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MELISSOPALYNOLOGY AND FORAGING ACTIVITY OF STINGLESS BEES, *LEPIDOTRIGONA TERMINATA* (HYMENOPTERA: APIDAE) FROM AN APIARY IN BESUT, TERENGGANU

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Abstract: Melissopalynology or study of pollen is crucial in understanding the crops and plants that are foraged by bees as their food source because bees have species-specific preferences of pollens. Pollen analysis is also important to the sustainable development of apiculture industry especially for the premium marketable honey and honey products. The aims of this study were to identify the pollens collected by stingless bees, *Lepidotrigona terminata* (Hymenoptera: Apidae) in an apiary of Besut, Terengganu from November 2012 until February 2013 and to investigate the effective time of foraging activity by the stingless bees. A total of 11 types of pollens were collected from the *L. terminata* foragers, however only 9 types of the pollens were successfully identified. The identified pollens were *Murraya paniculata, Citrus hystrix, Calophyllum inophyllum, Ixora coccinea*, *Bougainvillea glabra* (Type 1 and 2), *Mimosa pudica, Asystasia gangetica* and *Suregada multiflora*. *Ixora coccinea* was the most dominant pollen collected by *L. terminata*. Morning (0800-1100) and late afternoon (1400-1800) were found to be the most effective times for foraging activity of *L. terminata*. Our findings provide information on the favored bee plant species which is clearly an important pre-requisite for launching apiary industry in any locality. It is hoped that this study will enhance the knowledge of beekeepers on crop preferences for stingless bee cultures.

Keywords: Pollen, stingless bee, *Lepidotrigona terminata,* foraging, apiary.

Introduction

Melissopalynology or study of pollen is well established and has been used to determine floral sources, geographical origin and genus of the plants that the bees visited (Ponnuchamy *et al*., 2014). Previous studies suggested that numerous pollen types commonly found on honey combs and bee hives affords the possibility of identifying the botanical and geographical origin of the honeys as well as the biochemistry and quality determination of honeys (Herrero *et al*., 2002; Montenegro *et al*., 2010). Therefore, pollen analysis is important to the apiculture industry in developing the premium marketable honey and honey products.

Malaysia hosts a great number and diverse of honey bees and stingless bees species that forage on various plants and vegetation zones including grasses, herbs, forest trees and cultivated plants. However, information on the pollens collected by bees in Malaysia is poorly understood. Microscopic analysis of the pollen contents of seasonal honeys and pollen loads supplemented with phenology and floral biological study will provide reliable information regarding to the floral types which serve as major or minor nectar and /or pollen sources for the bees.

Malaysia is home to diverse species of stingless bees which consists of ~33 species (Mohd Norowi *et al*., 2008). Stingless bees are known to be important pollinator in tropical rainforest (Eltz *et al*., 2003) and also good candidates for providing pollination services in agricultural ecosystem such as starfruits, mango, durian, watermelon, guava and coconut (Slaa *et al*., 2006). The value of insect pollination service of these crops was estimated more than USD 19 million. However to date, very little attention has been made to study the melissopalynology and foraging activity of the stingless bees, specifically in Malaysia region.

Beekeeping endeavor in Terengganu has been taken up on a modest commercial scale by beekeepers in some districts. Besut district has great potential of beekeeping; however, reliable information on the bee plants in this district is as yet highly limited and inadequate. Therefore, in the present study, the main objectives were (1) to identify the pollens collected by *Lepidotrigona terminata,* one of the potential candidates of domesticated stingless bees in an apiary of Besut, Terengganu; and (2) to investigate the active period of foraging activity of *L. terminata.* Outcome from this study is useful to develop formulations or application strategies suitable for future use in apiculture industry and honey production.

Methodology

Study Sites

This study was conducted in an apiary of Besut, in the Terengganu state. The apiary chosen is located at N05°40'38.6", E102°43'03.2" and the area covered approximately 2.5 hectares. The apiary is selected as there are active stingless beekeeping and honey production activities by the local people. Approximately 10 colonies of stingless bees, *L. terminata* were reared there. The area was an orchard that contains different species of crops such as mango (*Mangifera indica*), rambutan (*Nephelium lappacaum*), jackfruit (*Artocarpus heterophyllus*), lime *(Citrus hystrix),* and langsat (*Lansium domesticum*) trees. Approximately 27 species of crops; a large numbers of trees, climbers, lianas, shrubs, herbs, epiphytes and saprophytes can be found in the area. Besides orchard, the area includes a mixture of rural residential, horticulture and grazing land. In general, the climate in Besut is hot and humid, with average year-round temperatures of ~25^{o}C. There was a rainy season from November to March, and another relatively dry season from July to September.

Reference Sample of Pollens

The reference samples of pollens (N = 21 pollen types) were collected using the methods of Wahizatul *et al*., (2012). Flower bud from the dominant tree species found in the apiary were plucked carefully and preserved in vial containing 70% ethanol to make sure that flower buds were in good condition. Pollen samples were then prepared on the slides using micropipette and cover slide. The prepared slides were observed under a Moticam 1300 light microscope (40X magnification) and pollen image was captured using Dino-Eye Digital Microscope Eyepiece Camera 2.0 (AM4023X). The length and width (in mm) of pollens were measured and used as reference in order to compare with the pollen type identified from the body of the stingless bees.

Sampling of Stingless Bees and Foraging Activity Observation

Sampling of the stingless bees was carried out from November 2012 to February 2013 to examine any differences in pollen preferred by the stingless bees for each month. Stingless bees were collected using a light insect net of 60 cm long and 25 cm diameter open-mesh net (250 mm) with little air resistance by swinging it rapidly. At least five individuals of stingless bees were collected at different time between morning (from 0800 am to 1100 am), mid-day (from 1100 am to 1400 pm) and late afternoon (from 1400 pm to 1800 pm). This was done by briefly closing the entrance to the hives and randomly capturing five arriving foragers that had pollen loads with insect net. Each individual of collected stingless bees was transferred into properly labeled vials, preserved in 70% ethanol and taken back to the laboratory for analysis. The vials then were labeled with date of collection, time of collection and number of individual. Foraging activity of stingless bees was observed throughout the sampling time (0800-1800) by recording using camcorder Panasonic HC-V100. The parameter measured for foraging activity was the total number of arriving foragers to the hives with pollen loads.

Preparation of Pollens Collected from L. terminata and Pollens Identification

The pollen loads were removed from the pollen baskets of the captured *L. terminata* foragers with a blunt needle. The pollen clusters from each individual were then preserved in the vials that containing 70% ethanol and the vials were properly labeled. Micropipette was used to transfer 1 mL of pollens that have been preserved earlier in the vial to the haemocytometer slide. Ten replicates of slides for each vial were prepared in order to provide representative coverage pollens that were collected by each individual of the stingless bees. The haemocytometer slide was observed under light microscope. The pollen image was captured and the length and width (in mm) of the pollen were measured. In the microscopic pollen analysis and identification, reference samples and available keys were used (Kiew and Muid, 1991; Hesse *et al*., 2009).

Data Analysis

For the frequencies of pollen grains in honey, the system adopted by Louveaux *et al*., (1978) was used. The following frequency classes that have been used in estimates of pollen grain frequencies comprised the following: "Dominant pollen" (more than 45% of the pollen grains counted); "Accessory pollen" (16-45%), "Isolated pollen" (3-15%) and "Occasional pollen" (less than 3%). Analysis of variance (ANOVA) was used to determine the differences of pollen types and abundances collected by *L. terminata* among months (November, January and February), and total number of arriving *L. terminata* foragers with pollen load among times of foraging activity (morning, mid-day and late afternoon), followed by Bonferroni test. All dependent variables were tested for normality of distribution (Kolmogorov–Smirnov test) before it was proceeded to one-way ANOVA statistical test. Data were log(x+1) transformed to ensure normality in calculations of means and ANOVAs. All the analyses were performed using the SPSS 19.0 statistical software.

Results and Discussion

Overall, a total of 11 types of pollens were collected from the *L. terminata* foragers. However, only nine types of the pollens were successfully identified (Table 1). The most frequently collected pollen was from the following plant species: *Ixora coccinea* (36.78%), followed by *Citrus hystrix* (24.27%) and *Murraya paniculata* (21.05%) (Figure 1). All the three pollens were considered as accessory pollens. The other pollen types were categorized as isolated pollens, which consist of: *Mimosa pudica* (13.56%), *Callophyllum inophyllum* (7.15%), *Asystasia gangetica* (4.3%), *Bougainvillea glabra* (Type 1) (3.73%) and unidentified pollen 1 (3.65%). However, unidentified pollen 2 (1.86%) and *Bougainvillea glabra* (Type 2) (0.86%) were considered as occasional pollens. The accessory pollens morphology and images are presented in Figure 2.

The highest percentage of pollen was in November 2012 from *Citrus hystrix* (1234 pollens; 67.17%), which indicates that lime was the favourite source of pollen at that time. *Calophyllum inophyllum* constituted the second highest percentage of pollen (241 pollens; 13.12%), followed by *Murraya paniculata* (232 pollens; 12.63%) and *Ixora coccinea* (130 pollens; 7.08%). Unfortunately, no sampling was done in December 2012 due to higher rainfall patterns which jeopardized the handling and sampling of *L. terminata* from the hives. In January 2013, the most predominant pollen collected was *Calophyllum inophyllum* (547 pollens; 44.44%). The second highest pollen collected was *Bougainvillea glabra* (Type 1) (411 pollens, 33.39%), followed by *Asytasia gangetica* (178 pollens; 14.46%) and the lowest was *Bougainvillea glabra* (Type 2) with only 95 pollens (7.72%). Interestingly, many pollen of different plant species were collected in February 2013. Seven of them were identified and the most frequent pollen collected was from *Ixora coccinea* (3,926 pollens, 49.32%). *Mimosa pudica* constituted the second highest pollen collected (1,495 pollens, 18.78%), followed by *Citrus hystrix* (1,442 pollens, 18.12 %), unidentified pollens 1 (402 pollens, 7.63%), *Asystasia gangetica* (296 pollens, 3.72%), unidentified pollens 2 (205 pollens, 2.54%), and the lowest was *Suregada multiflora* with only 194 pollens (2.44%).

Table 1: Occurrences of pollen class percentages collected by the stingless bees, *L. terminata* from an apiary in Besut, Terengganu. Notes: D = dominant pollen (> 45%); A = accessory pollen (15%-45%); I = isolated pollen (3%-15%); O = occasional pollen (< 3%); - = Absent

Habit	Pollen types (Order/ Family/ Species)	Common local name	Months		
			November 2012	January 2013	February 2013
Shrub	Sapindales / Rutaceae *Murraya paniculata*	Orange jasmine	I	-	-
Shrub	Sapindales / Rutaceae *Citrus hystrix*	Kaffir lime	D	-	I
Tree	Malpighiales / Calophyllaceae *Calophyllum inophyllum*	Balltree	I	A	-
Shrub	Gentianales / Rubiaceae *Ixora coccinea*	Ixora	I	-	D
Shrub	Caryophyllales / Nyctaginaceae *Bougainvillea glabra* (Type 1)	Paper Flower	-	A	-
	Bougainvillea glabra (Type 2)	Paper Flower	-	I	-
Perennial	Fabales / Mimosoideae *Mimosa pudica*	Sensitive plant	-	-	A
Herb	Lamiales / Acanthaceae *Asystasia gangetica*	Creeping Foxglove	-	I	I
	Malpighiales / Euphorbiceae *Suregada multiflora*	False lime	-	-	O
	Unidentified pollen 1		-	-	I
	Unidentified pollen 2		-	-	O

In this study, the most frequent pollen collected was *Ixora coccinea* which possess several characteristics that might influence the preference of pollen by *L. terminata*. *Ixora coccinea* or locally known as 'Jejarum' is a shrub that had been used extensively in traditional medicine. According to Gilman (1999), *Ixora coccinea* have four to six-inch globular clusters of bright red, orange, yellow, pink, or white tube-shaped flowers that bloom continuously under ideal conditions in full sun. Though not yet supported by statistically data in our experiment, there were several studies done by other researchers who found that flower attractiveness may have a positive influence on the abundance and frequency of the flower visitors. For examples, Brody and Mitchell (1997) reported that *Ipomopsis aggregata* could attract hummingbird and other flower visitor with the bright red color of the flower. Similar finding was revealed by Bosch and Waser (2001) who found that *Aconitium columbianum* could attract pollinator due to the flower attractiveness. Steven *et al*., (2003) and Harder *et al*., (2004) also claimed that flower attractiveness may have a positive influence on the number of pollinators.

Instead of colour, previous studies also showed that shape and odor of flowers play important roles as the signals that facilitate the recognition of rewarding resources.

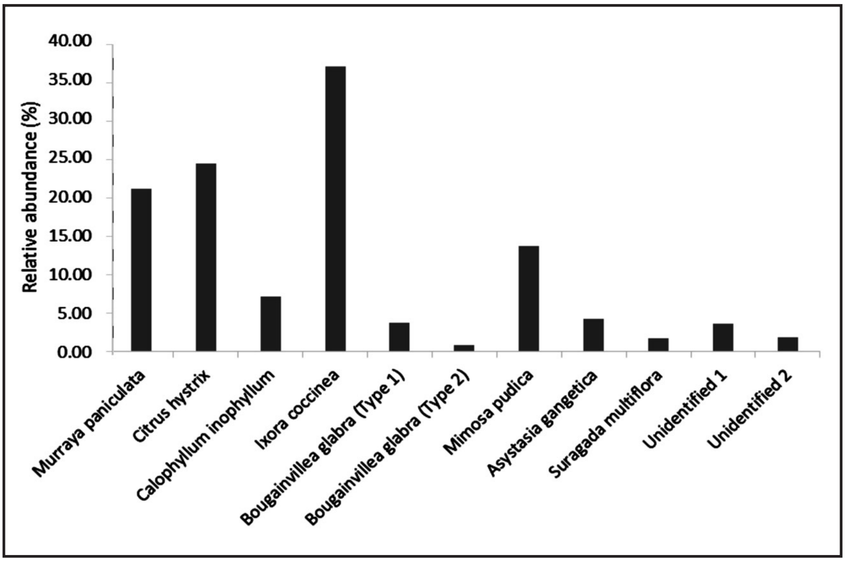

Figure 1: Relative abundance (%) of pollen types collected by the stingless bees, *L. terminata* from an apiary in Besut, Terengganu

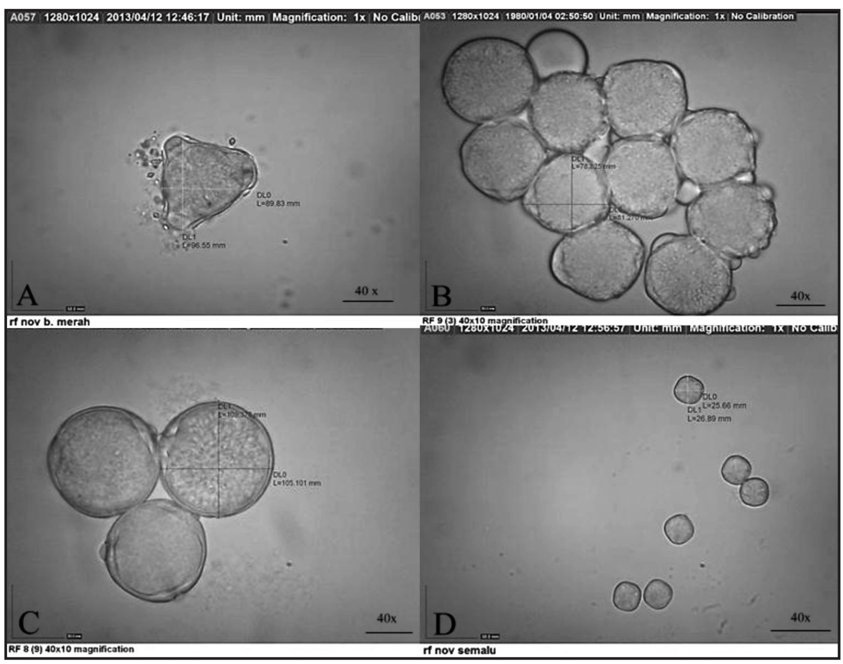

Figure 2: Light photomicrographs of accessory pollen types collected by the stingless bees, *L. terminata* from an apiary in Besut, Terengganu. Notes: A = *Ixora coccinea*, B = *Citrus hystrix*, C = *Murraya paniculata*, D = *Mimosa pudica*

Ixora coccinea, *Citrus hystrix* and *Murraya paniculata* have strong and nice odor. This would suggest why these three flowers were preferred by *L. terminata* in this current study. According to Azuma *et al*., (2002), flower scent and scent chemical profiles is one of the important factors in attracting the pollinators. This finding also was supported by Reichle *et al.,* (2010) who suggested that a bee species, *Scaptotrigona pectoralis* could learn a series of mixtures of odors which were linalool, phenylacetaldehyde, geraniol, and eugenol from the nest atmosphere.

The other factor of pollen preferences is probably the size of flowers. For example, the size of *Ixora coccinea* pollen is very small which might explained why *L. terminata* pollinated this flower more often compared with the other flowers. This postulation was in accordance to Wille *et al*., (1983) who revealed that crop or flower preferred by stingless bees were usually small flowers. This trend could be observed in three other flowers, which were *Citrus hystrix*, *Murraya paniculata* and *Mimosa pudica* which also possess the same characteristic of small size flowers (< 1 inch in size). The ability of *L. terminata t*o enter the corolla or calyx tube, even though the tubes are small or narrow, suggested that *L. terminata* is one of the most effective pollinator due to this exclusive ability which does not occur in other pollinators such as butterflies, honey bees and birds.

From the results, it could be observed that *L. terminata* preferred to collect small pollen grains, such as *Ixora coccine*a (~69.66 mm), *Murraya paniculata* (~92.29 mm), *Citrus hystrix* (~72.72 mm), and *Mimosa pudica* (~28.72 mm). However, they still showed preference for the medium to large size of grains such as *Suragada multiflora* (~257.29 mm) and unidentified pollen 1 (~198.60 mm). This implied that *L. terminata* is a flexible pollinator in which they could collect small grains from small flowers which could not be pollinated by other pollinators such as bats and birds, and also could collect larger size of pollen grains. This flexibility is an advantage for *L. terminata* as they could always survive to collect different sizes of pollens in any area. This ability is indirectly strengthening the belief that *L. terminata* is an effective and efficient pollinator of many plant species.

Time of the day had a significant influence on the total number of *L. terminata* foragers ($F = 35.034$, $p < 0.05$). Morning (0800-1100) and late afternoon (1400-1800) were the most effective times for foraging activity of *L. terminata*, while mid-day (1100-1400) was not effective time for the *L. terminata* foragers (Figure 3).

The current result obtained suggested that most visitors were noted in the morning (~100 individuals) and most frequently in bright weather. This could be associated by the flower anthesis concept which is the period during which a flower is fully open and functional. Most the dominant flowers in the apiary started to open between 0800 and 1030 hours, whereas the closing of the flowers occurred around 1230 hour. This might explain why the foraging activity of *L. terminata* was higher during morning and lower during mid-day.

The effective time for pollination of *L. terminata* could also be influenced by the energy preservation and nutrient achieved. The highest foraging activity in the morning was due to the need to fulfill their energy requirement. In the mid-day, when the temperature was the highest (~32ºC ± 0.5), less *L. terminata* were observed possibly to preserve their energy or to prevent dehydration. The number of foragers then increased in the late afternoon after the temperatures dropped to ~29ºC ± 0.5. In general, hot sunny days encouraged the *L. terminata* to venture out for foods compared with rainy days which mostly occurred in December. Roubik (1998) reported that response to weather and seasonal patterns of activity could affect the foraging biology of stingless bees. Also, Vicens and Bosch (2000) conducted a research on weather-dependent pollinator activity in an apple orchard, with special reference to *Osmia cornuta* and *Apis mellifera* (Hymenoptera: Megachilidae and Apidae). They found that bee activity was significantly dependent on temperature, solar radiation and wind speed. In this study, it is suggested that seasonally changing weather patterns could be associated with the foraging behavior of the stingless

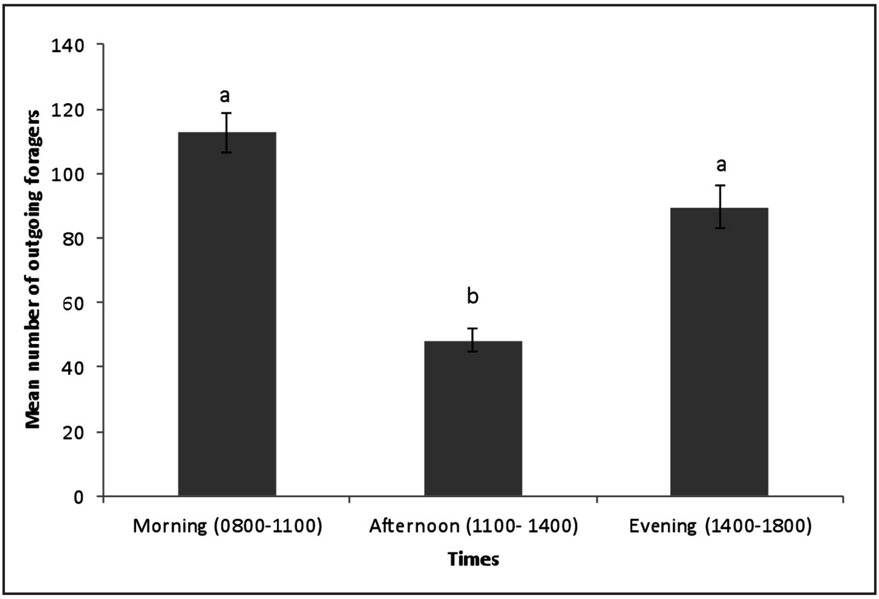

Figure 3: Foraging activity of *Lepidotrigona terminata* in the morning, afternoon and evening peak. Bars represent the means ± standard error of three measurements made during the study. Different letters indicate significant difference between the number of outgoing foragers and time (Bonferroni test: $p < 0.05$)

bees and thus, it is important to understand the characteristics of the tropical climate (i.e. rainfall and temperature) which play a critical role in the foraging preferences of stingless bees in the east coast of peninsular Malaysia.

Conclusion

In conclusion, this study shows that melissopalynological analysis has provide a useful information on the favored plant species and foraging preferences of stingless bees, *L. terminata* from an apiary in Besut, Terengganu. This present study also has provided information on the most effective foraging activity of *L. terminata,* which were in the morning (0800-1030) and late afternoon (1400-1800). Our observations show that colour, odor and size of the flowers and pollen grains were the main factors in attracting the *L. terminata* foragers. Therefore, this melissopalynological investigation is important to understand which plants are utilised as sources of bee forage which provides the information needed to assess the potential for beekeeping in an area. Melissopalynological and bee behaviour study are thus helpful in bee management and in promoting sustainability of apiculture industry.

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