Abstract

The integration of stingless bee in rubber estate and study of pollen is crucial in understanding the crops and plants interaction as stingless bee has species-specific preference toward pollens. Peninsular Malaysia has been the world's most important rubber cultivation area, and the present wealth of this area was largely based on the production of natural rubber. However, the price of latex drops recently due to reduce demand from the rubber industry. Therefore, the integration of stingless bee (Heterotrigona itama) in the rubber plantation can bee seen as an alternative activity to supplement the income of rubber smallholders while at the same time as an important pollinator agent for the crop. The aims of this study are to identify pollens collected by the stingless bee, Heterotrigona itama in rubber smallholding at Tepoh, Terengganu. The study was carried out from January 2017 until December 2017. Pollen distribution in March to June showed the highest varieties the plants start to flower. Results also showed that stingless bee has specific preference for the flower such as Ixora coccinea. Small flowers with less or no petals are most preferred by stingless bees. This study could help stingless bee keeper to understand and appreciate their surrounding and also managing a suitable landscape for stingless bee rearing.

Keywords: Stingless bee, integration, pollen distribution, pollen profile, rubber smallholding

Introduction

Hevea brasiliensis (Muell.) Arg. is a major crop for rubber smallholders in Malaysia and an important commercial crop in most of the countries in Southeast Asia. It is grown for latex production, while rubberwood is considered as a secondary product especially for furniture production. Therefore rubber is regarded as an important economical agricultural crop. Rubberwood has enjoyed an environmentally friendly reputation as a raw material, because it is a by-product of latex production, and when grown in renewable plantations, it can substitute timber from natural forests. Other than that, rubber estates also contribute to the world economic expansion. In recent years, the world market prices of rubber undergo fluctuations and become unstable due to the low demand from the the rubber industries. Many rubber estates was abandoned or replaced by other more lucrative crops such as oil palm. Therefore, an integration of stingless bee in the rubber estate can be an alternative source of income to the rubber smallholders during the dearth period of rubber and the diminishing hectarage of rubber plantation.
Stingless bees have been proven to play as major role as pollinators in their natural habitat especially in the Tropic region. This is an advantage to the economic importance for the rubber smallholders to increase the pollination and fruit set. Throughout pollination, fertilization occurs then fruits and seeds are formed. Previous studies have shown that the feral Africanized bees increase the coffee production in Central America (Roubik, 2002). Eltz et. al. (2003) stated stingless bees can be a good candidate for providing pollination services in agricultural ecosystem. It also good candidates for providing pollination services in agriculture ecosystem such as starfruits, mango, durian, watermelon, guava and coconut (Slaa et al., 20016). The goal of every living organism, including plants, is to create offspring for the next generation. Therefore, the diversity of flowering plants has been attributed, in part to adaptations to animal pollinators (Stebbins 1970; Pellmyr 2002).

The stingless bees are one of the most diverse, attractive, fascinating, and useful of all the insect groups of the tropical world. Stingless bees can be found in tropical and subtropical regions, and are adapted to different types of vegetation including forests, savannas, fields, and mountains (Andreia et al., 2005). Stingless bees are the large group of bees which lack a functional sting. According to Michener (2007), the bees are divided into seven families. The stingless bees belong to the family Apidae and tribe Meliponini.

Melissopalynological studies dealing with the microscopic analysis of the pollen contents of seasonal honey and pollen loads from a locality, when supplemented with critical field's studies involving phenology and floral biology, provide reliable information regarding the floral types which serve as major or minor nectar and pollen sources for the bees. Furthermore, these studies highlight various types of honey that can be obtained in different seasons, as also the flow dearth periods of a locality, thereby helping the beekeepers in the proper management of bee colonies. The pollen spectrum of a honey is also an expression of its geographical origin (Ramanujan, C.G.K., and Khatija, F., 1990) due to varied nature of floral component and local spread.

The study of pollen is well established and has been used to determine the geographical origin, floral sources, and genus of the plant that the stingless bees visited (Ponnuchamy et al., 2014). Previous studies suggested that numerous types of pollen found on honeycombs and beehives helps in identifying the botanical and geographical origin of the honey as well as the biochemistry and quality determination of honey (Herrero et al., 2002; Montenegro et al., 2010). Therefore, pollen analysis is important to identify the pollens collected by the stingless bee, Heterotrigona itama in relation to the quality of the honey in rubber estate and to the apiculture industry in developing premium marketable of honey and honey product.

Methodology

Study Sites

The experiments were carried out at Tepoh which is located (°21’27.1"N 103°03’46.4"E) in Kuala Nerus, Terengganu. The selected sampling site was chosen based on the ideal size of rubber smallholding (1/4 hectares) and its surrounding vegetation. Nine colonies of stingless bee (Heterotrigona itama) were set up in the rubber smallholding on the 10 October 2016. Besides rubber plantation, the surrounding vegetation include a mixture of rural residential, horticulture, and grazing land. In general, the climate in Tepoh is hot and humid, with the average year-round temperature of ~ 27-degree Celsius. The dry season started from July to
September and another relatively rainy season from November to February. The experimental design for this experiment is Systematic Randomized Complete Block Design (RCBD) or incomplete block design. The samples (H. itama log) were arranged in a row between three rubber tree for each log.

**Sample Preparation for Identification of Pollen**

**Pollen from Stingless bee’s Basket**

The sampling was carried out from November 2016 to October 2017 to examine any differences in pollen preferred by the stingless bees for each month. Pollen from stingless bee was taken twice in a month for 12 months. Pollen sample from nine different logs of *H. itama* was collected in the morning (from 0800 am to 1100 am,) and (1100 hours to 1400 hours). Pollen was obtained by collecting stingless bee with pollen loads using pooter. Pollen from the foragers then were transferred properly into labeled Eppendorf tubes. The eppendorf tube need to be labelled first before being transferred elsewhere. Then, the Eppendorf tubes were transferred to the laboratory for analysis.

Pollen was carefully separated from stingless bee’s corbicula (pollen basket) by using needles and diluted in 1ml of distilled water. A drop of pollen suspension was deposited onto a microscope slide and a coverslip was gently pressed onto the solution. The observation was done using an optical microscope Leica DM750 using 40 magnification objective lens and the image was acquired in order to compare with the flower pollen reference.

**Pollen as Flower Reference/database**

There are 70 types of flowers that were collected within a 500-meter radius from stingless bee's logs/colonies. The flower was plucked carefully and preserved at temperature 23 °C in order to minimize the respiration process. A small amount of pollen will be used and was transferred onto a microscope slide. A drop of distilled water was placed on the pollen sample and will cover by using coverslip. The prepared slide was observed under a light microscope at 40 magnification objective lens and the image was acquired. The length and width (in mm) of pollen were measured and used as a reference in order to compare with the pollen type identified from the body of the stingless bees.

**Results and Discussion**
Figure 1: Frequency of pollen types and distribution in one year by stingless bees, *H. itama* in Rubber Estate, Tepoh, Terengganu.
A total number of 37 types of pollens were collected throughout the experiment by *H. itama* foragers (Figure 1). However, only 29 types of pollen were successfully identified. The dominant pollen collected at rubber estate Tepoh is *Ixora coccinea* for the duration of ten months in a year. Followed by pollens from *Caudatus sulphureus* for eight months, then *Mimosa pudica* for seven months, *Pereskia scharosarosa* for six months, and followed by *Calliandra haematocephala, Malaleuca cajuputi, Cocos nucifera, Turnera subulata and Lantana camara* for five months pollen collected in a year. *Hibicus rosa sinensis, Clerodendrum thomsoniae, Melastoma malabathricum, Rhodomyrtus tomentosa, Combretum indicum, Jasminum polyanthum*, and *Ageratum conyzoides* were observed to be foraged by stingless bees for four months in a year. While, *Andrographis Paniculata, Averrhoa carambola, Nerium olendar, Asystasia gigantica, Hedyotis corymbosa, and Acacia auriculaeformis* were observed in three months throughout a year. The second lowest pollen collected is *Psidium guajava, Impatiens balsamina, Biden pilosa, and Adenium obesum* with two months pollen collected in a year. While the lowest pollen collected at rubber estate Tepoh is *Capsicum annum, Sida rhombifolia*, and *Carica papaya* with one pollen collected in a year respectively.

Pollen distribution varies every month depending on the surrounding and environment factors (Figure 5 - 16). The flower preference is important in determining the types of pollen collected by stingless bee. Studies based on the visitation of the pollinators of the flowers concluded that stingless bees are not specialized visitors in certain plant species, which only 7% of the plants in the rainforest are intensely visited by native bees; 77% of the plants are visited with less frequency, and 16% are not visited at all (Velthuis et al., 1997). In this study, the highest pollen collected by *H. itama* was *Ixora coccinea*. The local name for *Ixora coccinea* is “Jejarum”. *Ixora coccinea* is one of the pollen that has small in size, one of the reason for *Heterotrigona itama* visited this flower more frequent other than the other flower. Wille et al., (1983) reported that stingless bees usually preferred flower or crop with small in flower size. *Caudatus sulphureus* and *Mimosa pudica* also shown the same trend which also has the same characteristic of the small size of flowers and has pollen second higher of pollen collected in a year.

*Ixora coccinea* have several characteristics that influence the stingless bee (*H. itama*) to the pollen preference. *Ixora coccinea* consists four to six-inch globular clusters of orange, yellow, pink, bright red, or white and this flower can bloom continuously under ideal conditions in full sun (Gilman et al.,1999). There are several studies done by another
researcher who found that the abundance and frequency of the flower visitors influence by the flower attractiveness. For example, a study by Bosch and Waser (2001) found that *Aconitium columbianum* could attract pollinator due to the flower attractiveness. Besides that, *Ipomopsis aggregata* could attract hummingbird and another flower visitor with the bright red color of the flower (Brody et al., 1997).

Besides that, *Ixora coccinea* have a strong and nice odor which preferred by *H. itama* to forage for the pollen frequently. Apart from size of flower, previous study stated that odor also plays an important factor as the signals for the stingless bees to recognize the flower sources. A study by Azuma et al., (2002) found that flower scent is one of the important factors in attracting the pollinators. In this study, *Ixora coccinea* has the higher pollen collected due to the flower pollen size, flower attractiveness, and flower odor which have a positive influence on the number of pollinators. Interestingly, *Heterotrigona itama* does not forages from rubber flower as pollen from rubber flower was not observed throughout the experiment. This could be due to the height of plant which causes difficulties to the stingless bees to foraged for the pollen. The stingless bees most likely only collected the nectar from the extrafloral nectaries of *Hevea brasiliensis*.

![Unknown pollens distribution in 1 year](image)

**Figure 3:** Frequency of unknown pollen types distribution in one year by stingless bees, *H. itama* in rubber estate, Tepoh, Terengganu.
There are 8 types of unidentified pollen observed in the experimental plot and the rubber estate surrounding area that have been foraged by *H. itama*. The dominant unknown pollen that foraged by *H. itama* is Pollen D, which found seven months in a year. Then Pollen A, B, and E with three months in a year. Followed by pollen C and F with two months in a year and the lowers is pollen G and H with one month pollen collected in a year.
<table>
<thead>
<tr>
<th>Scientific name</th>
<th>Common name</th>
<th>Family</th>
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<tr>
<td><em>Pereskia sacharosa</em></td>
<td>Bunga duri tujuh</td>
<td>Cactaceae</td>
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<tr>
<td><em>Cocos nucifera</em></td>
<td>Kelapa</td>
<td>Arecaceae</td>
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<td><em>Ixora coccinea</em></td>
<td>Bunga jarum</td>
<td>Rubiaceae</td>
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<td><em>Caudatus sulphureus</em></td>
<td>Bunga kosmos kuning</td>
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<td><em>Mimosa pudica</em></td>
<td>Pokok semalu</td>
<td>Fabaceae</td>
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<td><em>Melaleuca cajuputi</em></td>
<td>Gelam</td>
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<td>Verbenaceae</td>
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<td><em>Adenium obesum</em></td>
<td>Bunga kemboja</td>
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<tr>
<td><em>Acacia auriculaeformis</em></td>
<td>Akasia</td>
<td>Leguminosae</td>
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**Table 1 : List of flower collected**
Pollen Distribution by Month

Figure 5: Percentage of pollen distribution in January 2017

Figure 6: Percentage of pollen distribution in February 2017
Figure 7: Percentage of pollen distribution in March 2017

Figure 8: Percentage of pollen distribution in April 2017
Figure 9: Percentage of pollen distribution in May 2017

Figure 10: Percentage of pollen distribution in June 2017
Figure 11: Percentage of pollen distribution in July 2017

Figure 12: Percentage of pollen distribution in August 2017
Figure 13: Percentage of pollen distribution in September 2017

Figure 14: Percentage of pollen distribution in October 2017
Figure 15: Percentage of pollen distribution in November 2017

Figure 16: Percentage of pollen distribution in December 2017
Conclusion

The annual bee calendar of January to December 2017 in the rubber smallholding environment was established. The most abundant pollen is *Ixora coccinea* from the Rubiaceae family which was visited for 10 months. Out of 37 types of pollen found, only 29 were successfully identified and another 8 types of pollen are unidentified. Pollen identification by using light microscope, enable us to identify types of pollen that are mostly foraged by stingless bee. The observation that has conducted showed stingless bee species has specific preference for flower. Stingless bees prefer to forage flower with certain characteristic such as its size, color and odor. Plant with small flower such as *Ixora coccinea* was recommended to be planted. During the rainy days, foraging activity decreases as they are unable to fly. This finding of this study could be used as a guideline in selecting suitable landscape for meliponiculture especially in the rubber environment.

Acknowledgment

I would like to acknowledge Faculty of Food Science and Technology, University Malaysia Terengganu, Central Laboratory (UMT) for the equipment for this study, and Mr. Muhammad Firdaus Sulaiman for assisting for site visit. This work was funded by Translational grant (53253) and Special Interest Group grant (53233).

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 have no disclosures to declare.

References


