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### **THE FORAGING BEHAVIOR OF** *Heterotrigona itama* **(COCKERELL) (HYMENOPTERA: APIDAE: MELIPONINI) IN CLOSE SYSTEM ROCK MELON (CUCUBITALES: CUCURBITACEACE) CULTIVATION**

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#### **ABSTRACT**

Stingless bees (Meliponini) exhibit intricate foraging behavior that is essential for their colony's survival and reproductive success. Stingless bees exhibit a diverse range of foraging strategies that are customized to their specific ecological niche. These strategies encompass both solitary and cooperative with individuals often specializing in particular tasks within the foraging process. Through sophisticated communication mechanisms, such as waggle dances and pheromone trails, stingless bees effectively coordinate foraging efforts, maximizing resource acquisition while minimizing energy expenditure. While hand pollination by humans is the typical method employed for pollinating rock melons, it is not the preferred option due to its labor-intensive and time-consuming. Through spatial analysis and community field work, this study was conducted to assess potential *Heterotrigona itama* (Cockerell) as pollinators in rock melon cultivation and to investigate the foraging behavior of *H. itama* in rock melon cultivation inside the glasshouse. The experiment was done by determining the foraging activities, floral visitation frequency and activities rate of stingless bee in rock melon cultivation inside glasshouse. Results show that *H. itama* flying out from hive were 1522 individuals, while 1034 individuals of stingless bees flew into hive were recorded for foraging activities. The peak hour for foraging activities by *H. itama* inside the glasshouse was in the morning, from 10 a.m. to 11 a.m. On floral visitation frequency, *H. itama* visited rock melon male flowers more frequently than female flowers. However, for activities rate, *H. itama* spent longer a period foraging the female flowers than the male flowers. Thus, the findings from the present study contribute to new and impactful knowledge gained by rock melon growers and are recommended as innovations in the cultural practice of modern rock melon industry.

**Keywords:** Activities rate, floral visitation, foraging activity, pollination stingless bees

### **ABSTRAK**

Lebah kelulut (Meliponini) mempamerkan tingkah laku mencari makanan yang rumit yang penting untuk kemandirian koloni mereka dan kejayaan pembiakan. Lebah kelulut mempamerkan pelbagai strategi mencari makanan yang disesuaikan dalam lingkungan ekologi khusus mereka. Strategi ini merangkumi kedua-duanya iaitu solitari dan bekerjasama dengan individu yang sering mengkhusus dalam tugas tertentu dalam proses mencari makanan. Melalui mekanisme komunikasi yang canggih, seperti tarian bergoyang dan jejak feromon, kelulut menyelaraskan usaha mencari makanan secara berkesan, memaksimumkan pemerolehan sumber sambil meminimumkan pengeluaran tenaga. Walaupun pendebungaan tangan oleh manusia adalah kaedah biasa yang digunakan untuk pendebungaan Tembikai Batu, ia bukanlah pilihan yang diutamakan kerana memerlukan intensif buruh dan memakan masa. Melalui analisis awal dan kerja lapangan komuniti, kajian ini untuk menilai potensi kelulut spesies *Heterotrigona itama* (Cockerell) sebagai pendebunga dalam penanaman Tembikai Batu dan untuk mengkaji tingkah laku mencari makan *H. itama* dalam penanaman Tembikai Batu di dalam rumah kaca. Eksperimen ini adalah bertujuan menentukan aktiviti mencari makan, kekerapan lawatan bunga dan kadar aktiviti kelulut dalam penanaman Tembikai Batu di dalam rumah kaca. Hasil menunjukkan bahawa *H. itama* yang terbang keluar dari sarang adalah 1522 individu, manakala 1034 individu daripada kelulut terbang masuk ke dalam sarang telah direkodkan untuk aktiviti mencari makan. Waktu puncak untuk aktiviti mencari makan oleh *H. itama* di dalam rumah kaca adalah pada waktu pagi, dari 10 pagi hingga 11 pagi. Pada kekerapan lawatan bunga, *H. itama* melawat bunga jantan Tembikai Batu lebih kerap daripada bunga betina. Walau bagaimanapun, untuk kadar aktiviti, *H. itama* menghabiskan lebih lama tempoh mencari bunga betina berbanding bunga jantan. Oleh itu, penemuan daripada kajian ini menyumbang kepada pengetahuan baharu dan berimpak yang diperoleh oleh penanam Tembikai Batu dan disyorkan sebagai inovasi dalam amalan kultura industri Tembikai Batu moden.

**Kata kunci**: Aktiviti mencari makanan, lawatan bunga, kadar aktiviti, pendebungaan, kelulut

# **INTRODUCTION**

Rock melon is an andromonoecious plant species, where male and hermaphrodite flowers occur separately on the same plant (Boualem et al. 2016). In cucurbits generally, the production of male flowers is more than female flowers, which is considered an undesirable trait of cucurbits (Prothro et al. 2013). The foraging behaviour of the insect pollinators differs depending on the characteristics and behaviour that influence the ability to pollinate. The successful rate of pollination by an insect can be determined as a pollinator's ability to compatibly deposit pollen grains on the stigma of the flowers (Horsburgh et al. 2011).

The foraging behaviour of stingless bee species may vary according to the species strategies, interspecific competition, floral preference, and plant phenology (Hilário et al. 2001). Their efficiency in pollen removal and deposition, and other behavioral factors affecting pollination effectiveness may differ among taxa and even individuals (Zych et al. 2013). Stingless bees can adapt to the surroundings and are able to forage uni-flowers. Flower constancy is an adaptive behaviour and the best strategy for bees to reduce the energy that would be redundantly spent for choosing other plants (Grüter & Ratnieks 2011).

The factors that influence flower constancy for pollinators include of odour or chemical compounds, colours, and shapes of flowers (Grüte & Ratnieks 2011). Stingless bees can be introduced as pollination agents for plants cultivated in residential areas and greenhouses. These bees have been introduced to improve pollination in greenhouse plants in The Netherlands and Japan. Stingless bees have been proven efficient pollinators in crop production (Slaa et al. 2006).

The contribution of stingless bee as a pollinator is more significant than the honeybees, since stingless bees are more effective in pollinating many cultivated plant species (Roubik 2014). Stingless bees can tolerate hot temperatures in comparison to honeybees (Remy et al. 2014). In the present study the stingless bees were place in a greenhouse where rock melon was cultivated. The pollinators should be able to transfer as many pollen grains as possible onto the stigmas since they can move from flower to flower (Rader et al. 2009). Effectiveness as a pollinator can also be measured based on its foraging behaviour, pollinating ability, and pollinator ecology (O'Neill 2010). Thus, the objectives of this study were to determine the foraging behaviour of *Heterotrigona itama* in rock melon cultivation in close system.

## **MATERIALS AND METHODS**

### **Study Area**

The study area was in a glasshouse located at Integrated Farm (2.995040420756378, 101.71418933878206), Faculty of Agriculture, Jalan Maklumat, Universiti Putra Malaysia (UPM), Serdang, Selangor, Malaysia. The area of glasshouse was about 7m x 25m with a maximum height of 4m, and was equipped with insect-proofed (using fine mesh) all around the glasshouse.

### **Sampling Area Preparation**

Rock melon seeds were planted in the glasshouse at Ladang 10, Universiti Putra Malaysia. Seeds were sown on peat moss in a seed tray and placed under a shelter of 70% light shade. After 10 days, germinated seedlings were transplanted into polybags (60 cm x 40 cm) containing coco peat and arranged inside the glasshouse. The plants were irrigated twice daily using drip fertigation controlled by a timer system. The rock melon plants were placed in four rows, and in each row 10 plants were placed. Supporting ropes were tied at each polythene bag to facilitate climbing by the plants. A hive of *H. itama* was placed inside the glasshouse and left to acclimatize for one week to adapt to the conditions of the glasshouse before data collection ensued. One hive of *H. itama* was adequate with the width of the glasshouse.

### **Sampling Period and Experimental Plot of Rock Melon Cultivation in Glasshouse**

Sampling activity was conducted from  $5$  May  $-25$  May 2019. The sampling period was three weeks, the data collection was done during the rock melon flowers were blooming. Data were recorded daily using a phone camera to observe and record the *H. itama* activities. The flight activities of stingless bees were recorded by observing the *H. itama*: (i) flying into the hive, (ii) flying out from the hive, and (iii) bringing pollens into the hive through the entrance of the hive. The individuals were counted using a manual hand counter. The observation was done for five minutes in each hour (Fidalgo & Kleinert 2007), starting from 8.00 a.m. to 11.00 a.m. based on the preliminary study and then data were cumulated for the three week experiment. The floral visitation frequency activity of *H. itama* was done by monitoring at each male flower, and female flower and how many times *H. itama* visited the flowers. While for activity rate, the landing duration of *H. itama* on each male and female flower was counted. The number of stingless bees visiting male and female flowers, was counted and recorded using phone camera. The experimental design implemented was a complete randomized design (CRD); five rock melon plants were selected randomly for data collection.

## **Data Analysis**

The data of flight activities within weeks and time, floral visitation frequency and activities rate were analysed using Two-Ways Analysis of Variance (ANOVA) at a 5 % of level of significance level using the Post Hoc Tukey test, from SAS Software 9.4 version statistical program.

## **RESULTS AND DISCUSSION**

### **Flight Activities of Stingless Bee** *Heterotrigona itama* **In Glasshouse**

The total number of stingless bees recorded flying out from the hive was 1522, while 1034 individuals flew into the hive. The total number stingless bees that brought pollens into the hive was 634, which was less than individuals flying into the hive and flying out from the hive, according to the caste system of the stingless bees, where by workers brought nectar, others brought resin and other substances. There was a significant interaction ( $F=41.35$ ;  $df= 6.156$ ; *P*<0.001) between the time and flight activities of stingless bees. The flight activities of stingless bees were influenced by the week of flowering period (F=6.02; df=4,156; *P*=0.0002).



Note: Means with the same letter within column are not significantly different (Tukey's test, *P*<0.05)

Based on the Table 1, the stingless bees flying into the hives were significantly differed between the time (F=15.04; df=3,56; *P*<0.0001). The time at 11 a.m. was highly significantly different within the time (16.60±0.72a). The time 10 a.m. and 9 a.m. was not a different number of stingless bees flying into the hive. While the 8 a.m. is the lowest number of stingless bees flying into hive.

The reason for the highest number of stingless bees flying out from the hive was due to the different castes and individual roles. According to Roubik et al. (2016) the stingless bee ranks, the duties assigned are according to the respective castes. The forager caste seeks for food sources like nectars and pollens the soldier caste does patrol duties inside and outside the hive. They are the housekeeping caste, which keeps the hive clean including bringing out the trash (Shamsul et al. 2017). A study by (Silva et al. 2010) reported that the peak of activity was between 9.00 a.m. and 11 a.m. After 11 a.m., the incoming of resources decreased until 1 p.m., and then remaining constant for the rest of the day.

This finding was supported by studies conducted by (Jaapar et al. 2016) and (Basari et al. 2018), the active period of *H. itama* was between 8 a.m. and 11 a.m. Russell et al. (2017) also reported that the availability of nectar was higher in the morning than other times during the day. Apart from nectars and pollens in the early morning, there is also a secretion of liquid by certain leaflets, called leaf exudate (Norowi et al. 2010). Inside a greenhouse, various factors contribute to temperature dynamics, including the type of glazing material, insulation, ventilation, and shading. While the greenhouse effect raises temperatures during the day, at night, without additional heating, the temperature inside can drop rapidly, especially if the greenhouse lacks proper insulation or thermal mass to retain heat (Soussi et al. 2022).

In this study, the stingless bee brought pollen into the hive was high from 9 a.m. until 11 a.m., this because of the surrounding environment. Generally, warmer temperatures and longer daylight hours promote earlier flowering. However, excessively high temperatures or drought stress can delay flowering (Kazan & Lyons 2016). In early morning, the pollen was not ready to be collected, as humid surroundings. After sunrise, the flower starts to fully bloom, and the pollen was not humid is an effective for stingless bees forage the pollen from flowers. The study done by (Gebremedhn et al. 2014), reported low correlations of air temperature and relative humidity with the frequency of foraging honeybee (*Apis melifera*) during the blooming period of *Guizotia abyssinica* in Ethiopia. Flowers produce a diverse array of volatile organic compounds (VOCs), which collectively create their unique scent profiles. These compounds are synthesized in specialized cells within the flower, particularly in structures such as petals, stamens, and pistils. The production of floral scents is often regulated by environmental factors, developmental, and circadian rhythms (Lo et al. 2024).



Note: Means with the same letter within column are not significantly different (Tukey's test, *P*<0.05)

Table 2 shows the first week was significantly different from week 2 and week 3 for the flying in foragers. There were no significant difference between weeks when flying out and foragers of stingless bees were brought in pollen.

### **Floral Visitation Frequency of Stingless Bee in Rock Melon Cultivation**

Table 3 shows, the mean number of stingless bees visiting the flowers on the foraging time within the weeks of flowering. The foraging time of stingless bees was no significant different throughout the weeks of flowering. Stingless bees visited flowers present in the greenhouse without discrimination.

Time	<b>Number Of Stingless Bee Visit Flowers</b>		
	Week 1	Week 2	Week 3
8 a.m.	$3.05 \pm 0.28$ <sup>a</sup>	$4.92 \pm 0.40^a$	$3.85 \pm 0.24$ <sup>a</sup>
9 a.m.	$2.75 \pm 0.21$ <sup>a</sup>	$4.90 \pm 0.43^{\text{a}}$	$4.42 \pm 0.34$ <sup>a</sup>
10 a.m.	$2.97 \pm 0.25^{\text{a}}$	$4.62 \pm 0.38$ <sup>a</sup>	$4.67 \pm 0.35^{\text{a}}$
11 a.m.	$3.05 \pm 0.23$ <sup>a</sup>	$5.02 \pm 0.23$ <sup>a</sup>	$4.70 \pm 0.34$ <sup>a</sup>

Table 3. Mean number±SE of stingless bees visiting rock melon flowers within the weeks

Note: Means with the same letter within column are not significantly different (Tukey's test, *P*<0.05)



Figure 1. The frequency of stingless bee on rock melon flowers within foraging time. Note: Means with the same letter within column are not significantly different (Tukey's test, *P*<0.05)

The stingless bees visit more frequent male flowers than female flowers (Figure 1). This is due to the male flowers is higher than the production of female flowers, with a sex ratio of 6-19 male flowers to 1 female flower (Abreu-Olivera et al. 2014). High visitation frequency may increase the chances of pollen delivery, hence increasing the chance that a flower matures into a fruit (Mayfield et al. 2001).

The male flower received more visits from the stingless bees than the female flowers. Flower morphology such as flower height and size of corolla facilitates the visualization of pollinators to approach the flowers. A study conducted by (Kiill et al. 2016) found that the nectar chambers of male melon flowers had larger dimensions for width and smaller in height when compared with female flowers. This difference is related to the flower structure since the gynoecium is absent in male flowers, and the nectar chamber forms a single cavity. Being shorter in the main stem of the plant also facilitates access of the pollinator to the resource. Further, pollen viability can decline with pollen age (Murren 2002) and environmental

conditions. Hence, slower moving insects may be less likely to transfer viable pollen over distance.

The high number of male flowers can attract more pollinators and increase reproductive success in subspecies. The findings from another other study, male flowers produced either more or larger grains, male pollen viability was higher and male flowers attracted more insect visitors than hermaphroditic flowers (Zhang et al. 2009). The production of many male flowers in an andromonoecious system increases the conspicuousness of the flowers and thus improves the pollination efficiency of the hermaphroditic flowers (Barrett 2002).

## **Activities Rate of Stingless Bees in Rock Melon Cultivation**

Figure 2 shows the trend of stingless bees staying on the male and female flowers. From 8 a.m. to 9 a.m. there is a slight increase in the time of stingless bees staying on male flowers. At 10 a.m., stingless bees only stay for a short duration, while at 11 a.m. the stingless bees stayed the longest duration on male flowers. The trend for staying on female flowers shows an increase from 8 a.m. to 10 a.m. but declined at 11 a.m. This trend indicated the increasing of staying duration of female flowers by increasing of time.



Figure 2. Mean landing duration of stingless bee on rock melon flowers. Note: Means with the same letter within column are not significantly different (Tukey's test, *P*<0.05)

The male flowers there was a slight variation in the preferred time of visiting by the stingless bees. In the first week of sampling, the longest time the stingless bees landed was at 9 a.m., while for the second and third week 11 a.m. was when the insects landed for the longest time. During the first week, it can be assumed that the stingless bees were still adapting to the environment and were still tracking the food sources, thus spending more time earlier in the morning. Later, when the foraging ports were identified and recognized, they were able to pollinate and forage the nectar later in the morning. Pheromone trails may play a role in guiding stingless bees to flowers, attraction to flowers is multifaceted and influenced by a combination of factors including visual cues, resource availability, flower morphology, foraging success, environmental conditions, and colony-level communication. These factors can help elucidate the complex dynamics of plant-pollinator interactions involving stingless bees.

The stingless bees spent more time in the female flowers than the male flowers. The average time for single stingless bee staying on a male flower is about 1 minute 49 seconds, while on female flowers it was about 1 minute 87 seconds before flying to another flower. The larger surface of the corolla may facilitate the visualization, the approach, and the landing of the visitor on the female flower (Tschoeke et al. 2015). Longer foraging times may translate into greater pollen removal and more pollen deposition on stigmas (Horsburgh et al. 2011). *Heterotrigona itama* has a larger corbicular area, and in bigger stingless bees this contributes to a higher collection of food resources (Veiga et al. 2012), resulting from spending a longer time on the flowers.

Alternatively, other factors including patch size of the crop and surrounding floral resources (Dieko''ter Leadbeater & Chittka 2009), edge effects and habitat quality at field margins (Marshall 2006) and habitat preferences associated with foraging, shelter, or nesting resource requirements (Svensson et al. 2000). Many eusocial insects start their foraging activity in the morning (Roubik 2006). In this study, the active time of stingless bees was around 9 a.m. to 11 a.m. coinciding with the anther dehiscence period (from 9 a.m. to 10 a.m.) for the female flowers. However, anther dehiscence of male flowers (from 7 a.m. to 12 noon) spanned longer than the female flower (Beaulieu & Grimm 2001). The abundance and quality of food sources from flowers also influence the foraging activities of the foragers (Goulson & Sparrow 2009). This was supported by the study by Fülöp & Manzel (2000), stating that food source volume had high impact on the foraging activities of *A. melifera*.

### **Fruit Set Production**

The fruit set produced after pollination was successful by stingless bees on the female flowers of rock melon plant. Normally, the fruit set protruding at the end of a two-week flowering period, having stingless bees pollinating the rock melon flowers, fruit setting begins by the third week of flowering. The fruit was kept for 60 days before harvest to achieve maximum weight of 1.0 kg to 2.0 kg for commercial purposes.



Note: Means with the same letter within column are not significantly different (Tukey's test, *P*<0.05)

The early appearance of female flowers on the lower nodes was the leading cause of early fruit formation (Kooner et al. 2000). Santos et al. (2008) reported that crops pollinated by stingless bee *Melipona quadrifasciata* produced higher quality, heavier, and larger fruits than crops pollinated by other species. It has been suggested that stingless bee behaviour can affect the weight and diameter of fruits, since they spend more time collecting pollens and nectars (Roselino et al. 2009) and supported by a previous study conducted in Malaysia by Wahizatul et al. (2016) on cucumber and chilies, reported that chilies produced from stingless bee pollination to be heavier, longer and contained greater number of seeds per fruit. Higher numbers of seeds developing inside the fruits produced from *M. subnitida* pollination would lead to larger and heavier fruits (Cruz et al. 2005).

The higher production of fruit sets determines the success of *H. itama* as a good pollinator. Even though the fruit that must be kept for harvest is only two fruits per plant maximum. *H. itama* can adapt and give excellent service even when inhabiting inside the glasshouse. *H. itama* can tolerate and survive elevated temperatures and high humidity, making excellent candidates for pollinators. While inhabiting inside the glasshouse *H. itama* can pollinate the rock melon flowers, they can be exceptionally good pollinator candidates for open environment crops, too.

Pollination of rock melon by stingless bees resulted in fruits of higher quality than those from hand cross-pollinated and self-pollinated plants (Wahizatul et al. 2018). These results are consistent with those from earlier studies that reported higher fruit sets in stingless beepollinated crop plants than in self-pollinated plants (Nunes-Silva et al. 2013). The present study also supported the finding by (Mehdi et al. 2010) where there was a significant increase in seed production of fruits after pollination from stingless bees.

## **CONCLUSIONS**

This study highlighted the importance of foraging behaviour of stingless bees as an efficient pollinator in rock melon cultivation in the glasshouse. Moreover, the ability to adapt and tolerate the temperatures inside the glasshouse, work well and found to be more efficient than pollination using manual labour. The production of rock melon fruit set is a major benchmark to suggest the stingless bee as a highly effective insect pollinator for rock melon cultivation in the glasshouse.

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# **AUTHORS DECLARATIONS**

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### **Conflict of Interest**

The authors declare that they have no conflict of interest.

### **Ethics Declarations**

No ethical issue required for this research.

## **Data Availability Statement**

My manuscript has no associated data.

### **Authors' Contributions**

Siti Asma' Samsudin (SAS), Syari Jamian (SJ) and Nur Azura Adam (NAA) conceived this research, SAS designed the experiments; SAS, Mohamad Syukri Tan Shilan (MSTS) and Wan Nur Asiah Wan Mohd Adnan (WNAWMA) do the research and collect the samples from the field. SAS, NAA, Norida Mazla (NM), and Puteri Edaroyati Megat Wahab (PEMW) participated in the interpretation of the data. SAS, SJ, and NAA wrote the paper and participated in the revisions of it. All authors read and approved the final manuscript

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