NEST CHARACTERISTICS AND PHEROMONE COMPOUNDS OF Heterotrigona Itama COCKERELL

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FBSB 2017 46

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By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

December 2017

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in the fulfilment of requirement for the degree of Master of Science

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December 2017

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Stingless bee is known as a potential insect for honey production and recently identified as a novel pollinator for Malaysia. However, the source of stingless bee colonies is entirely depending on feral colony hunting that potentially affects the ecosystem. As the stingless bee industry is growing in Malaysia, understanding their behaviour to support colony splitting, thus preventing feral colony hunting is crucial. To achieve this, specific objectives were outlined; i. To evaluate the nest characteristics of *Heterotrigona itama*. ii. To determine the correlation between number of queen cells and number of brood layers. iii. To describe the morphology of queen developmental stages. iv. To evaluate the compound in the dufour gland of the egg laying queen of *H. itama*. Firstly, the colonies were transferred from logs into hives. The colonies from the hives were then split into two. Nest characteristics of the daughter colonies (n=10) were analysed immediately and 12 weeks post-colony splitting based on the height of broods, number of brood's layer and number of queen cells. Then, the correlation between numbers of queen cell to brood's layer was performed. The morphology of the queen cell (n=15) was observed based on; i) length x width of the queen cell, ii) the outer layer of the cell, iii) the inner structure of the cell, iv) the comparison of both inner and outer structures, and v) size measurement of the cell. The volatile compounds from egg laying queens' dufour glands (n=7) were analysed through GC-MS using HP-5MS column.

Nest characteristics of daughter colonies immediately after colony splitting showed that the height of broods was 16.20 ± 4.29, followed by the number of broods' layer of 8.9 ± 26.7 and 5.2 ± 15.6 virgin queen egg. After 12 weeks, the height of broods became 16.41 ± 4.45 with 12.1 ± 36.3 broods' layer and 6.4 ± 19.2 virgin queen egg. For all the 10 colonies, the difference between two layers of broods showed a fixed value of 1.5 cm before and after 12 weeks observation. The numbers of queen cell and broods layer within the 12 weeks were significantly different where $F = 9.471$, $P < 0.0001$ and $F = 7.646$, $P < 0.0001$, respectively. However, the correlation between the two characters was significantly low (r = 0.421). Morphological analysis showed nine fully formed queens while six of them were still at development stage. Finally, from GC-MS analysis, results demonstrated that the area percentage of volatile compounds octadecane, heneicosane octacosane and pentacosane was more than 3% with octadecane and pentacosane, and heneicosane and pentacosane being statistically significant to one another. As a conclusion, nest characteristics and queen's morphology of *H. itama* have been successfully described with the compounds presented in the dufour gland of the egg laying queen that have been evaluated. Data from this study may provide a better understanding for the establishment of stingless bee colony splitting technique.

Keywords: Stingless bee, *Heterotrigona itama* species, egg laying queen, dufour gland.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Sarjana Sains

PENCIRIAN SARANG DAN KOMPAUN FEROMON *Heterotrigona Itama* COCKERELL

Oleh

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Kelulut dikenali sebagai serangga yang berpotensi dalam membantu proses penghasilan madu dan baru baru ini, kelulut dikenalpasti sebagai ejen pendebungaan baru di Malaysia. Walaubagaimanapun, koloni kelulut diperoleh daripada alam semulajadi, dan ia berpotensi untuk mengancam ekosistem. Maka, semakin berkembang industri kelulut di Malaysia, kefahaman tentang tingkah laku kelulut melalui proses pemecahan koloni adalah penting dalam menghalang aktiviti pemburuan kelulut secara berterusan. Untuk mencapai matlamat ini, terdapat beberapa objektif yang digariskan. i) menganalisa sifat sarang *Heterotrigona itama*, ii) memastikan korelasi antara sel ratu dan lapisan telur, iii) memberi deskripsi terhadap fasa pembesaran ratu, iv) mengenalpasti komposisi kimia kelenjar dufour dalam ratu *H. itama*. Pertama sekali, kesemua koloni dipindahkan daripada batang kayu kepada haif. Kemudian, koloni daripada haif dipecahkan kepada dua. Pencirian sarang koloni anak (n=10) dianalisa selepas proses pemecahan selama 12 minggu selepas pemecahan koloni berdasarkan tinggi telur, jumlah lapisan telur dan jumlah sel ratu. Selepas itu, kolerasi di antara jumlah sel ratu dan jumlah lapisan telur dijalankan. Morfologi sel ratu (n=15) dianalisa berdasarkan; i) panjang x lebar sel ratu, ii) lapisan luar sel, iii) lapisan dalam sel, iv) perbandingan antara struktur lapisan luar dan lapisan dalam, dan v) saiz ukuran sel. Kompaun daripada kelenjar dufour ratu bertelur (n=7) dianalisa melalui GC-MS menggunakan kolum HP-5MS.

Pencirian sarang koloni anak dijalankan secara terus selepas pemecahan koloni menunjukkan ketinggian telur sebanyak 16.20 ± 4.29, 8.9 ± 26.7 jumlah lapisan telur dan 5.2 ± 15.6 jumlah sel ratu. Selepas 12 minggu, tinggi telur menjadi 16.41 ± 4.45 dengan jumlah lapisan telur sebanyak 12.1 ± 36.3 dan jumlah sel ratu sebanyak 6.4 ±

19.2. Untuk kesemua 10 koloni anak, perbezaan di antara dua lapisan telur menunjukkan nilai yang tetap iaitu 1.5 cm sebelum dan selepas 12 minggu pemerhatian. Jumlah sel ratu dan lapisan telur selama 12 minggu menunjukkan perbezaan yang ketara dengan masing menunjukkan nilai $F = 9.471$, $P < 0.0001$ dan $F = 7.646$, $P < 0.0001$. Walau bagaimanapun, kolerasi antara kedua pemboleh ubah ini menunjukkan perbezaan yang rendah ($r = 0.421$). Analisa morfologi menunjukkan sembilan sel ratu sudah terbentuk sepenuhnya manakalah enam lagi masih di dalam fasa pembesaran. Akhir sekali, untuk analisa GC-MS, keputusan menunjukkan peratusan kawasan kompaun volatile octadecane, heneicosane octacosane and pentacosane melebihi 3%, dengan octadecane dan pentacosane, dan heneicosane dan pentacosane menunjukkan perbezaan ketara antara satu sama lain. Sebagai konklusi, pencirian sarang dan morfologi sel ratu *H.itama* berjaya digambarkan dan kompaun yang hadir di dalam kelenjar dufour ratu bertelur sudah dinilai. Data yang disediakan di dalam kajian ini boleh digunakan untuk mewujudkan kefahaham yang lebih baik dalam membantu teknik pemecahahan koloni lebah kelulut.

Kata kunci: Kelulut, spesis *Heterotrigona itama*, ratu bertelur, kelenjar dufour

ACKNOWLEDGEMENT

Alhamdulillah.
Firstly, all praises to Allah swt.

Million thanks are not enough to thank my dearest husband Mohamad Zulhafiz Shafiq bin Zulhilmi Cheng. Definitely, I could not have done this without my husband's endless love and support. To my dear daughter Aleesa Sofia, thank you for behaving so well whenever I am too occupied doing my work.

I wish to express my sincere gratitude to my parents Ahmad Jailani bin Darus and Norkaspawati binti Kassim, my parents in law Zulhilmi Cheng bin Abdullah and Zauyah binti Jamaluddin. The never endless support they gave me even in a far distance, I truly appreciate each of them. To my sisters, Nur Athirah and Nur Nabila, thank you for taking care of Aleesa Sofia when I am busy with my writing and lab work. Their help totally encouraged me all the way up to this point.

Thanks to my supervisor Dr Mariatulqabtiah Abdul Razak for taking me on as a student and giving me this chance. Without your advice and help, I could not have been able to complete my studies. To the wonderful staff at Biotech especially Puan Hasnezah, thank you so much for helping me with everything I need and making my life so much easier.

I also wish to thank Universiti Putra Malaysia for providing me with allowance throughout my journey. The Graduate Research Fellowship (GRF) was truly the knight in the shining armour as it helped to ease my financial issue for every single month. A big thank you, my dear UPM.

Thank you so much.

I certify that a Thesis Examination Committee has met on 6 December 2017 to conduct the final examination of Nur Maisarah binti Ahmad Jailani on her thesis entitled "Nest Characteristics and Pheromone Compunds of *Heterotrigona itama* Cockerell" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U. (A) 106] 15 March 1998. The Committee recommends that the student be awarded the (master degree in science).

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CHAPTER 1

INTRODUCTION

Malaysia is known for its richness of nature as Malaysian forest itself comprises various flora and fauna. Among these, the stingless bee is one of the magical creatures found in the forest. It is the member of the meliponini tribe. Meliponini belongs to the order of Hymenoptera and subfamily Meliponinae, which is divided into two tribes namely Meliponini and Trigonini (Vit & Tomás-Barberán, 1998). Meliponini can be easily found in tropical regions globally (Heard, 1999). There are plenty species of stingless bee, and in Malaysia, there are about 45 species reported until 2014 (Mohd Fahimee et al., 2016). As Malaysia is one of the countries located in tropical regions, it becomes the factor stingless bee is possible to be reared successfully. The name stingless bee portrays that this species has no ability to sting. This is due to their undeveloped stinger (Shackleton et al., 2015). Children can easily come and see them in a short distance because of this speciality. Due to the harmless factor, a stingless bee can be reared at home and this activity has become a trend (Cortopassi-Laurino et al., 2002). Stingless bees are familiar visitors to flowering plants in the tropics (Heard, 1999). This is why honey and harvested pollen are different in their composition relying upon availability of plant species flowering (Do Nascimento et al., 2015). In Malaysia, among the favourite flowering plants for a stingless bee to visit are *Antigonon leptopus (*Mexican creepers, Coral vines) and *Passiflora edulis* (Passion Fruit). Plants stated above are favoured by the beekeepers to be planted at their compound for the nutrients of stingless bee.

The Meliponini colony structures are more complicated than those of Apini species. For stingless bee, the colony is constructed in shielding chambers including an empty tree or under the ground (Sommeijer, 1999). Usually, stingless bees will make their house at the tree trunk with a diameter of 60 cm (Mohd Fahimee et al., 2016). Due to that matter, it is hard to harvest the honey. Nevertheless, there is a traditional method used in Malaysia to harvest the honey from the log. One hole should been made using a wood stick with distance about 10 cm from the stingless bee entrance. Honey is harvested using steel to ensure the honey flow from the hole made earlier (Mohd Fahimee et al., 2016). The downside of using logs as the method of stingless bee beekeeping is that the observation process cannot be done due to the condition of the log itself. Food storage area and the colonies growth rate can be interrupted as the space inside the log is limited (González-Acereto, 1999). Even though the expansion of traditional meliponiculture gives an opportunity to the people living in the village to make extra income on the stingless bee products including honey, it can promote a massive deforestation due to illegal hunting for stingless bee logs (Sommeijer, 1999). A better method has been developed for the colony where the ecosystem will not be affected by this transferring and splitting process. The process needs to be done meticulously to make it easy to the humans and stingless bees. There are factors that need to be considered such as the survival of colony if the queen died during the process with no mature virgin queen to replace the deceased queen, or that the colony of stingless bee will make a new queen from the virgin queen cell once it is ready to take over the throne (Mohd Fahimee et al., 2016). By standardising the current technique on colony splitting, the rate of success can be increased. In Malaysia, meliponiculture is considered being in its infancy phase where the advantages from rearing are to be used for the beekeeper. Meliponiculture is the activity of stingless bee keeping where the colony of stingless bees is extracted from the forest for beekeeping purpose (Saufi & Thevan, 2015). Meliponiculture is an important process that creates awareness to conserve the stingless bee. It is a pursuit that supports the preservation of stingless bee by ensuring the occurrence of pollination and aiding in reducing the deforestation and destruction to the forest (Carvalho et al., 2014; Rasmussen, 2013).

Wide ranges of the prospect of commercialisation are yet to be fully used as the prevention of continuous feral colony hunting is essential. In order to achieve a successful domestication, skill is needed to cultivate hive (transfer from log to the hive) together with multiplication process to help sustaining the environment (Singh, 2013). Stingless bee is known as a potential insect for honey production and pollinators in Malaysia (Mohd Fahimee et al., 2016). However, the source of stingless bee colonies is entirely depending on feral colony hunting that potentially affects the ecosystem. Therefore, as the stingless bee industry is growing in Malaysia, understanding on the stingless bee behaviour to support the colony splitting is crucial. Stingless bees (Meliponini sp.) are eusocial insects that live in their colony consisting a single queen that will lead the colony along with workers and exhibit complex social, navigational and communication behaviours. To date, there was no patented or published methodology on colony splitting for stingless bee of *H. itama* species using Mustafa Hive system. Besides, there was no correlation between colony splitting and dufour gland contents studied before.

Heterotrigona itama (H. itama) and *Genotrigona thoracica (G. thoracica)* are mainly two of the top species preferred by Malaysian beekeepers since they can be found and reared easily. *H. itama* and *G. thoracica* collect nectar and resin from the surrounding environment. Nonetheless, only *H. itama* can be kept and reared inside the hive.

The specific objectives of this study are henceforth;

i. To evaluate the nest characteristics of *Heterotrigona itama*.
ii. To determine the correlation between number of queen cells and number of brood layers.
iii. To describe the morphology of queen developmental stages.
iv. To evaluate the compound in the dufour gland of the egg laying queen of *H. itama*.

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