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# Urine versus Pre-mix (Sugar: Salt): Baits for Stingless Bees (Hymenoptera: Meliponini)

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#### ABSTRACT

Stingless bees are dispersed throughout Malaysia and form an important group of pollinators in agriculture and natural ecosystems. A study was conducted at Lojing Highland in Kelantan, Malaysia on the preference of stingless bees towards pre-mix bait [sugar and salt (1:2) (sugar: water:v:v)] with 2.5g NaCl added per 500 ml of solution] and urine bait for two consecutive days. A total of 285 stingless bees of 15 species were sampled for this purpose. Overall, stingless bees showed no preference for either bait, but a closer examination showed species-level preferences. Five species preferred urine bait over pre-mix bait, and another eight preferred pre-mix bait over urine bait. No significant differences were found on stingless bees preferences towards pre-mix bait and urine baits (p>0.05). Five stingless bee species (*Lisotrigona scintillans, Pariotrigona pendleburyi, Lepidotrigona ventralis, Tetrigona apicalis,* and *Tetragonula collina*) were found to be frequently attracted to the urine bait compared with pre-mix bait. This study shows that stingless bees' preference for pre-mix bait and urine baits over species attracted to pre-mix bait compared with urine bait. This study shows that stingless bees' preference for pre-mix or urine baits depends on species.

Keywords: Stingless bee, pre-mix, urine, bait preference

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## **INTRODUCTION**

Compared to *Apis*, stingless bees (Apidae: Meliponini) have 50 times more species and are very different biologically (Roubik, 2006). The stingless bees are dispersed throughout most parts of Malaysia and form an important group of pollinators in agriculture and natural ecosystems (Hannah

et al., 2012). In Asia, there are 43 recognised species belonging to two genera, namely, Lisotrigona and Trigona (Michener, 2007). The genus *Trigona*, comprising of 120 species, were placed into 10 subgenera including *Homotrigona*, *Lepidotrigona* and *Heterotrigona* (Chinh et al., 2005). Recently, these subgenera have been upgraded to genera (Rasmussen, 2008). Malaysia hosts a rich diversity of stingless bees; 29 species have been identified so far (Eltz & Bru, 2003; Mohd Norowi et al., 2010; Hannah et al., 2012).

Many studies have been conducted in forest ecosystems to document the diversity of stingless bee species using different baiting methods (Boontop et al., 2008). Baits include mixture of honey, water and salt (Boontop et al., 2008), sugar solution with added lemon or rose essence (Hannah et al., 2012), diluted honey (Salmah et al., 1990) and odour baits (Pedro & Cordeiro, 2015). However, not all stingless bees are attracted to sugar baits. Trigona necrophaga, T. hypogea and T. crassipes have an obligate necrophagy habit (Camargo et al., 2012), whereas in Thailand, Lisotrigona cacciae, L. furva and Pariotrigona klossi (Meliponini, Apidae) workers drink tears from human eyes (Banziger et al., 2009). Thus, when studying the diversity of stingless bees, using just one type of bait would make the sample bias to specific groups and fail to capture a more comprehensive representation of the stingless bee community of the study area. In order to better understand how bait types may affect the type of stingless bees

sampled, this study examined the stingless bee preference towards pre-mix (sugar: salt) and urine baits.

#### **MATERIALS AND METHODS**

The study was conducted at Lojing Highland located in Kelantan, Malaysia, for the period of 2 days (23<sup>rd</sup> - 24<sup>th</sup> January 2014). This sampling site is located at an altitude of 800 to 1000 meters above the sea level, which is categorised as an upper dipterocarp forest or lower montane forest. Two parallel transects, each 100m long, were used. The distance between the parallel transect was 1.5 m. In each transect, 10 baiting stations were installed at the distance of 10 meter apart. This was made into two replicates [sampling location coordinates – (04° 38' 01.7" N 101° 30' 21.0" E) (04° 38' 09.4"N; 101°30' 19.2" E)].

The pre-mixed bait was prepared by mixing sugar and water (1: 2), added with 2.5g NaCl per 500 ml of solution (Boontop et al., 2008). The urine was collected overnight from a person. At each baiting station, the pre-mix solution and urine were sprayed ( $\sim 20$  ml). The bait station was  $\sim 1$ m in diameter. The spraying commenced between 0800-0900 hours. The bees attracted to these bait spots were sampled for a maximum duration time of five (5)minutes in each baiting station, twice daily (9 a.m. - 11 a.m. and 2 p.m. - 4 p.m.). The same observations were done for both days. Sweep net was used to capture and collect the stingless bees. The captured stingless bees were then placed in a killing jar and preserved in 70% alcohol. The specimens

were brought back to Biology Laboratory, Universiti Malaysia Kelantan; they were pinned and preserved and identified using taxonomic keys provided by Sakagami et al. (1990).

#### **Statistical Analysis**

Collected data were subjected for normality test and were found not normally distributed (Shapiro-Wilk test, p<0.05). Therefore, Wilcoxon Rank Sum was performed to analyse any significant differences in the frequency of stingless bee captured using both the baits. Statistical analysis was done using JMP 8.0 (SAS Ins.).

#### **RESULTS AND DISCUSSION**

A total of 285 stingless bee specimens of 15 species were sampled for the duration of 2 days. There was no significant difference in the frequency of the stingless bees sampled using the pre-mixed bait and urine (p > 0.05)although more specimens were sampled for the pre-mixed baits (see Table 1). In particular, Tetragonula geissleri attracted to the pre-mixed bait twice as much compared with urine bait, whereas Lepidotrigona ventralis was found to prefer urine bait compared to the pre-mixed bait (Table 1). Five stingless bee species (Lisotrigona scintillans, Pariotrigona pendleburyi, Lepidotrigona ventralis, Tetrigona apicalis and Tetragonula collina) were found attracted to the urine bait compared to the pre-mixed bait. Meanwhile, eight (8) species attracted to the pre-mixed bait compared to the urine bait (Table 1). Throughout the sampling period, the Lepidotrigona

ventralis was also found to be attracted to wet socks and sweat. Baiting stingless bees using pre-mix bait is a commonly accepted technique (Boontop et al., 2008; Hannah et al., 2012) with the assumption that all stingless bees are attracted to sugar. A recent study in Thailand showed that using 50% (v/v) honey solution was able to attract 12 species of stingless bees (Jongjitvimol & Petchsri, 2015). Others experimented with odour baits (cineole, vanillin, benzyl acetate, methyl salicilate, eugenol and benzyl benzoate) to attract Trichotrigona but failed (Pedro & Cordeiro, 2015). However, this study found that some species preferred urine compared with pre-mixed baiting. Therefore, stingless bees might get attracted to the salt content in both the baits tested. Nevertheless, the subject matter needs to be further tested and scientifically validated. In addition, it was noticed during the sampling that the baiting stations which were exposed to sunlight attracted more stingless bees compared to those baiting stations located under the forest canopy. This could be due to the light intensity as a number of stingless bee catches were found to be positively correlated with transmission of light (Boontop et al., 2008).

#### CONCLUSION

In sum, the stingless bees in our study did not show any preference for either urine or pre-mixed baits (p > 0.05). Certain species were sampled at one bait more than double the other, though statistical tests were not performed. For a well distributed stingless bee sample, we suggest that different baits Table 1

The frequency of stingless bees sampled from the pre-mixed baits and urine baits

Species	Pre-mix (sugar: salt)	Urine
Geniotrigona thoracica	1	1
Lisotrigona scintillans	2	12
Pariotrigona pendleburyi	7	13
Heterotrigona erythrogastra	13	5
Lepidotrigona trochanterica	5	0
Lepidotrigona ventralis	18	25
Heterotrigona itama	9	2
Tetragonula geissleri	61	15
Tetrigona apicalis	1	6
Trigonella lieftinicki	3	0
Trigonella moorei	9	0
Tetragonula collina	4	10
Tetragonula laeviceps	13	0
Tetragonula reepeni	37	12
Tetrigona atripes	1	0
Total	184	101

are to be applied. In addition, biotic and abiotic factors should not be ignored as different stingless bees might have different foraging time, weather condition, and foraging distance.

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