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Hygienic Behavior of Stingless Bees (*Heterotrigona itama*) in Cultivated Colonies in the Imbo Putui Customary Forest

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ABSTRACT

One of the important behaviors of stingless bees is hygienic behavior, which includes cleaning the nest from dirt, food scraps, and dead insect carcasses to keep the colony healthy and disease-free. The goal of this study was to determine the type and quantity of waste produced by *H. itama* in the nest, as well as the time spent by worker bees removing waste from the nest. The research method employed was direct observation and quantitative measurement of three active *H. itama* colonies. The findings revealed that the trash generated contained carcasses, feces, and food scraps. Feces and food scraps accounted for 89%, with carcasses accounting for 11%. The activity of flinging waste out of the nest decreased during the day due to *H. itama*'s high movement in and out of the nest in search of food, as well as high ambient temperatures. The busiest times for garbage disposal were in the morning (09.10 - 11.10 WIB) and afternoon (13.10 - 16.10 WIB). A clean nest with minimal waste suggests a healthy *H. itama* colony with good hygiene behaviors. In contrast, a messy nest with much excrement indicates an unhealthy H. itama colony with poor hygiene.

Keywords: *Heterotrigona itama; hygienic behavior; nest cleanliness; stingless bees*

INTRODUCTION

Stingless bees (*Heterotrigona itama*) are a social insect that lives in colonies (Garmaev *et al*., 2022). Bee colonies typically consist of thousands of bees who perform a variety of functions to ensure mutual survival (Shibao *et al*., 2022). Stingless bees engage in sanitary behavior, which includes cleaning the nest of debris, leftover food, and dead insect carcasses (Hidayat, 2019). This behavior is critical for colony health, illness prevention, and ensuring that the nesting habitat is safe to live in.

However, to date, research on hygienic behavior in stingless bees, especially Heterotrigona itama, is still very limited. Most of the existing studies focus more on honey bees *(Apis mellifera)* (Medina-Flores *et al*., 2022; Spivak & Danka, 2021), which shows that hygienic behavior plays an important role in reducing the risk of infection from pathogens and parasites. Research on stingless bees in various countries, such as Australia conducted by Le Gros *et al*. (2022), is also still limited, especially to certain species such as *Tetragonula carbonaria* and *T. hockingsi.* Meanwhile, environmental conditions and other factors influencing stingless bee hygiene vary by region, including Indonesia.

Stingless bee cultivation, also known as meliponiculture (Harion, 2023; Nuraeni *et al*., 2022), has been practiced in Indonesia, particularly in the Imbo Putui Customary Forest area of Riau Province, however, research on the hygienic behavior of stingless bees in this area is almost non-existent. Beekeepers in this area tend to prioritize increased honey yield over nest cleaning. In reality, proper sanitary

behavior can have a direct impact on colony health, which in turn affects honey production results.

The novelty of this study lies in its focus on the hygienic behavior of *H. itama* in the context of the cultivation environment in the Imbo Putui Customary Forest area, Riau Province. Furthermore, this study is likely to help produce more sustainable stingless bee farming by improving understanding of the importance of nest cleaning as a supportive element for colony health and productivity. The goals of this study were to define the types and quantities of waste produced by *H. itama* in the nest, as well as to assess the timing of trash disposal outside the nest by worker bees. This study is expected to contribute valuable information to the development of more successful and sustainable stingless bee cultivation practices.

METHODS

This research was conducted in May 2024. Observations were conducted on three colony boxes in the *H. itama* cultivation region of the Imbo Putui Customary Forest, Petapahan Village (**Figure 1**).

Figure1. The Research Map of *H. itama* Cultivation Area at Imbo Putui Customary Forest

The colony boxes were chosen based on the presence of *H. itama* colonies that were still alive, not in direct sunlight, and worker bees were already visible collecting waste from the *topping*. Based on these parameters, the boxes chosen were box 9 behind the seed house, box 11 behind the herbal cafe, and box 15 beneath the oil palm tree. Observations of the behavior of cleaning the nest and disposing of waste were conducted for 3 weeks, with 1 colony box observed each day and each colony box repeated 3 times.

The tools used in this study were a camera, stationery, scissors, a small saw, rope, a meter, a stopwatch, a digital analytical scale, a Thermohygrometer, a lux meter, a bee net, a magnifying glass, an insect net, a tally counter, and tweezers. The materials used were glass plastic, sample bottles, label paper, masks, gloves, nylon nets, small plastic buckets, glue, and clear tape. The research procedure included colony box preparation, environmental parameter measurement, and

observation of worker bees collecting waste for 10 minutes every hour from 06.00-18.00 WIB, followed by a 20-minute observation of waste disposal activities outside the nest by *H. itama*, and waste collection and weighing via direct capture and transparent funnel traps. The proportion of waste collected and removed from the nest was compared in three colonies. Average results are expressed as ± one standard deviation (SD).

RESULTS AND DISCUSSION

Types and Quantity of Waste Produced by Stingless Bees *(H. itama)*

Based on the research results, the waste produced by *H. itama* in the nest in three cultivation boxes is carcasses, feces, and food scraps. This is in line with research conducted by Medina *et al*. (2014), on the stingless bee Melipona beecheii produces waste in the nest consisting of feces, old nest cells (*Old Brood Cells*) that are no longer used, cocoons (where bee larvae undergo metamorphosis into pupae), adult bee carcasses and young bee carcasses. The types and quantities of waste produced by *H. itama* in the nest can be seen in **Table 1**.

Table 1. Type and quantity of rubbish produced by bees without sting *H. itama* in the den

Rubbish Type	Rubbish Quantity			Total	Percentage%
	Box 11	Box 15	Box 9		
Corpse	2	0	1	3	11%
Other rubbish *	9	10	5	24	89%
Total	11	10	6	27	

*Dirt and food leftover SD= 0

According to **Table 1**, the type of waste produced in the *H. itama* nest is 89% feces and food waste, whereas *H. itama* carcasses account for 11% of the waste. This demonstrates that *H. itama*'s increased feeding activity in digesting food results in higher metabolic waste in the form of feces. The availability of food supplies, such as flowering plants located near the *H. itama* growing area in the Imbo Putui Customary Forest, may contribute to bees' high activity in consuming food, resulting in more feces in the nests. **Figure 2** shows the accumulation of waste produced by *H. itama* in the nest of three cultivation boxes.

Figure 2. Rubbish pile (a) dirt and (b)foot leftover in the topping

H. itama produces a significant amount of food waste in the nest. This state is induced by the activity of *H. itama*, which is constantly collecting food for the survival of the bee colony in the nest. Food waste is produced in the form of pollen that is no longer edible or contaminated, as well as honey that has gone bad or fermented.

(a) (b)

Figure 3. Bee *H. itama* makes rubbish (a) dirt, (b) leftover food out of the den.

In addition to waste and food waste, *H. itama* generates small amounts of carcass waste in its nest. *H. itama*'s low mortality rate is attributable to the colony's active defensive systems against pests and diseases. This is demonstrated by worker bees' hygienic behavior in cleaning the nest by collecting waste. Worker bees pile debris on the nest's border and mold it into waste pellets, which are eventually flung out (see **Figure 3**). *H. itama* detects carcasses in the nest and swiftly throws them out (**Figure 4**). This is reinforced by Heard (2016), bees will immediately throw dead bees or dead larvae from the nest, to maintain the cleanliness of the nest and prevent contamination of egg cells. This hygienic behavior mechanism will prevent the transmission of disease in the *H. itama* colony which allows a reduction in the number of carcasses produced in the nest. Based on the results of research conducted by Walton *et al*., (2019), it was found that only around 31% of worker bees in the colony carried out carcass removal even when carcasses were abundant and only around 12% participated in repeated behavior to lift carcasses.

Figure 4. Bee *H. itama* throws away the corpse rubbish out of the den

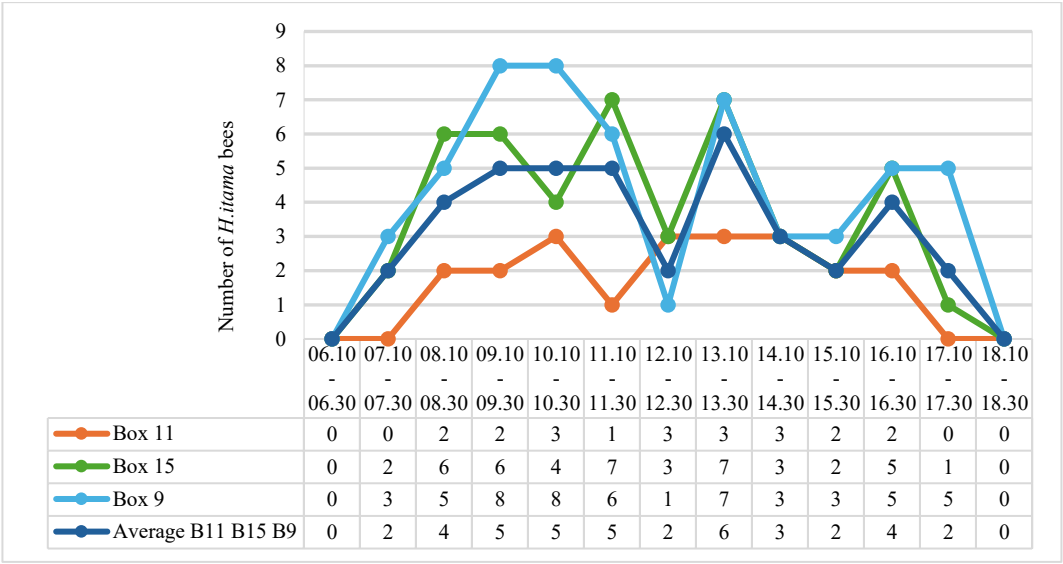
The quantity of waste produced by *H. itama* in the nest in the three observed cultivation boxes showed variations in the quantity of waste in each box (see **Table 1**). The waste with the highest quantity, totaling 11, was produced in box 11, which was dominated by feces and food waste 9, while the carcasses produced 2. This was due to the availability of food sources being closer to the nest of the stingless bee colony *H. itama* in box 11.

The total amount of waste in box 15 is 10, but it is just made up of feces and food waste, with no carcasses identified. This can be read as indicating that the *H. itama* colony in box 15 exhibits excellent sanitary behavior and is in good health, resulting in a low mortality rate, as evidenced by the absence of carcasses produced in the nest. Box 9 has the least amount of waste of the 3 cultivation boxes, with a total of six waste items consisting of 1 carcass waste, 5 excrement, and 1 food waste.

In the three cultivation boxes, the types of garbage and food waste had a standard deviation of zero, demonstrating that *H. itama* produces waste and food waste consistently. This suggests that the food source conditions in the three *H. itama* colony boxes were constant during the observation period. Therefore, *H. itama* bees do not lack or have excess food, remaining in a stable condition so that the condition of *H. itama* also remains healthy. According to Zaki and Razak (2018), the pollen profile of *H. itama* in small rubber farms varies with the flowering season, affecting the amount of feces produced due to changes in food sources. Furthermore, a study by Van Engelsdorp *et al*. (2017), found that bees with *Colony Collapse Disorder* (CCD) ate less, resulting in less fecal production than healthy bees.

Waste Disposal Time Out of the Nest by Worker Bees (*H. itama*)

The activity of bees disposing of waste out of the nest was observed in three cultivation boxes starting at 06.00 WIB to 18.00 WIB. The number of *H. itama* that came out each hour is presented in **Figure 5**.



	06.10 - 06.30	07.10 - 07.30	08.10 - 08.30	09.10 - 09.30	10.10 - 10.30	11.10 - 11.30	12.10 - 12.30	13.10 - 13.30	14.10 - 14.30	15.10 - 15.30	16.10 - 16.30	17.10 - 17.30	18.10 - 18.30
Box 11	0	0	2	2	3	1	3	3	3	2	2	0	0
Box 15	0	2	6	6	4	7	3	7	3	2	5	1	0
Box 9	0	3	5	8	8	6	1	7	3	3	5	5	0
Average B11 B15 B9	0	2	4	5	5	5	2	6	3	2	4	2	0

Figure 5. The Timing Graph of throwing away the rubbish out of the den by working bee *(H. itama)*

According to **Figure 5**, worker bees (*H. itama*) did not dump waste out of the nest in any cultivation boxes during the morning at 06.10 WIB and the afternoon at 18.10 WIB. Box 9 had the most *H. itama* worker bees that tossed waste, followed by boxes 15, and 11. According to the average of the three cultivation boxes, the peak of garbage-throwing activity happened in the morning (09.10-11.10 WIB) and in the afternoon (13.10-16.10 WIB). During the day, waste tossing activity is lower.

Environmental variables outside the *H. itama* nest can also influence worker bees' activity in removing garbage from the nest. This is supported by Asiah *et al*. (2015), findings, which show that *Heterotrigona itama* and *Geniotrigona thoracica* bees' flight activity reduces during the day. This is most likely owing to high temperatures, low relative humidity, and a paucity of pollen sources. Jones *et al*. (2024), found that waste removal rates were generally low. However, in a colony of 100 *Tetragonula carbonaria* bees, waste removal rates reached 73% on the first sunny day after a long period of wet weather.

A study conducted by Devkota *et al*. (2024), found that when the total activity of bees entering and leaving the nest rose, the number of workers engaging in waste disposal dropped or even ceased. High waste disposal activity occurred with periods of low colony admission and exit activity. Furthermore, the researchers discovered that the average number of bees involved in waste disposal activities was lower than those engaged in foraging trips for both species (*Plebeia droryana* and *Tetragonisca fiebrigi*). Other studies, Mateus *et al*. (2019) and Hammel *et al*. (2016), have also seen a shift in tasks as worker bees age, including waste removal and foraging flights.

CONCLUSIONS

H. itama produces waste in the nest, including carcasses, stool, and food scraps. The amount of waste produced is higher for feces and food scraps (89%), than for corpses (11%). A clean nest with minimal waste suggests a healthy *H. itama* colony with good hygiene behaviors. In contrast, a dirty nest with a lot of excrement indicates an unhealthy *H. itama* colony with poor hygiene. *H. itama* throws feces out of the nest most frequently in the morning (09.10 WIB-11.10 WIB) and afternoon (13.10 WIB-16.10 WIB).

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