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Investigation of the Best Artificial Propagation Technique for Stingless Bee *Heterotrigona itama* (Hymenoptera: Apidae: Meliponini)

Mohamad Syukri Tan Shilan[1,2], Nur Azura Adam[1]*, Syari Jamian[1,3], Wan Nur Asiah Wan Mohd Adnan[4] and Siti Asma' Samsudin[1]

[1]*Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM Serdang, Malaysia*
[2]*Division of Crop Industry Development, Department of Agriculture, 32020 Sitiawan,Perak, Malaysia*
[3]*Laboratory of Climate-Smart Food Crop Production, Institute of Tropical Agriculture and Food Security (ITAFoS), Universiti Putra Malaysia, Serdang Malaysia, 43400 Serdang, Selangor, Malaysia*
[4]*Faculty of Forestry and Environment, Universiti Putra Malaysia, 43400 UPM Serdang, Malaysia*

ABSTRACT

Meliponiculture (keeping stingless bees) is a practice that is growing rapidly in the tropical and subtropical regions of the world. A limited number of studies regarding the technique would be most accurate in propagating the colonies to increase their numbers. Three different artificial propagation techniques were investigated in Ladang 10, Universiti Putra Malaysia. Three artificial propagation techniques, namely splitting, bridging, and splitting bridging, were conducted for eight consecutive weeks. Honey pot quantity and pollen pot quantity were recorded weekly for eight consecutive weeks. The success of colony division under different artificial propagation techniques and all the parameters taken were observed and recorded weekly. A significant difference (F = 15.04, df = 2, P = <.0001) was detected in the number of pollen pots between the different artificial propagation techniques, but not for the honey pot quantity (F = 0.22, df = 2, P = 0.8054). The bridging technique recorded the lowest pollen pot quantity while there was no significant difference in splitting and splitting-bridging techniques. The result showed that the splitting technique obtained new brood cells and queen of *Heterotrigona itama*. The splitting-bridging technique developed new brood cells without a new queen, whereas the bridging technique produced only pollen and honey pots. A matured queen's presence can defeat the artificial propagation technique due to its pheromones function.

Keywords: *Heterotrigona itama*, meliponiculture, propagation technique

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E-mail addresses:
mohamadsyukri@doa.gov.my (Mohamad Syukri Tan Shilan)
nur_azura@upm.edu.my (Nur Azura Adam)
syari@upm.edu.my (Syari Jamian)
asiahwan@gmail.com (Wan Nur Asiah Wan Mohd Adnan)
asmasams@yahoo.com (Siti Asma' Samsudin)
* Corresponding author

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INTRODUCTION

Heterotrigona itama is one of the most commercial stingless bees reared in Malaysia (Mustafa et al., 2018). Deforestation reduces the colony of stingless bees and affects their actual role as forest pollinators (Eltz & Bru, 2003). The natural habitat of stingless bees could be destroyed by human activities of cutting down trees or hunting for bee colonies (Villamueva et al., 2005). Cortopassi-Laurino et al. (2006) stated that stingless bees colonies could survive for a long time, typically for more than 50 years. However, the number of swarming times and the queen’s lifespan remain unknown. Gradually, new colonies will begin to form as the old colony splits; this is when the new virgin queen leaves for a new house, escorted by a swarm of stingless bee workers (Nunes et al., 2014). A practical way to multiply the stingless bee colony is by constructing an artificial nest, where the process of stingless bee swarming can be performed naturally (Cortopassi-Laurino et al., 2006).

The stingless bee workers will transfer items such as cerumen, resin, and pollen from the old house needed for constructing a new house. Their activities would also aid in providing sufficient nutrients, which were originally transferred from the old house into the new house to develop a new colony (Kwapong et al., 2010). In addition, most stingless bee species have a steady supply of immature virgin queens as protection if the governing queen is killed (Sakagami, 1982). Therefore, the most typical technique for resolving the queen’s absence in a split colony is for one of the young genes to develop, fly, and take over the egg-laying duty (Imperatriz-Fonseca & Zucchi, 1995).

It is quite challenging for bee farmers to harvest their nest materials since the stingless bees’ nests are often found in tree hollows, dead logs, stems, branches of living trees, and cracks in the wall of houses. Therefore, alternative methods of rearing queen bees and propagating the colony need to be developed without altering the forest biodiversity by mimicking its initial habitat. Resultantly, moving the colony of stingless bees into the artificial hive facilitates the extraction of nest product, simpler to transfer and to propagate (Cortopassi-Laurino et al., 2006).

Splitting or dividing the colonies is another valuable technique. Many people use a crude way to separate their colonies by cutting down whole trees to reach the nests, which results in a lower success rate. However, scientific literature on colony transition and splitting strategies of economically important stingless bee species in Malaysia, such as *H. itama*, is comparatively scarce (Mohd Saufi & Thevan, 2015). This research aims to find the best artificial propagation technique for the stingless bee *H. itama* to expand its population.

MATERIALS AND METHODS

Sampling Site

The sampling site was in Ladang 10, Universiti Putra Malaysia, Serdang, Selangor, Malaysia, with a latitude of 2°59’28.7” N longitude 101°42’52.9” E.

Approximately 30 maintained colonies of *H. itama* were present on the farm. The area was surrounded by various fruits trees such as star fruits, mangoes, rambutan, cempedak, and dukong. Mangosteen trees farm was situated about 50 m from the sampling site. Flowering plant, *Antigonon* sp., (Mexican creeper), commonly known as "Air Mata Pengantin" in Malaysia, was also planted near the sampling site.

Sampling Period and the Hive

This study was conducted from February 2019 to March 2019 for eight consecutive weeks (two months). The hive model (25.5cm x 16.5cm x 16.5cm) was constructed with three compartments of the same size, and one 16 mm diameter entrance hole was located at the lowest compartment.

Sampling Method

Three different artificial propagation techniques were set up in the experimental plot: i) bridging technique, ii) splitting bridging technique, and iii) splitting technique. Each artificial propagation technique was replicated thrice in the experiment. As a result, the success of colony division (obtained new queen) under three different artificial propagation techniques was observed and recorded. In addition, the total number of honey pots and the number of pollen pots were recorded weekly for eight consecutive weeks in the empty boxes of the bridging technique and both (parent and daughter) colonies in the splitting and splitting-bridging techniques.

Bridging Technique. A well-developed and maintained *H. itama* hive's logs in the study site were selected, and the empty medium vertical hive model was placed in front of the log, hooked. A 16 mm hole was drilled in the empty box as an entrance hole to allow foragers to go through it. Stingless bees were only allowed to use the new artificial single way to access their colony in the bridging technique (Klumpp, 2007).

About 10 cm of 16 mm in diameter black poly irrigation pipe was used as a connector between the log's hive and the hive model. Half of an empty 500 ml drinking water bottle was carved and used to cover the log entrance tube before the connecter was attached and secured in place with black duct tape. The roof was provided on top of the hive model. The log and the hive model were kept above the ground using plastic chairs to avoid predators such as ants, termites, toads, and lizards, especially when the nests were situated close to the ground (Kajobe & Roubik, 2006) (Figure 1).

Figure 1. Bridging technique. A black poly irrigation pipe with a diameter of 16 mm was used as a connector between the log's hive and the hive model

Splitting Technique. The maintained colonies of the *H. itama* bees were obtained by cutting off the log carefully using the Stihl M210 chain saw (Stihl, Germany). The mature stingless bee colony consisted of 9 to14 layers of brood cells (Jaapar et al., 2016). Ten layers of brood cells were transferred into each new hives of which the food sources had been removed (honey and pollen). The layers of the brood cells were placed at the centre of the bottom compartment of the box before closing the lid. A colony was divided into two hive boxes, with one of the hives containing matured brood cells (pupa stage, light brown) and at least two of the virgin queens' cells, while another hive contained young brood cells (larval stage, dark) and a mature queen (Figure 2).

The hive containing the queen was marked with a permanent marker. The hive that contained young brood cells (larval stage, dark brown) was placed at the original position while another hive was placed five meters away from the other box (Quezada-Euán, 2018). The hives were kept above the ground using plastic chairs, so termites were prevented from entering. Tiles (2' x 2') were used as the roof and were placed on top of the hives.

Splitting Bridging Technique. The colonies of the *H. itama* were obtained by carefully cutting off the maintained log using the Stihl M210 chain saw (Stihl, Germany). There were two entrance holes sized 16 mm in diameter of each hive. Despite the hive entrance hole, 10 cm length of 16 mm in diameter of black poly irrigation pipe was used as a connector to attach the two hives at the back. The mature stingless bee colony consisted of 9 to 14 layers of brood cells (Jaapar et al., 2016). Furthermore, ten layers of brood cells were transferred into each new hive, of which the food sources were removed (honey and pollen) to avoid attack from natural enemies.

After that, ten layers of the brood cells were placed at the centre of the bottom compartment of the box before closing the lid. A colony was divided into two hive boxes, with one of the hives containing mature brood cells (pupal stage, light brown) and at least two of the virgin queen's

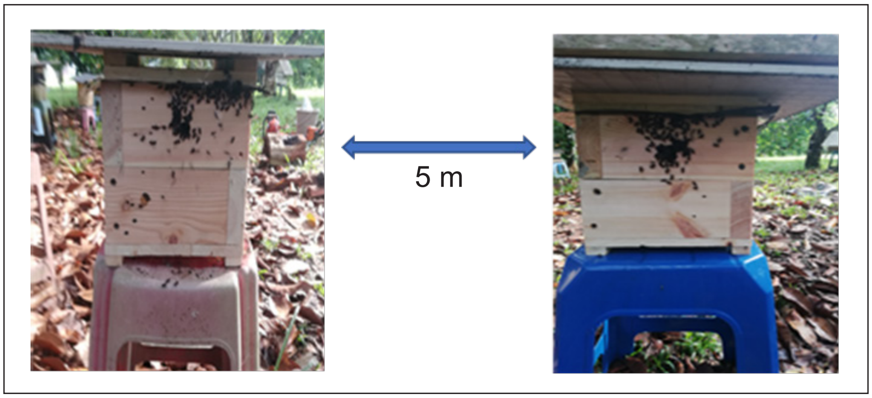

Figure 2. Splitting technique. Five-meter distance of each medium hive model

cells. In contrast, the other hive contained young brood cells (larval stage, dark brown) and a queen was placed at their original positions. The hive containing a queen was marked using a permanent marker. The hives were kept above the ground using plastic chairs to deter the predators from entering, and 2 feet × 2 feet tiles were used as a roof on top of the hives (Figure 3).

Data Analysis

All recorded data were subjected to one-way analysis of variance (ANOVA), and the least significant difference (LSD) mean separation was used at a significant level of 5%. All the analyses were conducted using SAS 9.4 version.

Figure 3. Splitting-bridging technique. Two entrance holes sized 16 mm diameter of each hive, 10 cm length of 16 mm in diameter of black poly irrigation pipe was used as a connector to attach the two boxes

RESULTS AND DISCUSSION

The Observation and Success Rate of Colony Division under Three Different Artificial Propagation Techniques

Splitting Technique. All three colonies used in the splitting technique were successfully divided and obtained a new queen. In the splitting technique, a colony of stingless bees was successfully divided into two colonies. One of the colonies contained a mature queen, while the other contained a new queen that emerged from the virgin queen cell. New queens and brood cells were obtained in the box containing mature brood cells and virgin queen cells (Figure 4). In this study, the emergence of the *H. itama* virgin queen was observed for two weeks after the splitting process. At the same time, the new brood cells were constructed as early as three weeks after the splitting process. The result is consistent with the swarming activities of *Tetragonula laeviceps* reported by Inoue et al. (1984).

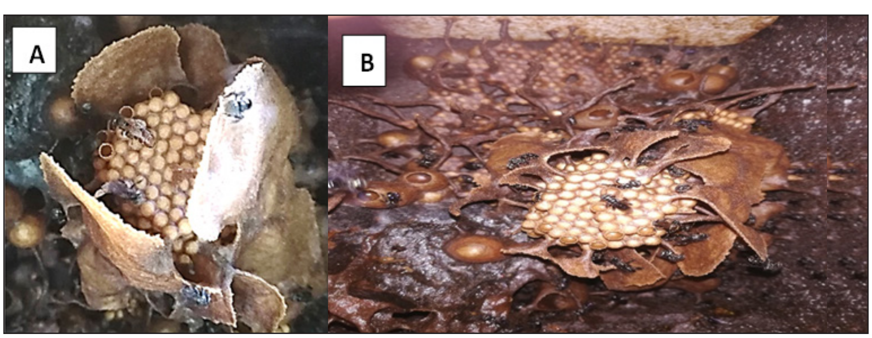

Figure 4. Observation after eight weeks in the splitting technique
Notes. A colony of stingless bees was successfully divided into two colonies, containing a matured queen (A) and a new queen that emerged from the virgin queen cell (B)

The authors reported that swarming was a rapid process and discovered that a week after the virgin queen's arrival, the daughter colony was independent of the mother's colony. The attractiveness of virgin queens changed after mating. The workers normally produce the brood cells constantly, only if the queen is present.

According to Ahmad Jailani and Abdul Razak (2018), colony splitting is a term used to describe the process of forming two colonies in a specific hive from an established colony to maximise the hive's productivity and separating or splitting the size of bee colonies. When a colony is divided, one of the daughter colonies will have no queen, and most stingless bees' propagation techniques rely on artificially dividing a colony into two daughter colonies (Nunes et al., 2014). However, physically splitting the hive into two halves is considered the quickest and most utilised approach (Dollin, 2001).

Between February and late April is the best period in the Yucatan Peninsula to divide colonies, covering the dry season (Quezada-Euán, 2018). However, it is not advisable to divide colonies during the rainy season, which runs from late May to November, this is due to the increase in the breeding of flies, and there would not be enough food in the field to sustain the establishment of new colonies (González-Acereto et al., 2006). In addition, queen mating may take longer during the rainy season since male production reduces at this time (González-Acereto et al., 2006; Moo-Valle et al., 2000). The dry season, popularly known as the fruit season, is between February and July in Malaysia, but it might change due to weather conditions and the colonies' requirements (Jaapar et al., 2016).

Splitting-Bridging Technique. All three colonies used in the splitting-bridging technique were not successfully divided. Although new brood cells were developed in both (parent and daughter) colonies, a new queen was not obtained, and the mature queen controlled the new brood cells. The virgin queen of the daughter colony was unsuccessful to requeen in the splitting-bridging technique, which might be due to the bridge that acted as a tunnel or connector for the mature queen. The bridge or connector provided access to the mature queen to patrol from one hive to another. Regarding the emerging virgin queens, Imperatriz-Fonseca and Zucchi (1995) summarised all three possibilities that could have occurred: i) virgin queen being killed, ii) replaced by the dominant queen, and iii) workers gather to establish a new nest. The queen utilised pheromones to inhibit and monitor their workers (Fletcher & Ross, 1985). Moreover, pheromones indicate the presence of the queen (Nunes et al., 2014). Imperatriz-Fonseca and Zucchi (1995) also reported that the former queen of the colony would be replaced once she became less attractive to the workers. Workers become enraged by the virgin queens' appearance and beauty and begin hunting and murdering them by twisting off their heads and other body parts (Imperatriz-Fonseca & Zucchi, 1995).

Bridging Technique. No new queen and brood cells were developed in the empty hives of the bridging techniques. Bridging has become a new and popular method among many native beekeepers for spreading stingless bees (Dollin, 2001). Dollin (2001) also reported that the bridging technique was discovered by Tom Carter and further developed by Klumpp (2007). The bridging or budding technique is very helpful to create a new bud colony in a position where there is no access to remove it from the current parent colony (Heard, 2016).

The bridging technique also requires proper skills to reduce the chances of the parent colony trying to kill the daughter colony queen (Heard, 2016). The stingless bees can also be coaxed into a box using this approach from a natural nest location in a big tree or an inaccessible hole (Dollin, 2001). Several studies have reported that the development of new colonies took about four months in the bridging method (Dollin, 2001; Mythri et al., 2018; Vijayakumar et al., 2013) and could be prolonged until 45 weeks (Heard, 2016).

Comparison of Honey and Pollen Pot Quantity in Different Artificial Propagation Techniques

As shown in Figure 5, the honey pot quantity was not significantly different between the different artificial propagation techniques (*F* = 0.22, df = 2, *P* = 0.8054). In contrast, there was a significant difference (*F* = 15.04, df = 2, *P* = <.0001) of pollen pot quantity between the different artificial propagation techniques. Figure 6 shows that the bridging technique recorded the lowest pollen pot quantity while there was no significant difference in splitting and splitting-bridging techniques. The lowest number of pollen pot quantities in the

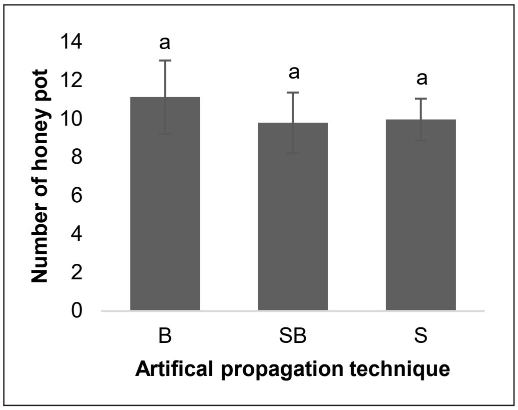

Figure 5. Mean numbers of honey pots for *Heterotrigona itama* in different artificial propagation techniques
Notes. B = Bridging technique; SB = Splitting-bridging technique; S = Splitting technique. Means with the same letters are not significantly different (*P*>0.05)

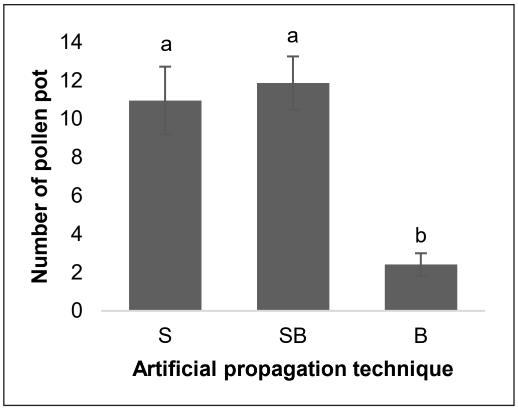

Figure 6. Mean numbers of pollen pots for *Heterotrigona itama* in different artificial propagation techniques
Notes. B = Bridging technique; SB = Splitting-bridging technique; S = Splitting technique. Means with the same letters are not significantly different (*P*>0.05)

bridging technique indicated slow growth of the colony development.

It might be due to the availability of the existing food storage in the parent colony since there were no brood cells in the empty boxes. The empty box may be accepted as part of their nest and food pots because, in the bridging technique, the parent colony was not removed or transferred from its original location. Pollen was gathered in huge amounts by stingless bees for supplying brood cells or storing pollen pots (Ghazi et al., 2018). Pollen and nectar harvesting efficiency impact a colony's survival, growth, and reproductive success (Maia-Silva, 2014). Thus, pollen is essential for the initial stage of colony development. Most stingless bees get their nitrogen source from pollen, which was gathered in huge amounts by workers for supplying brood cells or storing in colony pollen pots (Ghazi et al., 2018). Roubik and Wheeler (1982) reported that brood production was influenced by the amount of pollen stored in a stingless bee colony.

CONCLUSION

This study successfully investigated three different artificial propagation techniques for stingless bees, *Heterotrigona Itama*, with the splitting technique being the only successful one. The bridging technique took a very long time (>4 months) for a colony to propagate and needed proper skills to reduce the chances of the parent colony trying to kill the virgin queen of the daughter colony. New brood cells were developed in the splitting-bridging technique but no new queen. The distance between two colonies once divided influenced the success of colony division. The presence of a mature queen can defeat the artificial propagation technique due to its pheromones function.

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