throughout the year. Eighty plant species were identified based on pollen references collected at the sampling site. *L. terminata* and *T. fuscobalteata* showed similar preferences in flowers (*Citrofortunella microcarpa*), while pollens preferred by *G. thoracica*, *H. itama*, *T. minor* and *T. testaceitarsis* were *Emilia sonchifolia*, *Muntingia calabura*, *Ardisia crispa* and *Lantana camara*, respectively. The preferred plants flower all year round. The results from this study could help beekeepers in managing the bee escape that provides food sources for stingless bees, thus ensuring a sustainable meliponiculture in Malaysia.

Keywords: Stingless bee, pollen, eusocial, relative abundance, meliponiculture

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Introduction

A stingless bee is a eusocial insect from the Meliponine family that lives in a perennial colony consisting of a queen, workers (sterile female bees) and drones (male bees). Stingless bees are also known as generalist insects [1] as they are assumed to be extremely generalised in pollen foraging. This is because the foragers in their colonies are actively foraging on a large diversity of floral resources throughout the year [2]. Pollen, nectar and resin are foraged by stingless bees for their provision. Nectar and pollen are used as the main sources of nutrition, while resin and latex are used to build and protect their nests [1]. However, little attention has been given to studying palynology and the foraging activity of stingless bees in Malaysia [3].

Palynology refers to the study of pollen and spores, both structural and functional [4], while melittopalynology is the analysis of pollen samples from bees that aids to assess which plants are visited by the foragers [5]. The morphological characteristics of pollen grains guide palynological nomenclature [4]. Pollen is the principal protein source for larval development among most bee species [5]. Stingless bees store pollen in oval pots made of wax and resin. Pollen grains may be found not only in the pollen storage pots but also in the honey pots. This is mainly because foragers dislodge some of the flower's pollen when landing on the flower to collect nectar, which falls into the nectar [5].

Pollen grains often have very specific morphology associated with the plant species from which they originate. Therefore, through careful examination of their morphology, it is possible to determine the botanical origin of the resources collected by the bees [5]. While foraging for pollen, stingless bees also act as pollinators. Cholis et al. [1] stated that stingless bees are effective pollinators. Stingless bees have been proven to play a major role as pollinators in their natural habitat, especially in the tropic region [6]. The corbicula (pollen basket) that functions as a pollen collector also helps the transfer of pollen to other flowers [1].

Various families of plants have been visited by stingless bees to collect pollen for their colonies. Acanthaceae, Begoniaceae, Cucurbitaceae, Ciperaceae, Poaceae, Malvaceae, Myrtaceae, Rutaceae, Euphorbiaceae and Leguminosae are examples of plant families visited by stingless bees [1]. According to Norita et al. [7], other plant families visited by stingless bees to collect pollen include Rubiaceae, Sapindaceae and Solanaceae. This current study also focuses on identifying pollen sources from stingless bee species at Taman Pertanian Sekayu, Terengganu. The problem addressed in this study is the lack of ideal food sources for stingless bees that could be provided by beekeepers to increase their colonies' health and honey production. This pollen analysis would help in identifying the plant species visited by stingless bees, which could be provided by beekeepers on their farms in the future to increase the colonies' longevity and honey production.

Materials and Methods

Sampling Site

This study was conducted at Taman Pertanian Sekayu located in Hulu Terengganu, Terengganu (4.98°N, 102.93°E). The duration of this study was 12 months (from October

2019 until September 2020). There were six stingless bee species involved in this study; *Geniotrigona thoracica*, *Heterotrigona itama*, *Lepidotrigona terminata*, *Tetragonula fuscobalteata*, *Tetragonula minor* and *Tetragonula testaceitarsis*.

Materials

The field apparatus utilised during pollen sampling were a pooter, 1.5 ml centrifuge tube, needle and gloves. In the laboratory, instruments and consumables used were glass slides, coverslips, distilled water and an optical microscope. Flowers around the sampling site were collected to observe and record their pollens. The recorded pollen from the flowers became reference materials for this study.

Methods

To catch stingless bee foragers, their hive's entrance was closed with a leaf or other material to stop them from entering the hive. The returning foragers were then sucked into a pooter container. Once sucked, some pollen would fall off the corbicula while some would still be attached to the corbicula. Any fallen pollen was collected directly after releasing the foragers. Pollen that was still attached to the corbicula was then collected using a needle without killing the stingless bees. All pollen samples were placed in their respective centrifuge tubes and diluted in 0.5 mL of distilled water. The samples were stirred by shaking and tapping on the tube to disperse the pollen. A drop of pollen suspension was deposited onto a glass microscopic slide and a cover slip was gently pressed onto it. Pollen observation was done using a Leica DM750 optical microscope with a 40X-magnification objective lens. The acquired pollen images were compared with those of identified flowers in Taman Pertanian Sekayu.

Then, percentage of relative abundance of each plant species was calculated using the following equation:

$$\text{Relative abundance} = (\text{TS}/\text{TP}) * 100 \quad (1)$$

Parameters used in this formula are the TS; the number of stingless bee frequently visited a plant species over TP; the total number of plant species that were visited by all stingless bee species at the sampling site.

Results and Discussion

In this study, 720 pollen grains were successfully acquired during the 12 months of observation. In total, 120 pollen samples were collected from each stingless bee species. Out of 720 pollen samples, 32 unidentified pollens did not match any of the pollens in the pollen database at the sampling site. The relative abundance of identified pollen grains was counted for all plant species.

Figure 1 displays the collection of pollen from the stingless bee *Geniotrigona thoracica* (*G. thoracica*) collected from foragers upon arriving at their hives. A total of 32 types of pollen grains were collected by *G. thoracica* for 12 months. The most collected pollen was from *Emilia sonchifolia* (*Bayam peraksi*), followed by *Ruellia brittoniana*

(Ruella), *Bulbine frutescens* (Asphodelaceae), *Combretum indicum* (Akar Dani), *Ageratum conyzoides* (Rumput Tahi Ayam) and *Tetracera indica* (Mempelas). *E. sonchifolia* pollen grains were of the highest percentage of relative abundance (8.33%). Meanwhile, the other five flowers had the same percentage of 5%, respectively.

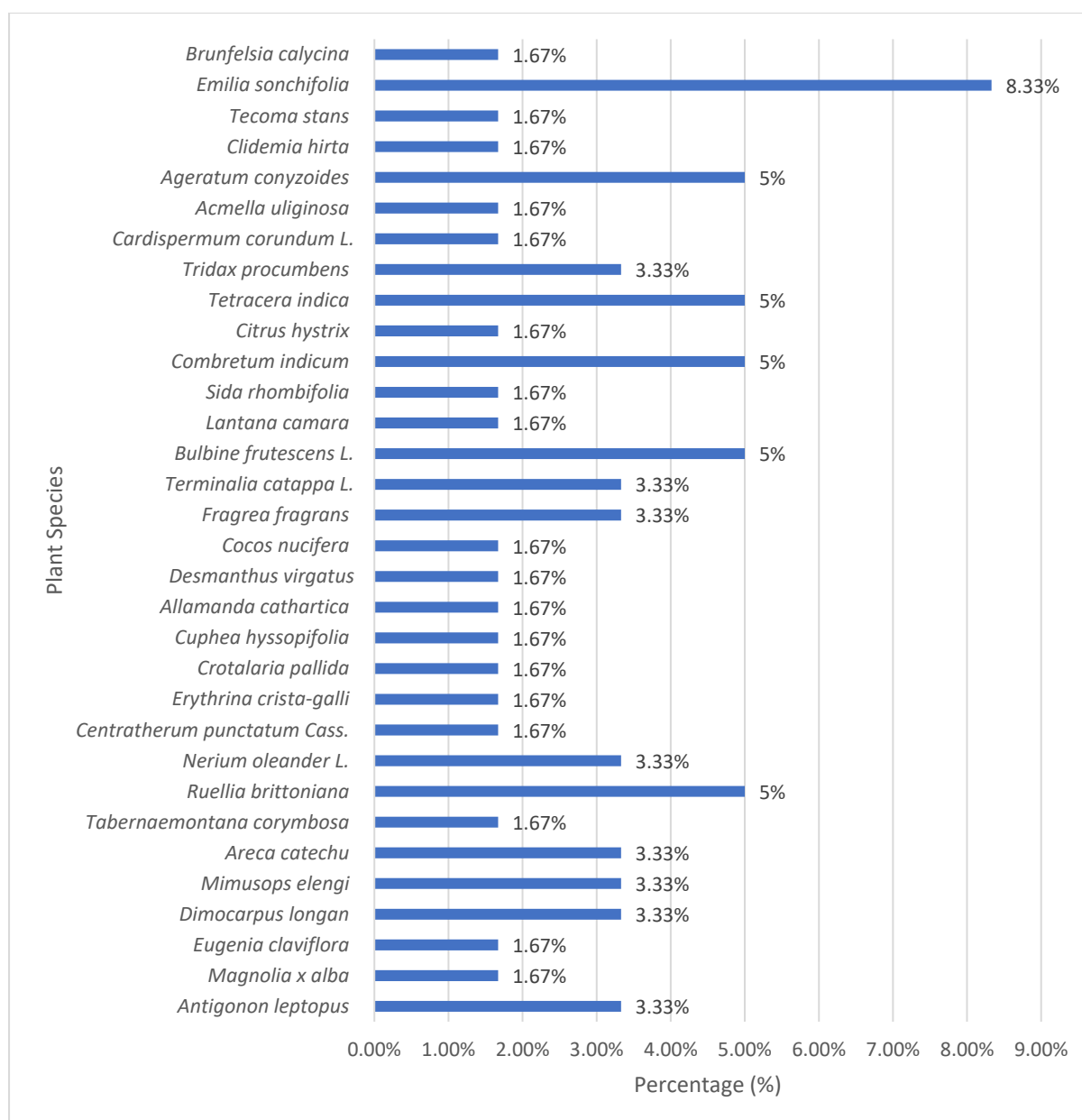


Figure 1: Pollen grains collected by *Geniotrigona thoracica* from Oct 2019 to Sept 2020

E. sonchifolia is a type of weed that grows 30-40 cm in height and belongs to the family of Asteraceae. The weed is commonly known as the lilac tassel flower or Cupid's shaving brush. *E. sonchifolia* is a glabrous, slender herb [8]. Its leaves are obovate with purplish flowers [8]. The pollen shape of *E. sonchifolia* is circular with an echinate surface as seen by the macro-size thorn on the pollen surface (Figure 7(A)) [9]. According to Neethu and Gangaprasad [8], *E. sonchifolia* can be found in waste grounds or moist areas. In Malaysia, this plant is regarded as a weed since it can grow in any moist soil. In Taman

Pertanian Sekayu, *Emilia sonchifolia* plants are dispersed randomly, especially at the sampling site.

Figure 2 illustrates the pollens collected by the *Heterotrigona itama* (*H. itama*) stingless bee over 12 months. *Muntingia calabura* (*Kerukup siam*) was the most collected pollen by *H. itama* during the period of this study, followed by *Tridax procumbens* (*Tridax daisy*). There were 32 types of plant species identified from pollen grains collected by *H. itama*. Among them, *M. calabura* demonstrated the highest percentage of relative abundance (8.33%). The second most collected pollen of *H. itama* was *T. procumbens* with a percentage of 6.67%.

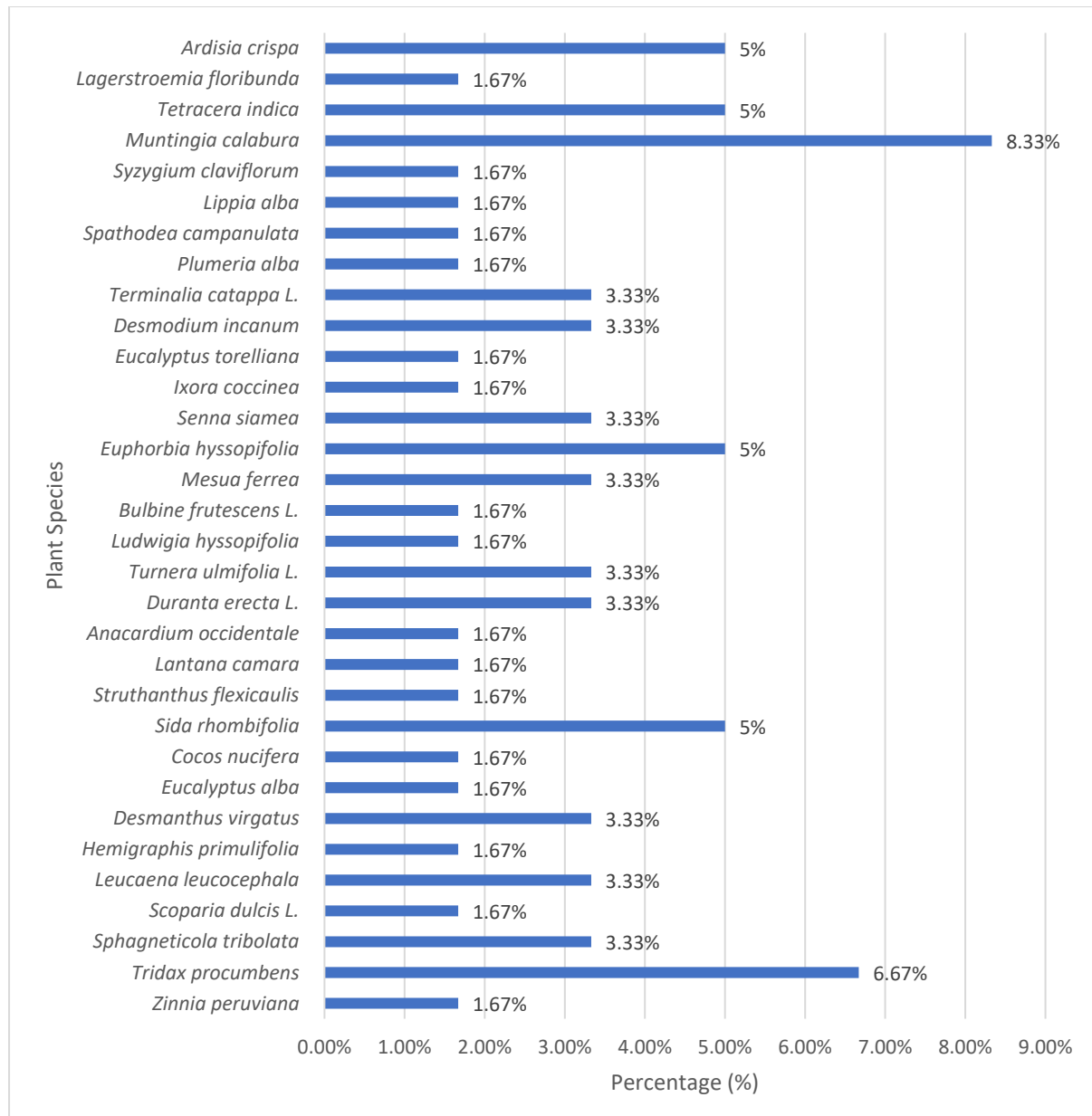


Figure 2: Pollen grains collected by *Heterotrigona itama* from Oct 2019 to Sept 2020

M. calabura is widely cultivated in warm areas such as Malaysia, Indonesia and the Philippines [10]. This plant is a fast-growing tree of slender proportions with a height of

approximately 7.5-12 m. The flowers are approximately 1.25-2 cm wide; borne singly or in 2's or 3's in the leaf axils, with 5 green sepals and 5 white petals, as well as many prominent yellow stamens [10]. The pollen shape of *M. calabura* is small and spherical (Figure 7(B)). Its surface is viewed as smooth under a compound microscope.

M. calabura plants from the Muntingiaceae family have been gaining attention among researchers throughout the world owing to their medicinal uses. For example, the flowers and barks were used as an antiseptic to reduce swelling in the lower extremities in Peruvian folklore. The infusion of the flowers was used as a tranquilliser and tonic in Colombia, while in the Philippines, the flowers were used to treat headaches and incipient colds or as tranquillisers, antispasmodics and antidyspeptics [10]. *M. calabura* was seen to disperse randomly with no predictable pattern in Taman Pertanian Sekayu.

Figure 3 shows the pollens collected by *Lepidotrigona terminata* (*L. terminata*) stingless bees. The pollen from *Citrofortunella microcarpa* (*Limau kasturi*) was the most collected during the 12-month duration, followed by *Tetracera indica* (*Mempelas*) (13.33%) and *Tabernaemontana corymbosa* (*Jelutong Badak*) (11.67%). *C. microcarpa* was the most preferred pollen by *L. terminata* with the highest relative abundance percentage of 18.33%, whereas *Citrus hystrix* (Kaffir lime) (1.67%) was the least preferred pollen shown in the bar graph from Figure 3. Pollen from *C. microcarpa* are spheroid to prolate-spheroidal shaped and have reticulated surfaces (Figure 7(C)) [11]. *C. microcarpa* plants are widely grown in India, Southern Asia and Southeast Asia (Vietnam and Malaysia) [12].

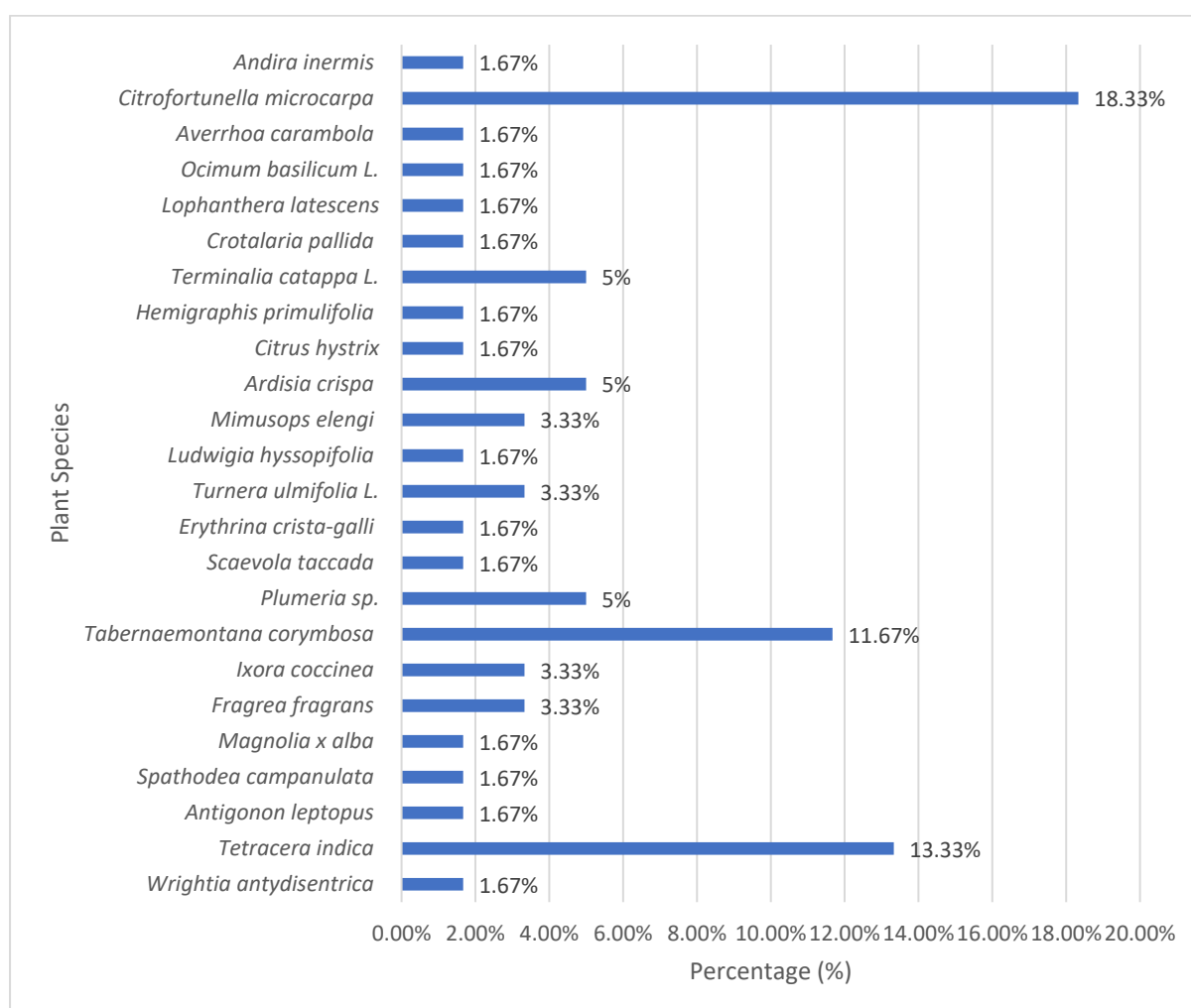


Figure 3: Pollen grains collected by *Lepidotrigona terminata* from Oct 2019 to Sept 2020

Figure 4 shows the collection of pollen by *Tetragonula fuscobalteata* (*T. fuscobalteata*). The highest percentage of pollen collected was 10% from *Citrofortunella microcarpa* (*Limau kasturi*), followed by pollen from *Combretum indicum* (*Akar Dani*) (8.33%), *Terminalia catappa* L. (*Ketapang*) and *Wrightia antydisentricta* (*Anting Puteri*) with the same percentage of 6.67%, respectively. Manila-Fajardo et al. [17] stated that pollens from *C. microcarpa* are spheroid to prolate-spheroidal shaped and have reticulated surfaces (Figure 7(D)).

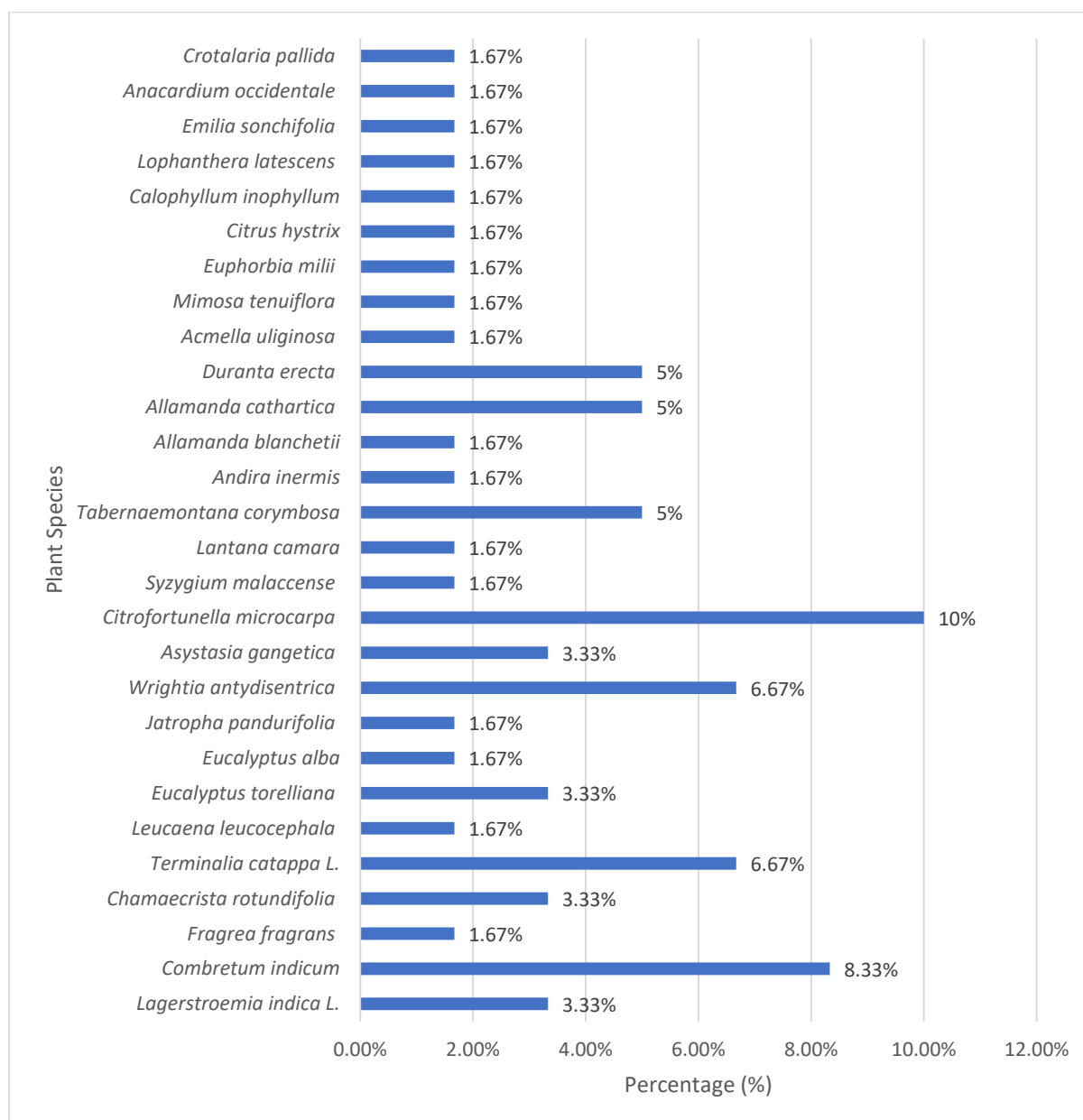


Figure 4: Pollen grains collected by *Tetragonula fuscobalteata* from Oct 2019 to Sept 2020

C. microcarpa is a type of fruit plant in the Rutaceae family. Nguyen et al. [12] stated that the genus *Citrus* from the Rutaceae family consists of the most popular and important fruit crops like orange, grapefruit, pomelo, lime and lemon. A study conducted on essential

oil extracted from the flower of *C. microcarpa* by Nguyen et al. [12] showed that essential oil can be used as a natural antioxidant source. Based on the finding, it can be assumed that the pollen from *C. microcarpa* collected by the foragers is rich in antioxidants. The dispersion of *C. microcarpa* plants was in a uniform pattern in Taman Pertanian Sekayu. These plants were grown as a landscape near the location of the sampling site.

Figure 5 shows the pollen collected by *Tetragonula minor* (*T. minor*). Twenty-five plant species were visited by *T. minor* foragers during the 12 months of observation. The pollen with the highest percentage of relative abundance collected by *T. minor* was *Ardisia crispa* (Mata Ayam) (16.67%). The second most collected pollen was from *Averrhoa carambola* (Belimbing) (13.33%) followed by *Euphorbia milii* (Sesudu) (8.33%).

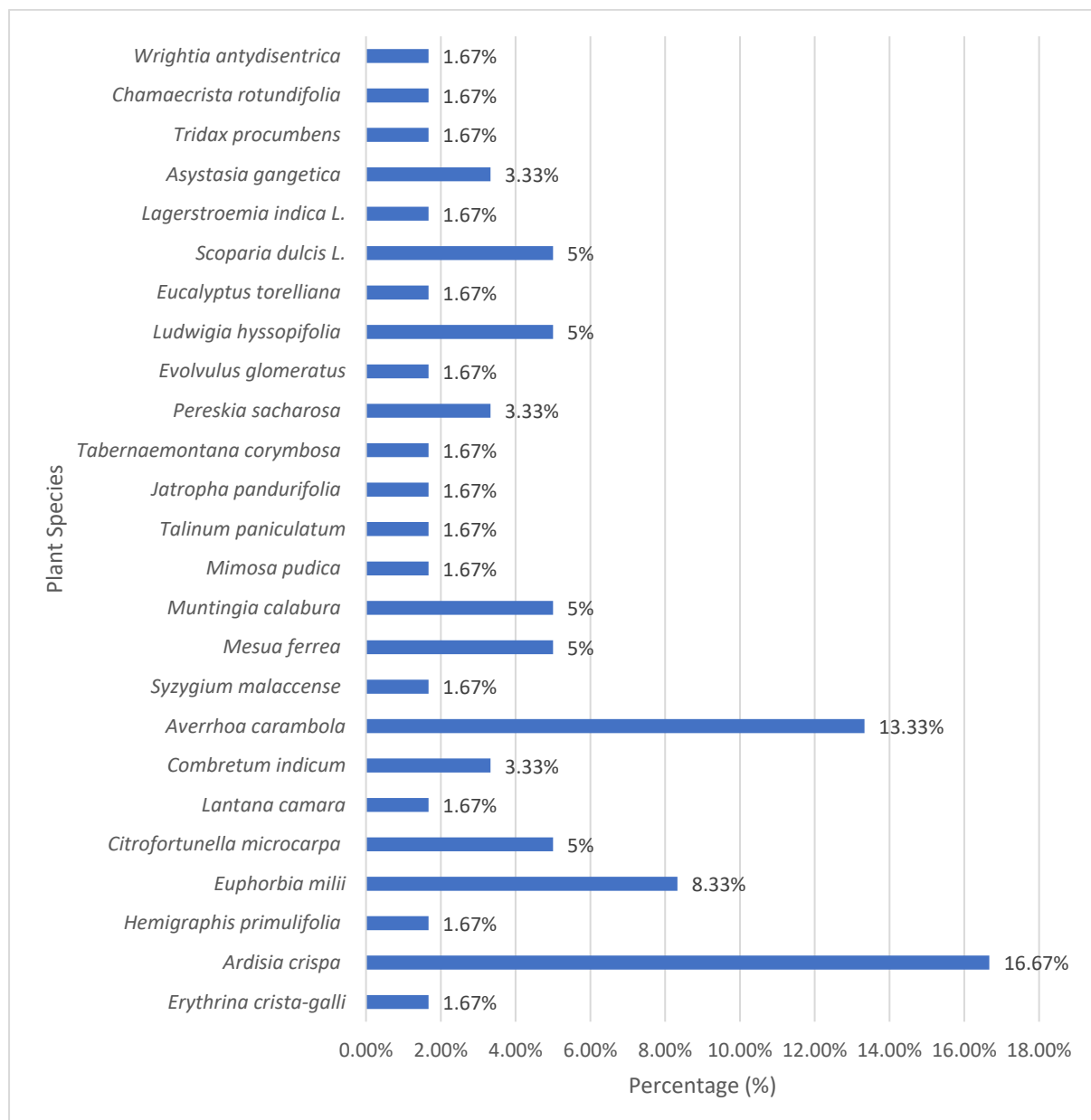


Figure 5: Pollen grains collected by *Tetragonula minor* from Oct 2019 to Sept 2020

Ardisia crispa (*Mata Ayam*) belongs to the Myrsinaceae family, and the genus *Ardisia* is the largest in the family. Some plant parts of *A. crispa* possess medicinal properties. For instance, the roots are used to reduce fever and stop excessive salivation in China, while in Malaysia, juice from the leaves is used to treat scurvy [14]. Pollens of *A. crispa* are spherical in shape and small in size (Figure 7(E)).

Averrhoa carambola (*A. carambola*) is a fruit tree commonly known as the starfruit tree. It is a plant in the family of Oxalidaceae. Its flowers are small and purplish with five sepals, five petals and five stamens [15]. The flowers bloom all along the trunk, branches, and twigs [15]. According to Gowrishankar et al. [15], flowers can be used as medicine to cure diseases such as fever, subcalorism, and malaria in India, while in Java, flowers are added to salads.

In Taman Pertanian Sekayu, *A. carambola* plants were dispersed in a uniform pattern in a reserved area. The shape of pollens of *A. carambola* is elongated or spherical [16]. In this study, the pollen of *A. carambola* was found in an elongated shape with a rough-looking surface.

Figure 6 shows the percentage of relative abundance of pollen collected by *Tetragonula testaceitarsis* (*T. testaceitarsis*) in the 12 months of observation. *Lantana camara* (*Bunga Tahi Ayam*) was the pollen with the highest percentage of relative abundance of 11.67% collected by *T. testaceitarsis*. The second most collected pollen was from *Duranta erecta* (*Duranta*), followed by *Magnolia x alba* (*Cempaka putih*) and *Desmodium incanum* (*Fabaceae*) with the same percentage (6.67%), respectively.

L. camara, a plant from the Verbenaceae family, is a vigorous shrub with a triangular stem, stout recurved pickles and a strong odour of black currants [17]. The *L. camara* plants can grow up to one to three metres high. Its leaves and stem are covered with rough hair, while the flowers are small and held in clusters called umbels. The colour of the flowers varies from white to red in various shades but according to Sanjeeb et al. [17], the flowers of *L. camara* plants are usually orange in colour and change colours as they aged. This observation is supported by Aluri and Subba Reddi [18] discussing that *L. camara* undergoes changes in flower colour from the time of opening to the time of dropping.

The pollens of *L. camara* are spheroidal in shape (Figure 7(F), equatorial view). Munsif et al. [19] stated in their study that the pollen of *L. camara* has a semi-angular shape in the polar view and a spheroidal shape in the equatorial view. The distribution of *L. camara* in Taman Pertanian Sekayu was randomly dispersed with no predictable pattern.

Figure 7 presents the pollen grains that have the highest percentage of relative abundance and preference by each of the six species of stingless bees. These pollen micrographs were taken under a 40X-magnification objective lens. The pollen grains varied in colour and size but were similar in shape characteristics. All pollens were spheroid to prolate-spheroid shaped, while their surfaces differed such as echinate (Figure 7(A)), smooth (Figures 7(B), (E) and (F)) and reticulated (Figures 7(C) and (D)) surfaces.

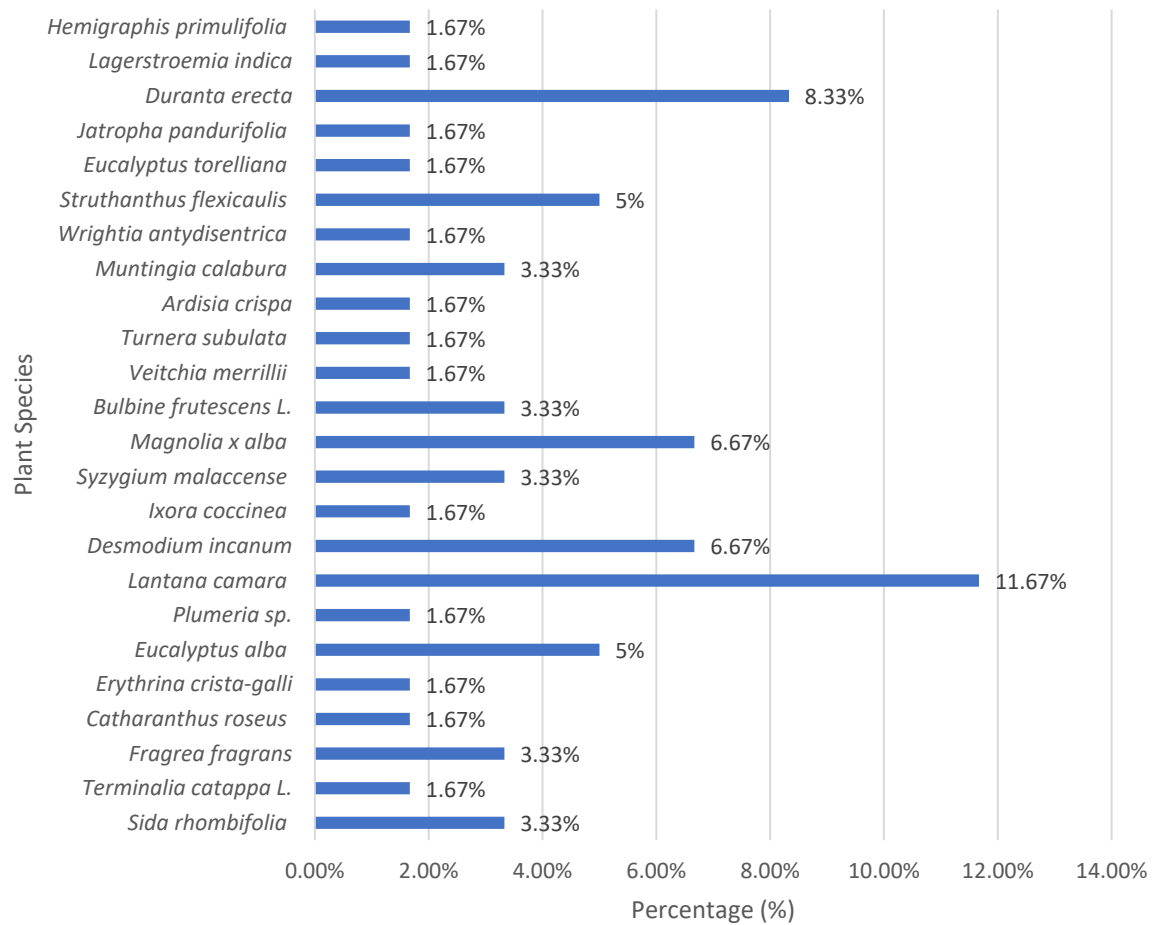


Figure 6: Pollen grains collected by *Tetragonula testaceitarsis* from Oct 2019 to Sept 2020

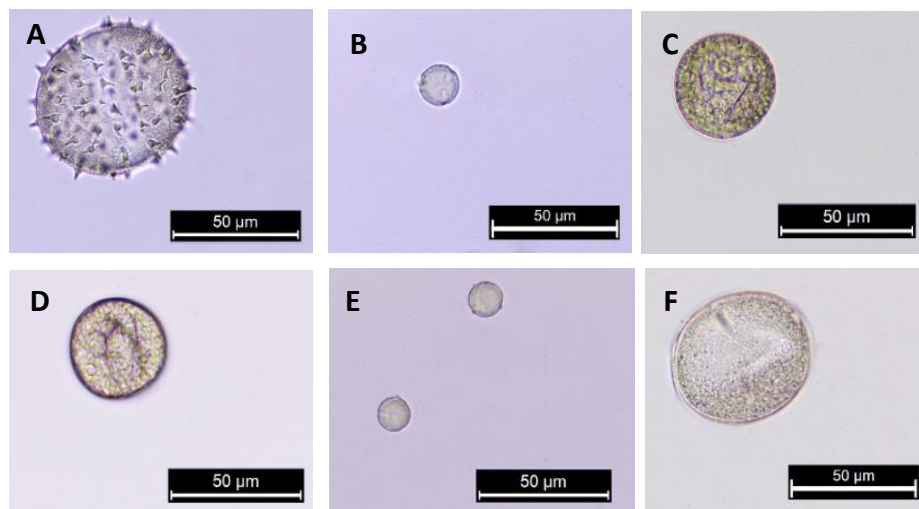


Figure 7: Micrograph of pollen grains based on the highest percentage of relative abundance of the plant species for each stingless bee species; (A) *Emilia sonchifolia* by *Geniotrigona thoracica*, (B) *Muntingia calabura* by *Heterotrigona itama*, (C) *Citrofortunella microcarpa* by *Lepidotrigona terminata*, (D) *Citrofortunella microcarpa* by *Tetragonula fuscobalteata*, (E) *Ardisia crispa* by *Tetragonula minor*, and (F) *Lantana camara* by *Tetragonula testaceitarsis*.

Table 1 shows the variety of flowers most visited by foragers to collect pollen every month from October 2019 to September 2020. This calendar would help beekeepers in planting flowering plants favoured by different species of stingless bees in their farms. The availability and diversity of plants will help stingless bees gather the sources needed by the colony.

Table 1: Calendar of flowers of six stingless bee species based on their pollen preferences every month

	<i>G. thoracica</i>	<i>H. itama</i>	<i>L. terminata</i>	<i>T. fuscobalteata</i>	<i>T. minor</i>	<i>T. testaceitarsis</i>
Oct 2019						
	<i>Antigonon leptopus</i>	<i>Tridax procumbens</i>	<i>Antigonon leptopus</i>	<i>Lagerstroemia indica</i> L.	<i>Ardisia crispa</i>	<i>Sida rhombifolia</i>
Nov 2019						
	<i>Ruellia brittoniana</i>	<i>Turnera ulmifolia</i> L.	<i>Citrofortunella microcarpa</i>	<i>Combretum indicum</i>	<i>Lantana camara</i>	<i>Eucalyptus alba</i>
Dec 2019						
	<i>Ruellia brittoniana</i>	<i>Lantana camara</i>	<i>Citrofortunella microcarpa</i>	<i>Citrofortunella microcarpa</i>	<i>Combretum indicum</i>	<i>Desmodium incanum</i>

Jan 2020						
	<i>Combretum indicum</i>	<i>Turnera ulmifolia</i> L.	<i>Tabernaemontana corymbosa</i>	<i>Citrofortunella microcarpa</i>	<i>Ardisia crispa</i>	<i>Lantana camara</i>
Feb 2020						
	<i>Cocos nucifera</i>	<i>Duranta erecta</i>	<i>Turnera ulmifolia</i> L.	<i>Combretum indicum</i>	<i>Ardisia crispa</i>	<i>Lantana camara</i>
Mar 2020						
	<i>Bulbine frutescens</i> L.	<i>Sida rhombifolia</i>	<i>Citrofortunella microcarpa</i>	<i>Combretum indicum</i>	<i>Ludwigia hyssopifolia</i>	<i>Lantana camara</i>
Apr 2020						
	<i>Sida rhombifolia</i>	<i>Sida rhombifolia</i>	<i>Tetracera indica</i>	<i>Citrofortunella microcarpa</i>	<i>Ardisia crispa</i>	<i>Sida rhombifolia</i>
May 2020						
	<i>Emilia sonchifolia</i>	<i>Duranta erecta</i>	<i>Citrofortunella microcarpa</i>	<i>Duranta erecta</i>	<i>Euphorbia milii</i>	<i>Ardisia crispa</i>

June 2020						
	<i>Emilia sonchifolia</i>	<i>Lippia alba</i>	<i>Tetracera indica</i>	<i>Citrofortunella microcarpa</i>	<i>Citrofortunella microcarpa</i>	<i>Duranta erecta</i>
Jul 2020						
	<i>Ageratum conyzoides</i>	<i>Muntingia calabura</i>	<i>Duranta erecta</i>	<i>Euphorbia milii</i>	<i>Tridax procumbens</i>	<i>Lantana camara</i>
Aug 2020						
	<i>Tetracera indica</i>	<i>Muntingia calabura</i>	<i>Lophanthura lactescens</i>	<i>Duranta erecta</i>	<i>Asystasia gangetica</i>	<i>Duranta erecta</i>
Sept 2020						
	<i>Combretum indicum</i>	<i>Ardisia crispa</i>	<i>Crotalaria pallida</i>	<i>Duranta erecta</i>	<i>Averrhoa carambola</i>	<i>Duranta erecta</i>

Pollen preferences among stingless bee species differed even though they were placed in the same location. The results of this study displayed that some species of stingless bees in the same location preferred similar pollen or visited similar flowers. For both *L. terminata* and *T. fuscobalteata*, they preferred *Citrofortunella microcarpa* (Limau Kasturi). The other four stingless bee species (*G. thoracica*, *H. itama*, *T. minor* and *T. testaceitarsis*) preferred *Emilia sonchifolia* (Bayam peraksi), *Muntingia calabura* (Kerukup siam), *Ardisia crispa* (Mata ayam) and *Lantana camara* (Bunga Tahi Ayam) respectively.

Among the six species of stingless bees, *G. thoracica* had the largest body size (mean: 8.44 mm), while the smallest was *T. testaceitarsis* (mean: 1.67 mm). According to Md Zaki et al. [6] stingless bees usually prefer crops or plants that produce small flowers. The observation corroborated in this study showed flowers that were relatively small, such as *Lantana camara* with a flower diameter of 0.5 cm were the most preferred. The diameter of flowers identified in this study ranged from 0.5 – 4.4 cm.

The flowers visited by the stingless bees were varied in colour. There were white (*C. microcarpa* and *M. calabura*), red (*A. crispa*), purple (*E. sonchifolia*) and orange (*L. camara*) colours. Chittka and Raine [20] reported that pollinators use the traits of flowers (odour, shape, size and colour) as a signal to locate sources of pollen and distinguish between flower species.

Stingless bees used pollen as one of their main sources of food [7]. Pollen can be found in perennial and annual types of flowers. The plants identified in this study all had one thing in common: they bloomed all year. This could mean that the stingless bee would have food all year round, thus ensuring their food storage during the rainy season.

In Malaysia, the weather can change quickly from sunny days to rainy days, especially towards the end of the year. Malaysia is located on the Equator line or “*Garisan Khatulistiwa*” and does not experience the four seasons. Stingless bees are found in abundance in the Tropic regions.

In Terengganu, the distribution of rainfall is relatively high at the end of the year during the monsoon period in the Northeast of Peninsular Malaysia (November – March). During this monsoon period, Terengganu would experience heavy rain, making it difficult for the stingless bee to forage for food (pollen). Rain can be described in three situations: sprinkling/drizzling (lightly raining), pouring (heavy rain) and sun showers (raining with the sun out). Stingless bees still forage for pollen during sprinkling and sun showers, but would not go too far since the rain would wet their wings.

Stingless bees cannot forage for pollen in heavy rain. During this time, they would stay inside their hive and mostly do housekeeping activities. In addition, rain also affects flowering plants. Lawson and Rands [13] stated that pollen grains that come in contact with free water become inviable through osmotic disruption causing a reproductive disadvantage to the plants.

The osmotic disruption would interfere with pollen transfer from the stigma to ovary. However, this condition does not occur in all plant species as some plant species utilise rain for self-pollination [13]. Flowering plants that bloom all year round would be helpful as food sources for stingless bees since stingless bees could store more food (pollen) in their hives, especially during the monsoon period.

Conclusions

A pollen calendar of different species of stingless bees has been successfully produced for every month of the year in this study. Stingless bees forage for a variety of flowers for pollen. Every stingless bee species preferred different kinds of flowers as their food source, although some species (*Lepidotrigona terminata* and *Tetragonula fuscobalteata*) prefer the same kind of flowers, including *Citrofortunella microcarpa* (*Limau kasturi*).

Stingless beekeepers could take an initiative by creating a bee garden or planting plants that are bee-friendly, especially plants that are not seasonal for bees to forage. This could help maintain the longevity of the colonies, increase productivity and produce good-quality honey.

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Author Contributions

All authors contributed toward data analysis, drafting and critically revising the paper and agree to be accountable for all aspects of the work.

Disclosure of Conflict of Interest

The authors report no conflicts of interest in this work.

Compliance with Ethical Standards

For this type of study, formal consent is not required.

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