Malaysian Applied Biology
https://doi.org/10.55230/mabjournal.v54i2.3364

Research Article

Melissopalynological Analysis and Plant Preferences of Stingless Bee, *Heterotrigona itama* (Hymenoptera: Apidae) at The Malaysian Agricultural Research and Development Institute (Mardi), Serdang

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ABSTRACT

Melissopalynology plays a pivotal role in ecological studies by identifying floral sources for honeybee nutrition and contributes to a better understanding of plant-pollinator interactions. It is essential for biodiversity monitoring, conservation efforts, and assessing the quality and authenticity of honey products based on pollen analysis. This study investigates the melissopalynological of honey samples collected from stingless bee species *Heterotrigona itama* (Hymenoptera: Apidae) at the Malaysian Agricultural Research and Development Institute (MARDI). A total of 17 flower samples and 10 mL of honey were collected from one beehive and further analyzed using a light microscope. The analysis identified eight pollen types from eight distinct plant species from the honey sample. *Clematis crispa* was the most prominent, representing 21.74% of the total pollen count, followed by *Combretum indicum* (17.39%) and *Cucumis sativus* (17.39%). *Lagerstroemia speciosa* contributed 13.04%, *Ageratum conyzoides* and *Averrhoa bilimbi* each accounted for 8.70%, while *Ipomoea purpurea* and *Clitoria ternatea* contributed 8.70% and 4.35%, respectively. The results highlight the floral preferences of *H. itama* bee at MARDI. This comprehensive melissopalynological analysis contributes to biodiversity monitoring and conservation efforts by providing a better understanding of the floral resources used by stingless bee species in the MARDI ecosystem.

Key words: Floral resources, *Heterotrigona itama,* honey authenticity, melissopalynology, pollen

INTRODUCTION

Melissopalynology, a branch of palynology, refers to the study of pollen found in honey. Melissopalynology offers valuable insights into the flora that bees frequent and the nectar and pollen they collect for honey production. Melissopalynological studies have been frequently employed to determine the geographical and floral origins of honey (Rodopoulou *et al*., 2018). It is also an important field of study as it allows for the identification and analysis of pollen grains found in honey to determine the floral sources of the nectar used by bees to produce the honey. This information can be used to determine the geographical origin of the honey and to assess its quality and authenticity (Bogdanov & Martin, 2002).

In Malaysia, stingless beekeeping (meliponiculture) has recently gained popularity. Many beekeepers now manage stingless bees on a large scale (Suhaizan *et al*., 2017). Moreover, the beekeeping industry in Malaysia is a significant and essential part of the agricultural sector, giving farmers additional income and indirectly feeding the populace through pollination services. Ramadani *et al*., (2021) suggested that studies on stingless bee species' melissopalynology can shed light on how these bees forage and the plants they visit. Researchers can identify the plant species bees visited and figure out the relative importance of different kinds of plants to the bees by studying the pollen grains in honey samples.

In Malaysia, MARDI has had a renowned stingless bee species honey farm since 2016. MARDI first introduced stingless beekeeping in 2004, with the aim of offering an alternative species that could supplement current beekeeping projects in terms of honey production and pollination services (Majid *et al*., 2020). Only two species of stingless bees, *H. itama* and *Geniotrigona thoracica,* have been widely reared for commercial honey production. In the southern part of Malaysia, *H. itama* is a species that is easily found in the forest and is highly sought after by beekeepers (Majid *et al*., 2020). Despite their small body size, *H. itama* can collect a wide variety of nectar and pollen while foraging (Benedick, Gansau, & Ahmad, 2021).

Pollen, a fine powder produced by the male organs of flowers in seed plants, is essential for plant fertilization and fruit and vegetable production (Faegri & Iversen, 1989). Pollination occurs when pollen is transferred between plants through various means, including wind, insects, and animals (Buchmann & Nabhan, 1996). The study of pollen morphology and viability has

Article History
Accepted: 9 April 2025
First version online: 30 June 2025
Cite This Article:
Rahmatullah, S., Ahmad Juhari, M.A.A., Jaapar, M.F., Mohamad, B. & Samion, M.N. 2025. Melissopalynological analysis and plant preferences of stingless bee, *Heterotrigona itama* (Hymenoptera: Apidae) at the Malaysian Agricultural Research and Development Institute (Mardi), Serdang. Malaysian Applied Biology, 54(2): 65-73. https://doi.org/10.55230/mabjournal.v54i2.3364

been used to identify potential crop pollinators and support the development of sustainable agricultural practices. For pollen identification, various microscopic techniques are used. Light microscopy (LM) uses morphological (pollen shape) and surface structures (exine) identification (Pospiech *et al*., 2021). Scanning electron microscopy (SEM) is another microscopic technique that allows for a more accurate determination of the type of pollen grain based on differences in surface structures (Pospiech *et al*., 2021). The use of transmission electron microscopy (TEM) can provide information on the morphology of internal structures, such as the cytoplasm, nucleus, and organelles within the pollen grain, but due to the processing complexity, it is primarily used for descriptive studies in the field of palynology. Other LM techniques, such as phase contrast (Ph), Nomarski phase, and polarization, can be used to highlight surface structures (Pospiech *et al*., 2021). In this research, a LM was used to identify the pollen structure from honey and floral resources. While SEM is more accurate in revealing fine surface details, the LM method was chosen for its simplicity, cost-effectiveness, and ability to quickly assess a large number of pollen samples. However, SEM could be considered in future studies for more detailed surface analysis of specific pollen types.

Honey is a natural product that is widely used by humans due to its sweet taste and health benefits that bees produce from nectar and honeydew of various plants. To enhance honey production, it is essential to first understand the plants that play a role in its production (Rosdi *et al*., 2021). However, honey containing pollen from poisonous or toxic plant species is not suitable for human consumption. Pollen sensitivities can cause allergic reactions to honey because bees transport pollen through the air (Helbling *et al*., 1992). According to Agashe and Caulton (2019), pollen of *Lasiosiphon* sp is not harmful to bees, but honey contaminated with this pollen is extremely dangerous and poisonous for human consumption. *Euphorbia geniculata* pollen, on the other hand, is highly toxic and almost lethal to bees, but honey contaminated with this pollen does not affect human health (Gela, 2017). In contrast, *H. itama* pollen contains various beneficial compounds, including fatty acids, phenolic compounds, and other nutrients, which contribute to its health benefits. Linoleic acid (omega-6) is the most abundant fatty acid in bee pollen, followed by α-linolenic acid (omega-3) and palmitic acid (Mohammad *et al*., 2021). Pollens selected by *H. itama* contain a high concentration of phenolic compounds, such as flavonoids and tannins, which have antimicrobial and antioxidant properties. *H. itama* bee bread is a good source of amino acids, minerals, polyphenols, carotenoids, flavonoids, and phytosterols, all of which contribute to its antioxidant properties. These compounds contribute to the potential health benefits of *H. itama* pollen, such as lower Lee obesity index and total cholesterol, low-density lipoprotein (LDL), and high-density lipoprotein (HDL) levels (Mohammad *et al*., 2021). Based on this consideration, melissopalynology studies of stingless bee species are an important tool for understanding the ecology and behavior of these bees, as well as for promoting their conservation and sustainable use.

Despite the significance of stingless bees in Malaysian agriculture, limited research has been conducted on their pollen sources (Ghazi *et al*., 2018). Thus, this study aims to provide detailed information on the pollen preference of *H. itama* at MARDI, contributing to a broader understanding of stingless bee ecology and supporting conservation and sustainable beekeeping practices.

MATERIALS AND METHODS

Materials

Honey and flower samples were collected from the Stingless Bee farm located at Malaysia Agro Exposition Park Serdang (MAEPS), MARDI Serdang, Malaysia (Figure 1). The sampling was conducted during the period from July to August 2023, specifically during daytime hr between 12:00 p.m. and 1:00 p.m., when most flowers were in full bloom and accessible to foraging bees. A total of 10 ml of honey was extracted directly from one *H. itama* beehive for microscopic analysis to identify the pollen types. Additionally, 17 different floral samples were collected from different plant species within a 50-meter radius of the beehive to compare with the pollen in the honey collected (Azmi *et al*., 2015). For pollen reference analysis, flowers from dominant tree species in the apiary were carefully plucked and preserved in Eppendorf tubes containing glacial acetic acid to ensure proper preservation.

Methods

Microscopic analysis for pollen identification involved acetolysis treatment of the floral samples following Erdtman (1963) and Juhari *et al*., (2021). Approximately 1–2 ml of pollen sample was placed in a centrifuge tube and treated with a mixture of 9 parts acetic anhydride to 1 part concentrated sulfuric acid. The mixture was incubated in a 70°C water bath for 10–15 min to dissolve organic material and enhance pollen wall visibility. Samples were then centrifuged at 3000 rpm for 5 min, the supernatant discarded, and the pellet rinsed twice with glacial acetic acid to remove residual chemicals. Cleaned pollen grains were suspended in glycerine jelly and mounted on slides with coverslips for microscopic analysis. The acetolysis facilitated the removal of unwanted organic material and improved the visibility of pollen characteristics (Jones, 2014). After acetolysis, pollen grains were observed under an Olympus BX43 Biological LM using the single-grain technique following Zetter (1989). In this technique, individual pollen grains are isolated and examined one at a time to allow precise identification based on detailed morphological features, such as size, shape, aperture type, and exine patterns.

For honey samples, raw honey was centrifuged, and the sediment was left overnight before being examined and photographed using the Olympus BX43 LM, equipped with Toupview software. The analysis was repeated three times to ensure accuracy in pollen identification. All the pollen grains present in the honey sample were examined and subsequently matched with pollen types from the collected floral samples. To obtain the average size measurement, at least 10 pollen grains from each species were measured in terms of their polar and equatorial diameters using a calibrated light microscope. The mean values were then calculated to represent the typical pollen size for each species. These procedures allowed for a comprehensive examination of pollen present in both floral and honey samples, enabling accurate identification and comparison. Although LM is more affordable and widely used for this study, SEM could provide more detailed results in future research, improving the accuracy and resolution of pollen identification.

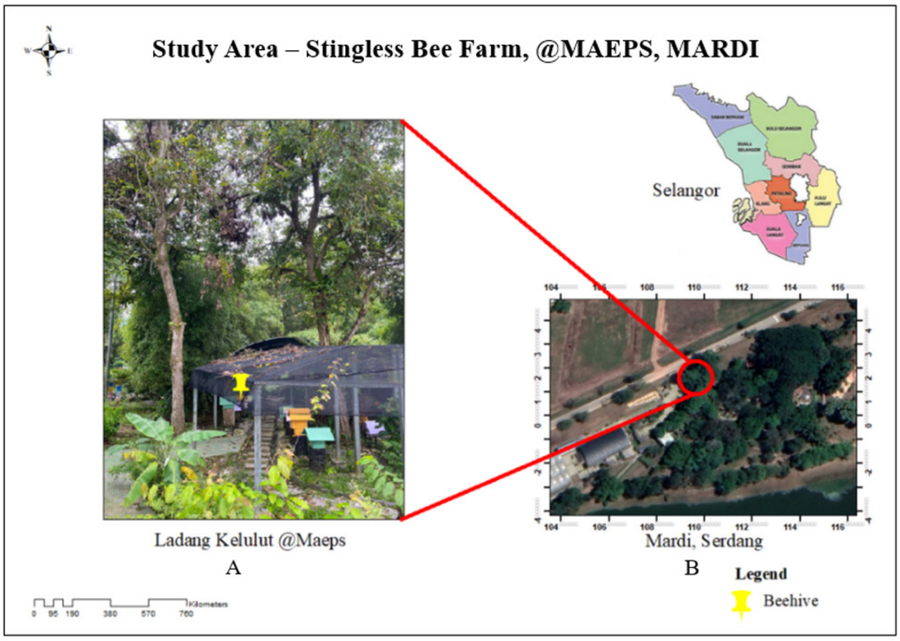

Fig. 1. A) Study Area Map of Ladang Kelulut @MAEPS. B) MARDI, Serdang, Selangor.

RESULTS

Pollen composition

The analysis of honey samples revealed a diverse composition of pollen types, enhancing our understanding of the floral resources utilized by *H. itama*. A total of eight pollen types, each corresponding to a different plant species identified in the honey samples: *Clematis crispa* L., *Combretum indicum* (L.) DeFilipps, *Cucumis sativus* L., *Lagerstroemia speciosa* (L.) Pers., *Ageratum conyzoides* L., *Averrhoa bilimbi* L., *Clitoria ternatea* L., *and Ipomoea purpurea* (L.) Roth (Figure 2). All identified pollen types matched plant species within the 17 floral samples collected, indicating a strong correlation between local floral availability and bee foraging behavior. The pollen morphology of these eight species is shown in Figure 3, while a summary of pollen morphological information for each species is presented in Table 1.

The relative frequency (%) of each pollen type was determined using the following formula:

$$Relative \quad frequency \quad \left(\%\right) = \frac{number \quad of \quad grains \quad of \quad specific \quad pollen \quad type}{the \quad total \quad number \quad of \quad pollen \quad grains \quad counted} \times 100$$

Among the identified pollen types, *C. crispa* was the most dominant, constituting 21.74% of the total pollen content. *C. indicum* and *C. sativus* followed closely, each contributing 17.39%. *L. speciosa,* contributed 13.04%, further enriching the floral diversity accessed by the bees. *A. conyzoides, I. purpurea,* and *A. bilimbi* were comparable at 8.70%, indicating their significance in the foraging behavior of local honeybees (Figure 4). Although *C. ternatea* was present in a lower proportion at 4.35%, it nonetheless adds to the complexity of the honey's botanical origins.

This nuanced distribution of pollen types reflects the diverse plant species that *H. itama* interacts with, emphasizing the intricate relationship between bees and their floral environment. Such interactions ultimately shape the unique characteristics of the honey produced.

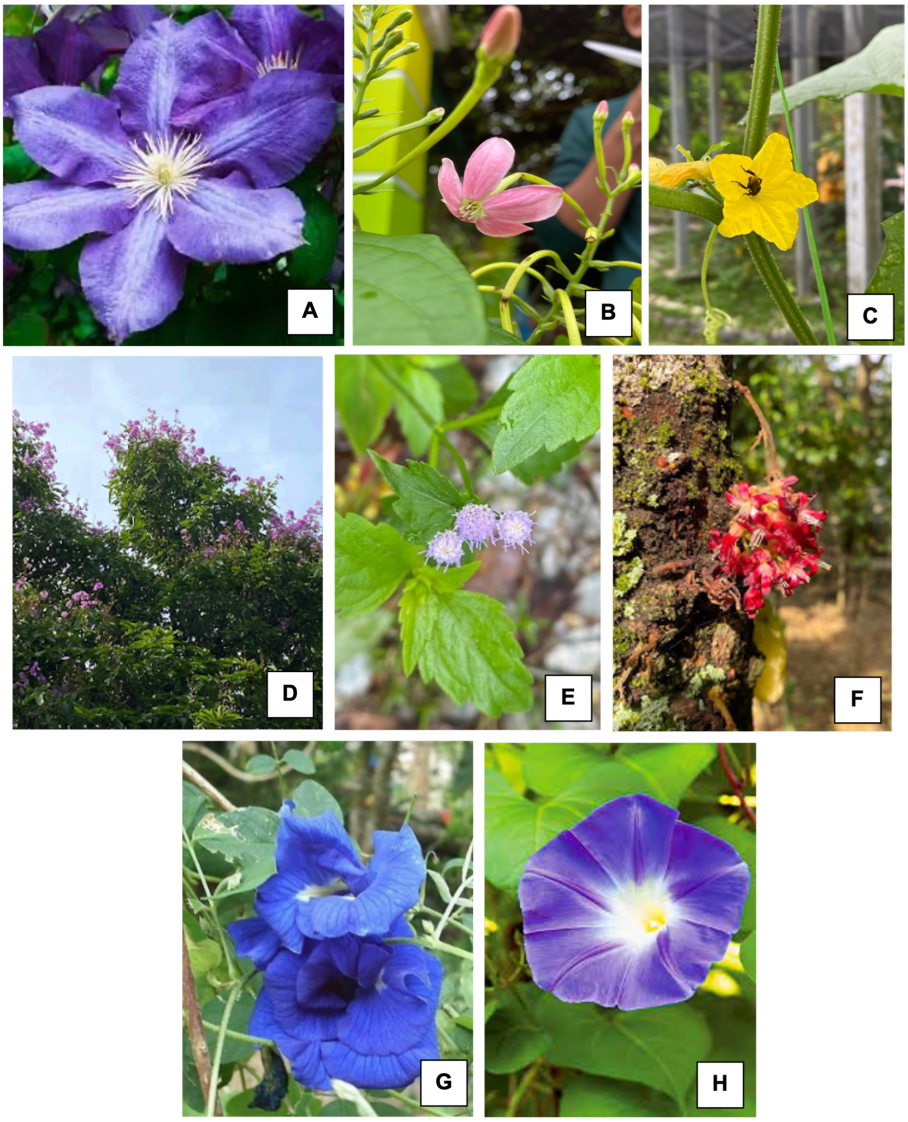

Fig. 2. Flowers visited by *H. itama*. (A) *Clematis crispa* (B) *Combretum indicum* (C) *Cucumis sativus* (D) *Lagerstroemia speciosa* (E) *Ageratum conyzoides* (F) *Averrhoa bilimbi* (G) *Clitoria ternatea* (H) *Ipomoeia purpurea*

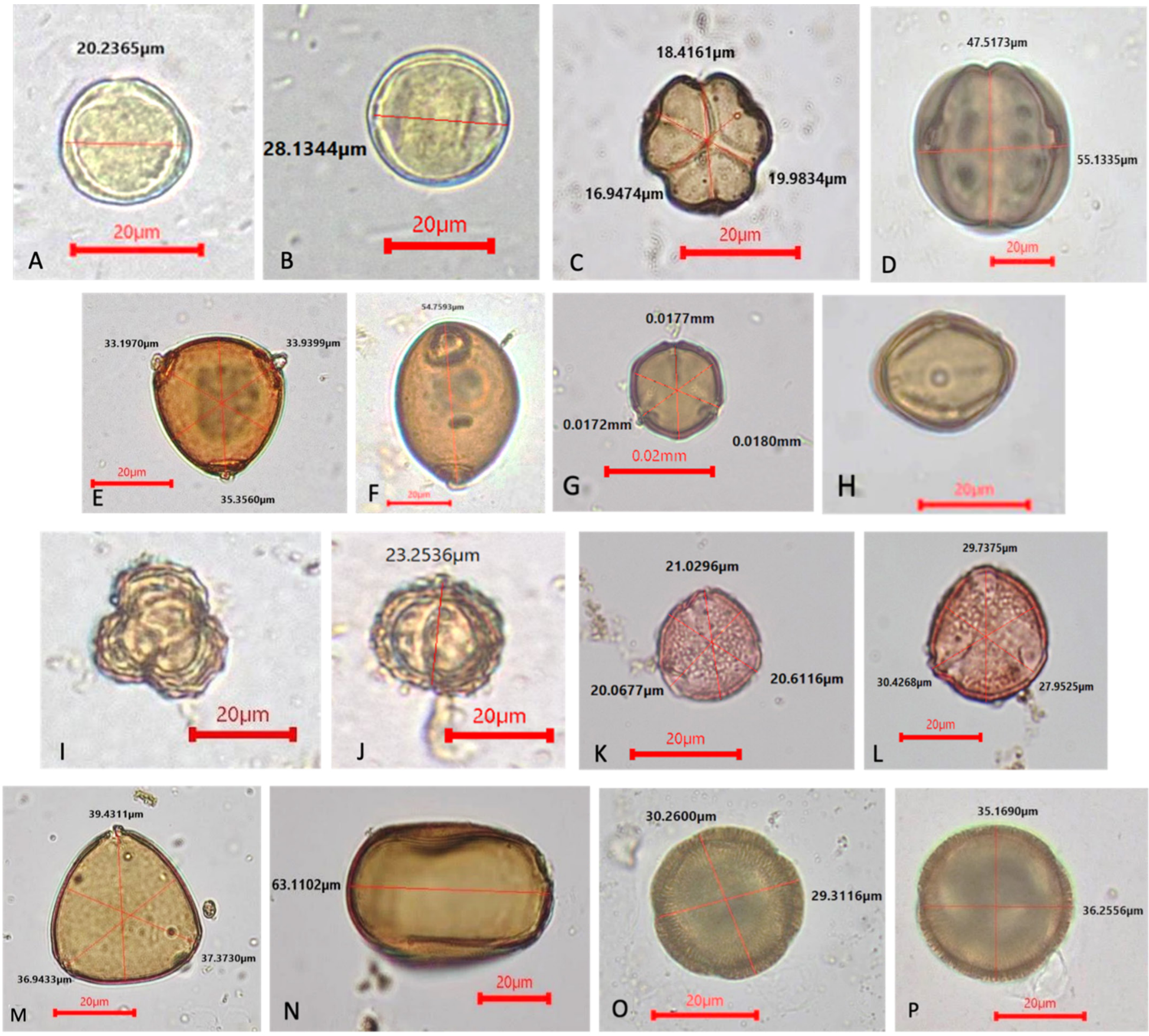

Fig. 3. Pollen morphology under light microscopy. A-B) *C. crispa. (Polar view)* C-D) *C. indicum. (Polar and Equatorial view)* E-F) *C. sativus. (Polar and Equatorial view)* G-H) *L. speciosa. (Polar and Equatorial view)* I-J) *A. conyzoides (Polar and Equatorial view).* K-L) *A. bilimbi. (Polar view)* M-N) *C. ternatea. (Polar and Equatorial view)* O-P) *I. purpurea. (Polar view)*

Table 1. Summary of pollen morphological characteristics under the light microscope

Species	Class	Aperture	P/E Index	Shape	Polar measurements (P) (µm) (Mean)	Equatorial measurements (E) (µm) (Mean)
C. crispa	Colpate	Tricolpate	0.90	Oblate-Spheroidal	20.23	22.32
C. indicum	Colpate	Tricolpate	0.71	Suboblate	19.24	26.88
C. sativus	Porate	Triporate	0.65	Spheroidal	44.73	54.75
L. speciosa	Colporate	Tricolporate	0.58	Prolate spheroidal	17.63	30.15
A. conyzoides	Colporate	Tricolporate	0.87	Spheroidal	20.30	23.26
A. bilimbi	Colpate	Tricolpate	0.95	Spheroidal	27.88	29.19
C. ternatea	Porate	Triporate	0.80	Suboblate or oblate-spheroidal	51.08	63.11
I. purpurea	Porate	Pantoporate	1.00	Spheroidal	35.70	34.80

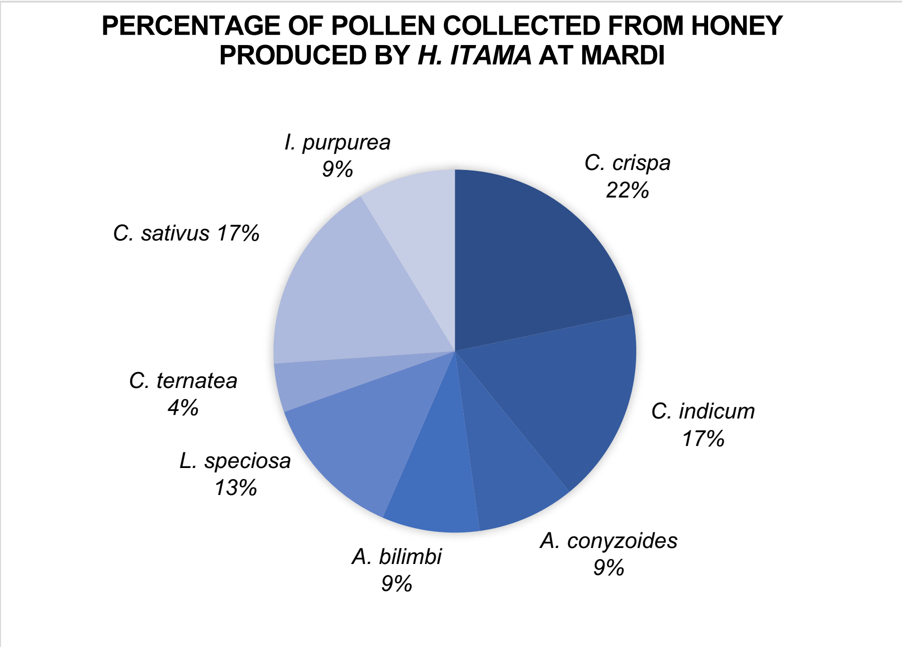

Fig. 4. The percentage of pollen collected from honey produced by *H. itama* at MARDI.

DISCUSSION

The analysis of the honey sample from MARDI provides valuable insights into the diverse pollen sources contributing to its composition. The identified pollen types and their respective percentages reveal the floral composition of the area and elucidate the foraging behavior of *H. itama*.

H. itama exhibits a preference for foraging in proximity to its nesting sites, primarily selecting areas rich in diverse food sources. Notably, *C. crispa* and *C. indicum* emerge as significant nectar and pollen sources in the result. Research indicates that *H. itama* favors nectar with a sugar content exceeding 35% (Basari *et al*., 2018), highlighting the importance of sugar concentration in foraging decisions, particularly as ambient temperatures rise. While the availability of food near the beehives allows many foragers to exploit the food sources, foraging activity decreases as the ambient temperature rises toward midday between 12:00 pm and 1:00 pm (Basari *et al*., 2018). Active foraging resumes in the early afternoon when food with a high sugar concentration is abundant. The plant species available in *H. itama*'s environment influence its foraging behavior. These plant species' availability and distribution may influence bee foraging behavior and plant preferences (Jaapar *et al*., 2021). The most preferred flowers show different colors such as *C. crispa* is purple, while *C. indicum, C. sativus,* and *L. speciosa* are red, yellow, and purple serially.

The flowers observed during the research were around a 50-meter area of the beehive and the preferred flower results also were closely located to the beehive. This gives a piece of knowledge about *H. itama*, that it might take distance and color into account. According to Jaapar *et al*. (2018), *H. itama* prefers to forage in areas close to their nesting site, where there are a variety of food sources. The ideal temperature for *H. itama* foraging behavior was discovered to be 29°C to 32°C. *H. itama*'s peak foraging activity was discovered to be between 8:00 am to 5:00 pm. These findings can help researchers broaden their understanding of stingless bee foraging behavior, as well as farmers manage chemical toxins within meliponiculture.

Seasonal changes can have a significant impact on *H. itama* bees' melissopalynological activities. Wahyuningtyas *et al*. (2021) suggested that during the rainy season, bee activity in search of nectar, pollen, and resin decreases dramatically, resulting in a "death period" with no honey flow. The study in MARDI also shows that, during the rainy season the activity of bees and flowers might decrease since flower availability decreases during the rainy season. The highest contributor flower *C. crispa* is not much available during the rainy season while in summer, the flower availability is rich in number and all the flowers are available during the season. The bees can have a healthy ecosystem for their food and for collecting nectar and pollen, they can visit more flowers since all the flowers are available. The ideal temperature for *H. itama* foraging behavior is affected by seasonal changes in floral resources. Adnan *et al*. (2021) suggested that high environmental temperatures may necessitate more energy for foraging, and high humidity may result in bee dehydration. However, in response to these conditions, bees may modify their foraging activities to deal with the difficulties. Seasonal changes can affect a variety of aspects of *H. itama* bee behavior, such as foraging activities, temperature and humidity preferences, water foraging, stress levels, and urbanization-related resource availability. Understanding these behavioral changes can assist beekeepers and researchers in developing strategies to support *H. itama* bee health and survival in a variety of environments.

In the study of *H.itama* honey, a distinct relationship between pollen size and the percentage of pollen collected by the bees was observed. Larger pollen grains, such as those of *C. ternatea* (4.35%), were found in lower percentages, likely due to the greater energy required for collection and transport (Majid, Ellulu, & Abu Bakar, 2020). In contrast, smaller pollen types like *C. crispa* (21.74%) were more abundant, suggesting they are easier to gather and carry. This indicates that bees may prioritize smaller pollen grains for efficiency (Hao, Tian, Wang & Huang, 2020). In addition, pollen collection is also influenced by nutritional factors, such as protein content, which is crucial for brood development and overall colony health (Ghosh *et al*., 2020).

In addition to pollen size, *H. itama* demonstrated a clear preference for brightly colored flowers, including purple *C. crispa*, red *C. indicum*, yellow *C. sativus*, and purple *L. speciosa*. These preferences align with findings that stingless bees are drawn to bright hues like purple, yellow, and red, which serve as visual cues to guide them toward rich pollen and nectar sources (Gerten Née Papiorek *et al*., 2015). For instance, *H. itama* frequently visits brightly colored plants such as *Antigonon leptopus* and *Portulaca grandiflora*, further supporting the idea that flower color plays a key role in foraging behavior (Shamsul & Samsudin, 2015).

Bees respond to visual, olfactory, and mechanosensory signals, such as flower color, fragrance, pollen size, and the amount of pollen a flower releases (Bisrat & Jung, 2022). They also suggested that bees primarily rely on floral scents to locate blooming flowers, as their visual cues are limited, with floral scents serving as key attractants that elicit behavioral responses critical for maintaining plant-pollinator relationships. These cues help bees determine which flowers are most worth visiting. Bees also modify their preferences based on feedback from the colony and prior foraging experiences, which suggests that foraging behavior is both a learned and adaptive process (Nicholls & Hempel de Ibarra, 2017). Pollen size, flower color, and proximity to the hive are all important factors in foraging efficiency (Ghosh, Jeon & Jung, 2020). Smaller, nearby pollen grains are typically favored for energy conservation, but during peak flowering periods, bees may expand their foraging range to collect a wider variety of pollen types. This combination of factors including pollen size, nutritional value, color, and distance, helps optimize *H. itama*'s foraging strategy (Azmi *et al*., 2016).

To support the long-term viability of *H. itama* and enhance melissopalynological research, MARDI should implement comprehensive conservation strategies. These include establishing and maintaining diverse flowering habitats, particularly within a 50m radius of beehives, prioritizing key species such as *C. crispa*, *C. indicum,* and *C. sativus*. Monitoring population dynamics and engaging communities in sustainable beekeeping practices are also critical. Collaborative research with institutions and NGOs on environmental impacts, pesticides, and climate change will inform adaptive management strategies, ensuring that the foraging needs of *H. itama* are met and contributing to their health and productivity.

CONCLUSION

The melissopalynological investigation of honey from *H. itama* at the MARDI has identified significant floral preferences, with *C. crispa*, *C. indicum*, and *C. sativus* emerging as key contributors. This finding enhances our understanding of plant-pollinator interactions and underscores the vital role of *H. itama* in supporting local biodiversity. The insights gained are pivotal for conservation efforts, emphasizing the need to preserve these key plant species to sustain bee populations and maintain ecosystem health. Additionally, this research provides a framework for assessing honey quality and authenticity, linking floral diversity directly to honey composition. Such connections support both ecological balance and agricultural sustainability in Malaysia, emphasizing the importance of preserving the intricate relationships between pollinators and their floral resources.

Future studies could focus on expanding the geographical scope of melissopalynological investigations across diverse regions of Malaysia, such as the highland and coastal ecosystems. This broader approach will yield a more comprehensive understanding of the floral preferences of *H. itama* and how these may vary across different habitats. Furthermore, conducting longitudinal studies could reveal temporal variations in plant-pollinator interactions, particularly in response to seasonal changes in floral availability and bee foraging behavior. Such research endeavors will deepen ecological insights and inform conservation strategies aimed at preserving both pollinator species and their essential floral resources.

ACKNOWLEDGEMENTS

This work was supported by the Ministry of Higher Education (MOHE) for funding under the Fundamental Grant Scheme (FRGS) (FRGS/1/2021/WAB02/UPM/02/3). We also would like to extend our gratitude to MARDI Serdang for their generous support in facilitating this research.

ETHICAL STATEMENT

Not Applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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