



UNIVERSITI PUTRA MALAYSIA

***FORAGING BEHAVIOURS OF STINGLESS BEES *Heterotrigona itama*
Cockerell AND *Geniotrigona thoracica* Smith AS POTENTIAL
POLLINATORS TO STAR FRUIT *Averrhoa carambola* L.***

WAN NUR ASIAH BINTI WAN MOHD ADNAN

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By

WAN NUR ASIAH BINTI WAN MOHD ADNAN

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in
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March 2021

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

FORAGING BEHAVIOURS OF STINGLESS BEES *Heterotrigona itama* Cockerell AND *Geniotrigona thoracica* Smith AS POTENTIAL POLLINATORS TO STAR FRUIT *Averrhoa carambola* L.

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March 2021

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Star fruit, *Averrhoa carambola* is Malaysia's most exported exotic fruit and is mostly marketed overseas. The demand for this fruit increases yearly and sometimes exceeds the supply capacity. The low production of this fruit due to the pests and diseases also decreased the cultivation land. To improve the productivity of this fruit to prevent insect pest visitation and other diseases, star fruit trees have been planted in netting structure and there is a need to find a potential pollinator to enhance the fruit production. Stingless bee could be one potential pollinator to be introduced in the netting structure. Unfortunately, there is still a lack in knowledge on the foraging behaviours of *Heterotrigona itama* and *Geniotrigona thoracica* in order to confirm their potential as efficient pollinators of star fruit flowers. To fill the knowledge gap, this study was conducted at the Integrated Farm, Universiti Putra Malaysia (UPM) to i) investigate the foraging activities of two selected stingless bee species, *G. thoracica* and *H. itama* in relation to seasonal and abiotic factors, ii) determine the pollen resources taken by *G. thoracica* and *H. itama*, and construct the pollen profile as a baseline data, iii) assess the efficacy and compare their visitation rate to the flowers of star fruit. The observation of bees that flew in and out from their nest was made hourly from 0800 to 1600 hours. Pollens from their corbiculae legs were collected, examined and counted under light microscope and SEM. The flower visitation rates of these bees were calculated based on visitation frequency on star fruit flowers. Results showed that the daily foraging activities and foraging pattern of these two bee species were significantly different at various times of the day. The two bees were observed to be more actively foraging in the morning during 0900 hours to 1100 hours after which they slowed down until the end of the day. Nectar collection for *G. thoracica* (67,363 individuals) and *H. itama* (53,518 individuals) were significantly higher compared to pollen collection which were 11,075 individuals (*G. thoracica*) and 6,803 individuals (*H. itama*). Bee activities also varied at different time and seasons (wet and dry) of the year and was demonstrated by significant fluctuations in the number of foraging bees among all months of observations. The highest foraging activities were recorded in January 2015 (wet

season) for both species, i.e., 12,303 individuals (*G. thoracica*) and 12,979 individuals (*H. itama*). The lowest foraging activities were recorded in September 2014 for *G. thoracica* (7,517 individuals) and in August for *H. itama* (1,960 individuals). Variations in abiotic factors such as temperature, relative humidity and light intensity at the study area significantly affected the bees' foraging activities. Composition of plant diversity at the study site influenced the foraging activities of the two bee species. Three most preferred plant families were Oxalidaceae (1,342 individual pollens or 18% of the total pollens collected), Arecaceae (1,064 individual pollens or 15%) and Fabaceae (722 individual pollens or 10%). Star fruit being a tree is the most preferred vegetation category from which the main pollen and nectar resources were obtained by the bees followed by shrubs, grass, herbs and vegetables. Pollens of star fruit were highly attractive to these two bees due to the aromatic odor produced by this tree. Performances of flower visitation rate of these bees were slightly higher for *G. thoracica* compared to *H. itama* ($I_{VR} G. thoracica = 0.47 \pm 0.02$; $I_{VR} H. itama = 0.12 \pm 0.02$). The number of bees and their visitation (*G. thoracica*) to the star fruit panicles proportionately increased with the increase in the number of flowers on the panicles. The visitation rates of *G. thoracica* and *H. itama* remained low and quite similar to each other due to similar resources and foraging activities, and total dependence on one resource in the net cage. In conclusion, *G. thoracica* and *H. itama* were more actively foraging in the morning and during wet season. *G. thoracica* preferred to visit bigger panicles with more flowers and their visitations also increased when the size of the panicles increased. *Geniotrigona thoracica* could be a potential pollinator of the star fruit tree compared to *H. itama* based on its slight advantage of having higher frequency of visitation to the star fruit flowers.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

PERILAKU Mencari Makanan Lebah Kelulut, *Heterotrigona itama* Cockerell DAN *Geniotrigona thoracica* Smith SEBAGAI AGEN PENDEBUNGA BERPOTENSI KEPADA BELIMBING *Averrhoa carambola* L.

Oleh

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Lebah kelulut (Apidae, Meliponini) telah menarik minat yang mendalam di kalangan penyelidik dan petani sebagai agen pendebunga alternatif disebabkan oleh taburannya yang meluas dan kekurangan populasi lebah madu sedunia. Di Malaysia, industri lebah kelulut telah menjadi terkenal dan mendapat perhatian dari kerajaan untuk meningkatkan pendapatan sosioekonomi di kalangan petani. Walau bagaimanapun, masih terdapat kekurangan pengetahuan tentang beberapa aspek perilaku mencari makanan oleh *Heterotrigona itama* dan *Geniotrigona thoracica* untuk mengesahkan potensi mereka sebagai agen pendebunga yang cekap. Untuk mengisi jurang pengetahuan tersebut, kajian ini telah dilakukan di Ladang Bersepadu, Universiti Putra Malaysia (UPM) untuk i) menyiasat kegiatan mencari makanan dua spesies lebah kelulut, *G. thoracica* dan *H. itama* dan hubungannya dengan faktor musim dan abiotik, ii) menentukan sumber debunga yang diambil oleh *G. thoracica* dan *H. itama*, dan membina profil debunga sebagai satu data asas, iii) menilai keberkesanan dan membandingkan kadar lawatan bunga oleh *G. thoracica* dan *H. itama* pada bunga buah belimbing (*Averrhoa carambola* L.). Kajian lebah terbang masuk dan keluar dari sarang mereka di Ladang Bersepadu, UPM dicatatkan untuk setiap jam dari jam 0800 hingga 1600. Debunga dari corbiculae dikumpulkan kemudian diperhatikan dan dihitungkan di bawah mikroskop cahaya. Kadar lawatan bunga lebah ini dihitungkan berdasarkan kekerapan kunjungan pada bunga buah belimbing. Hasil kajian menunjukkan bahawa kegiatan mencari makanan harian dan corak mencari makanan kedua-dua spesies lebah ini berbeza secara signifikan pada pelbagai waktu dalam sehari. Kedua-dua lebah tersebut dilihat lebih aktif mencari makanan pada waktu pagi pada jam 0900 hingga jam 1100 dan selepas itu mereka menjadi kurang aktif sehingga penghujung hari. Pengumpulan nektar bagi *G. thoracica* (67,363 individu) dan *H. itama* (53518 individu) lebih tinggi secara signifikan berbanding pengumpulan debunga iaitu 11,075 individu (*G. thoracica*) dan 6,803 individu (*H. itama*). Kegiatan lebah juga bervariasi pada waktu dan musim yang berlainan (hujan dan kering) tahun itu dan ditunjukkan oleh turun naik jumlah lebah mencari makanan yang signifikan di antara bulan-bulan yang dikaji.

Kegiatan mencari makanan tertinggi dicatatkan pada Januari 2015 (musim hujan) untuk kedua-dua lebah, 12,303 individu (*G. thoracica*) dan 12,979 individu (*H. itama*). Kegiatan mencari makanan terendah dicatatkan pada bulan September 2014 bagi *G. thoracica* (7517 individu) dan pada bulan Ogos bagi *H. itama* (1960 individu). Variasi faktor abiotik seperti suhu, kelembapan relatif dan intensiti cahaya di kawasan kajian mempengaruhi kegiatan mencari makanan lebah secara signifikan. Komposisi kepelbagaian tanaman di Ladang Bersepadu, UPM sangat mempengaruhi kegiatan mencari makanan dua spesies lebah tersebut. Tiga keluarga tanaman yang paling disukai adalah Oxalidaceae (1,342 individu debunga atau 18% daripada jumlah debunga yang dikumpulkan), Arecaceae (1,064 individu debunga atau 15%) dan Fabaceae (722 individu debunga atau 10%). Lebah tersebut lebih menyukai Oxalidaceae (*A. carambola*) daripada kategori tanaman berkayu sebagai sumber utama debunga dan nektar mereka diikuti oleh pokok renek, rumput, tumbuh-tumbuhan dan sayur-sayuran. Debunga *A. carambola* adalah sangat menarik bagi kedua-dua lebah ini kerana aroma yang terhasil daripada debunga dan nektar. Prestasi kadar lawatan bunga lebah-lebah ini sedikit tinggi bagi *G. thoracica* berbanding *H. itama*. ($I_{VR} G. thoracica = 0.47 \pm 0.02$; $I_{VR} H. itama = 0.12 \pm 0.02$). Lawatan mereka pada panikel belimbing meningkat secara berkadar dengan bertambahnya jumlah bunga pada panikel. Kadar lawatan serangga *G. thoracica* dan *H. itama* adalah rendah dan hampir sama antara satu sama lain disebabkan sumber dan kegiatan mencari makanan yang hampir sama, dan kebergantungan pada satu sumber di dalam sangkar jaring. Kesimpulannya, *G. thoracica* dan *H. itama* lebih aktif mencari makanan pada waktu pagi dan semasa musim hujan. *G. thoracica* lebih suka melawat panikel yang lebih besar dengan lebih banyak bunga. *G. thoracica* lebih berpotensi menjadi pendebunga belimbing berdasarkan kepada kekerapan melawat bunga belimbing yang lebih tinggi berbanding dengan *H. itama*.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
°C	Degree Celsius
%	Percentage
DOA	Division of Agriculture
DJPEN	Directorate General for National Export Development
FI	Flight In
FO	Flight Out
h	Hour
IVR	Insect Visitation Rate
LI	Light Intensity
MARDI	Malaysian Agriculture Research and Development Institute
MANOVA	Multivariate Analysis
MOA	Ministry of Agriculture
RH	Relative Humidity
SDR	Species Diversity Richness
SE	Standard Error
SPSS	Statistic Package for Social Science
UPM	Universiti Putra Malaysia

CHAPTER 1

GENERAL INTRODUCTION

1.1 Introduction

Stingless bees belong to the tribe Meliponini (Michener, 2013) which is relatively small in size. They are also called ‘sweat bees’ and ‘dimmer bees’ because they gather salt from human sweat (Vijaykumar, 2017). Stingless bees are social bees which possess sting which remains non-functional (Heard, 2016). They defend themselves by biting their enemies using mandibles, but the bite intensity is similar to the bite of an ant. They are able to produce honey similar to honey bee (Apidae). Nowadays, there are more than 600 species of stingless bee that can be found throughout the tropics. Their distribution is pan-tropical with the highest diversity found in tropical mainland like America, Africa, Madagascar, Australia and South East Asia (Heard, 2016). In Malaysia, there are 35 identified species of stingless bee (Eltz & Bru, 2003; Hannah et al., 2012; Norowi et al., 2010; Farisya et al., 2015).

The cultivation of stingless bee on a commercial scale for honey production or pollination is known as meliponiculture. Such activities have been widely practiced in Brazil, Mexico, Africa, Australia and Thailand (Cortopassi-Laurino et al., 2006; Halcroft et al., 2013c; Sawatthum, 2004). In Malaysia, there is a keen interest on stingless bees among researchers and farmers recently as alternative pollinators due to their wide distributions. The stingless bee industry has become popular and gaining attention from the government for improving income among farmers. The diverse groups of stingless bees found in Malaysia could be potential pollinators of various crops. It was reported that stingless bees pollinate several crops in Australia and improve the productivity of these crops (Heard, 2016). Greco et al. (2011) reported that the Australian stingless bee, *Austroplebeia australis* Friese and *Trigona carbonaria* have improved the production of chilli, *Capsicum annum* in greenhouse. *Trigona carbonaria* has also been showed to improve the production of blueberry in Australia (Kendall et al., 2020). Thus, stingless bees in Malaysia also have similar potential that should be explored and studied so as to allow them to serve as efficient pollinators and further improve local fruit production.

1.2 Problem Statement and Justification of the Study

Star fruit (*Averrhoa carambola*) was introduced and commercialised for the world’s market over 100 years ago and is a tropical fruit that is well established in the market (Hashim et al., 2017). Star fruit is Malaysia’s most exported exotic fruit and is mostly

marketed overseas (Abdullah et al., 2007; Hashim et al., 2017). The demand of star fruit in the market is still higher but the production of this fruit has become low and insufficient to be exported (Hashim et al., 2017; DOA 2018). Many reasons have contributed to the low production of star fruit in Malaysia, such as pests and diseases as well as the decrease in cultivation land for this fruit. In 2003, star fruit tree had been cultivated in netting structure to prevent the insect visitation cause diseases to star fruit tree (Zabedah et al., 2003). Introducing potential pollinator to the star fruit in the netting system is needed to enhance the production of this fruit. Stingless bees are one potential pollinator that could be used in the netting system compared to honey bees due to their small foraging range and they are not harmful to the farmers due to the lack of sting and their resistant to diseases that are associated with honey bees (Heard, 2016).

The ideal candidates of an alternative pollinator in commercial pollination can be found in the diverse group of stingless bees (Heard and Dollin, 2015). However, not all species have an economic value despite high diversity of stingless bee species in Malaysia (Kelly et al., (2014)). For example, in Australia there are only two species of stingless bee dominating the stingless bee industry which are *Tetragonula carbonaria* and *Austroplebeia australis* (Halcroft et al., 2013a). In Thailand, there are four stingless bee species with economic values, namely *Trigona pegdeni*, *Tetragonula laeviceps*, *Lepidotrigona terminata* and *Lepidotrigona ventralis doipaensis* (Sawatthum, 2004).

A recent study showed that there are five species of stingless bee that have a commercial value in Malaysia. They are *Geniotrigona thoracica*, *Heterotrigona itama*, *L. terminata*, *Tetragonula fuscobalteata* and *T. laeviceps* (Kelly et al., 2014). Among the five species of stingless bee that have been commercialized in Malaysia, only two species were chosen as an alternative pollinator to the star fruit tree based on their high abundance and popularity among the stingless beekeepers in Malaysia (Salim et al., 2012; Farisya and Thevan, 2015; Hamid et al., 2016). This research mainly focused on the foraging behaviour of two stingless bee species, i.e. *G. thoracica* which is one of the largest stingless bee species in Malaysia and *H. itama* which is the most abundant stingless bees found in orchard. Moreover, there is still a lack in knowledge on many aspects of the foraging behaviours of these bees to confirm their potential as efficient pollinators of many crops especially star fruit.

In order to use stingless bee as a potential pollinator of the target crop, their biological and ecological aspects like foraging behaviours toward potential crops need to be further investigated as the related research is still very limited as compared to those done on honey bees (Heard and Dollin, 2015). Thus, the study of some aspects foraging behaviours of *G. thoracica* and *H. itama* as potential pollinators of star fruit was conducted.

Star fruit was chosen as the target crop in this study as there is a high preference for the crop by the two bee species (Norowi et al., 2010). However, Norowi et al. (2010) did not provide any statistical data to support such statement which was made solely based on

their observations in the field. Thus, this study was conducted to confirm whether these two bee species had a higher preference for the star fruit flowers and hence become the potential pollinators of this crop in open orchards and netting system.

In order to use stingless bees to serve as a potential pollinator of the target crop, like star fruit, their foraging behaviours need to be further investigated as the related research is still very limited especially in Malaysia as compared to those done on honey bees. Thus, this study was conducted to examine the foraging behaviour of two stingless bee species, namely *Heterotrigona itama* and *Geniotrigona thoracica* as a potential pollinators of star fruit.

1.3 Objectives of the Study

The general objective or scope of the study was to investigate some aspects of foraging behaviours of *G. thoracica* and *H. itama* bees as a potential pollinator of the star fruit crop. The specific objectives are:

- i. To investigate the foraging activities of two selected stingless bee species, *G. thoracica* and *H. itama* in relation to seasonal factors and abiotic factors;
- ii. To determine the pollen resources taken by *G. thoracica* and *H. itama* and construct the pollen profile as a baseline data;
- iii. To assess the performance and compare the flower visitation rate of *G. thoracica* and *H. itama* on the star fruit (*Averrhoa carambola*) flowers.

1.4 Significance of the Study

This research would contribute to the knowledge on foraging behaviour of stingless bee species in Malaysia, i.e., *G. thoracica* and *H. itama* as the potential alternative pollinators which can generate income to the bee farmers either in term of stingless bee honey production and increasing in their crop yields resulted from successful pollination services provided by these stingless bees. Mustafa et al. (2018) reported that 36% and 29% of stingless bee keepers have generate additional income of RM833 and RM1,666 monthly, respectively with stingless bee honey production. Hence, this encouraged the bee keepers to cultivate a variety of crops so as to provide continuous supply of nectar and pollen in a way to increase honey production. The result of this research will facilitate the bee farmers to develop a better management approach on these potential pollinators of their target crops. The daily and monthly patterns with respect to foraging activities of *G. thoracica* and *H. itama* will provide information to the beekeepers on the right time to apply insecticide or fungicide to their crops, if any, and to sustain the

stingless bees' colonies for pollination based on the number of individual bees hovering at the entrance of the colony. The result pertaining to seasonal changes in the bees' foraging behaviour will aid the beekeepers to pay more attention on colony sustainability. Melissopalynology or pollen analysis using pollen samples collected from corbiculae of the bees will provide information on the type of flowers favoured by stingless bees. The study on flower visitation rate of *G. thoracica* and *H. itama* on star fruit flowers will allow beekeepers to assess the suitable stingless bee species to be used as a pollinator of this crop.

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LIST OF PUBLICATIONS

Journal

Wan Nur Asiah, W. M. A., Sajap, A. S., Nur Azura, A. & Norowi, M. H. (2015). Flight intensity of two species of stingless bees *Heterotrigona itama* and *Geniotrigona thoracica* and its relationships with temperature, light intensity and relative humidity. *Serangga*, 20(1): 35-42.

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