EVALUATING THE COLONY DEVELOPMENT OF STINGLESS BEE (*LEPIDOTRIGONA TERMINATA*) USING THE MORY TECHNIQUE

by

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DECLARATION

I declare that this thesis entitled "Evaluating the colony development of stingless bee (*Lepidotrigona terminata*) using the MORY technique" is the result of my research except as cited in the references. This thesis has not been accepted for any degree and is not concurrently submitted in the candidature of any other degree.

Signature : 

Name : NURUL MASRETA BINTI MASRI

Date : 9 AUGUST 2024

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Evaluating the colony development of stingless bee (*Lepidotrigona terminata*) using the MORY technique

ABSTRACT

This study examines the effectiveness of different Colony treatments on the growth of brood, honeypots, and beebread in *Lepidotrigona terminata* Colonies. Conducted from October 21, 2023, to January 1, 2024, for Colony 1, and from March 5, 2024, to May 7, 2024, for Colonies 2 and 3, the experiment measured weekly growth to assess treatment impacts. This study was conducted at Kg Paloh, Tendong, Pasir Mas, Kelantan. ANOVA results showed significant effects on brood growth *(F* = 17.057, *df* = 2, $p < .001$), with Colonies 2 and 3 outperforming Colony 1. However, honeypot and beebread pot growth showed no significant differences ($F = 2.519$, $df = 2$, $p = .097$), suggesting these aspects may be influenced by factors like temperature, humidity, and light intensity, requiring longer observation. Post hoc tests confirmed Colony 2 as the most favorable for brood growth, likely due to optimal environmental and structural conditions. The MORY technique, integrating brood, beebread, honey pots, and queen stages from various *L. terminata* Colonies, was used to boost reproductive capacity. Strategic queen selection accelerates development and enhances new colony stability. A one-sample t-test showed no significant difference in lux values from the test value of 700 lux ($t(13) = 0.046$, $p = 0.964$). In a temperature study, the sample mean ($M = 26.44$, $SD = 1.13$) significantly exceeded the standard value of 20 ($t(13) = 21.350$, $p < .001$). Similarly, a relative humidity study found the sample mean ($M = 73.23$, $SD = 1.79$) significantly higher than the test value of 70 ($t(13) = 6.764$, $p < .001$). These findings highlight the importance of colony treatment selection for brood growth and provide insights for improving meliponiculture practices.

Keywords: *Lepidotrigona terminata*, propagation techniques, Colony development, ANOVA, one sample t-test.

Menilai perkembangan koloni lebah tanpa sengat (*Lepidotrigona terminata*) menggunakan teknik MORY

ABSTRAK

Kajian ini mengkaji keberkesanan rawatan Koloni yang berbeza terhadap pertumbuhan induk, honeypot, dan pasu roti lebah dalam koloni *Lepidotrigona terminata*. Dijalankan dari 21 Oktober 2023, hingga 1 Januari 2024, untuk Koloni 1, dan dari 5 Mac 2024, hingga 7 Mei 2024, untuk Koloni 2 dan 3, percubaan itu mengukur pertumbuhan mingguan untuk menilai kesan rawatan. Kajian ini dijalankan di Kg Paloh, Tendong, Pasir Mas, Kelantan. Keputusan ANOVA menunjukkan kesan yang ketara terhadap pertumbuhan induk (F = 17.057, df = 2, p <.001), dengan Koloni 2 dan 3 mengatasi Koloni 1. Walau bagaimanapun, pertumbuhan periuk honeypot dan beebread tidak menunjukkan perbezaan yang ketara (F = 2.519, df = 2, p = .097), mencadangkan aspek ini mungkin dipengaruhi oleh faktor seperti suhu, kelembapan dan keamatan cahaya, yang memerlukan pemerhatian yang lebih lama. Ujian post hoc mengesahkan Koloni 2 sebagai yang paling sesuai untuk pertumbuhan induk, mungkin disebabkan oleh keadaan persekitaran dan struktur yang optimum. Teknik MORY, menyepadukan induk, roti lebah, periuk madu, dan peringkat ratu daripada pelbagai Koloni *L. terminata*, digunakan untuk meningkatkan kapasiti pembiakan. Pemilihan ratu strategik mempercepatkan pembangunan dan meningkatkan kestabilan koloni baharu. Ujian-t satu sampel menunjukkan tiada perbezaan yang signifikan dalam nilai lux daripada nilai ujian 700 lux (t(13) = 0.046, p = 0.964). Dalam kajian suhu, min sampel (M = 26.44, SD = 1.13) secara signifikan melebihi nilai piawai 20 (t(13) = 21.350, p <.001). Begitu juga, kajian kelembapan relatif mendapati min sampel (M = 73.23, SD = 1.79) jauh lebih tinggi daripada nilai ujian 70 (t(13) = 6.764, p <.001). Penemuan ini menyerlahkan kepentingan pemilihan rawatan Koloni untuk pertumbuhan induk dan memberikan pandangan untuk menambah baik amalan meliponikultur.

Kata kunci: *Lepidotrigona terminata*, teknik pembiakan, perkembangan koloni, ANOVA, satu sampel ujian-t.

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LIST OF ABBREVIATIONS AND SYMBOLS

MORY	Mohd Rosli Bin Yaakub
%	Percentage
LUX	Unit of measurement of light level intensity
Rh	Relative humidity unit
°C	Temperature unit
m	Meter unit
Cm2	Centimetre square
MET	Malaysian Meteorological Department
Image J	Software to measure images in centimeter square unit
ANOVA	Analysis of Variance
SD	Standard deviation
N	Total number of observations
M	The mean or average of a set of data
P	p-value in statistical tests.
t	t-statistic in a t-test

CHAPTER 1

INTRODUCTION

1.1 Background of study

Lepidotrigona terminata is a species from the order of Hymenoptera in the family Apidae, belonging to the class Arthropoda (Smith, 1878). Stingless bees comprise a primarily tropical group, with over 500 known species, and potentially an additional 100 species yet to be formally described (Michener, 2013). Insects, which are the most numerous groups on Earth, encompass approximately 675,000 species distributed worldwide (Freitas & Paxton, 1996). Stingless bees are distinctive for their lack of a stinging organ, relying on the propolis they produce as a self-defense mechanism (Free, 1982). Propolis is a substance created by stingless bees from a blend of tree sap, beeswax, bee saliva, and bee enzymes, with bees collecting sap or resin from various plant sources and transforming it into propolis by combining it with other ingredients. Bees employ propolis to repair and safeguard their hives (Popova et al., 2021).

Stingless bees, particularly those found in Java, Indonesia tend to construct hives significantly smaller than those created by domesticated honeybees (Fisher, 2021). In many instances, the hives of stingless bees are discovered nestled within bamboo culms, wooden structures, or even the recesses of stone or brick walls (Fisher, 2021). Essentially, these remarkable creatures make use of any available small hollow space to establish their hives (Fisher, 2021). Stingless bees, also known as Indigenous Peoples' bees, play a crucial role as

vital pollinators in preserving biodiversity and supporting food stability. These species, including stingless bees (Meliponini), have evolved in harmony with local plant species over thousands of years (Anon, 2023). Therefore, deforestation reduces the colony of stingless bees and affects their actual role as forest pollinators (Eltz & Bru, 2003). The natural habitat of stingless bees couldbe destroyed by human activities of cutting down trees or hunting for bee colonies (Villamueva et al., 2005). It stated that stingless bee colonies could survive for a long time, typically for more than 50 years (Cortopassi-Laurino et al.,2006). 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 queenleaves 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). Moving the colony of stingless bees into the artificial hive facilitates the extraction of nest products, simpler to transfer and to propagate (CortopassiLaurino et al., 2006). Splitting or dividing the colonies is a valuable technique (Mohd Saufi & Thevan, 2015). a split of the hive will naturally accept the current queen, the other half must create a new queen in the hive (Poulsen, 2021).

All stingless bees live in colonies, typically comprising dozens to hundreds of workers and usually only one queen (Michener, 2013). These colonies are active every day (Hansell, 1993; Roubik, 1989). Stingless bees are recognized as the

smallest honey-producing bees, with a maximum body size of about 4 mm. As eusocial insects, they form colonies consisting of more than 3,000 adult individuals (Free, 1982). Inside the nest, there are various shapes and arrangements of brood cells and food storage containers. Honey and pollen are stored separately in different containers, with stored nectar or ripened honey located at the extreme ends of the nest cavity for storage during periods of heavy flowering, while pollen and some honey surround the brood area (Sakagami et al., 1993).

1.2 Problem statement

The MORY technique, crafted by Cikgu Mohd Rosli Bin Yaakub based on his five-year expertise in stingless beekeeping, introduces an inventive approach to boost *L. terminata* colonies. This method employs a log as an entrance for stingless bees, creating a welcoming pathway, and utilizes a topping to initiate the formation of a new colony. The log serves as a strategic gateway, while the topping provides the essential conditions for colony development. Cikgu Mohd Rosli bin Yaakub's extensive background in beekeeping adds a unique and insightful dimension to the MORY technique, making it a pioneering and effective methodology for the transplantation of *L. terminata*. Despite the potential benefits associated with the MORY technique in beekeeping practices, there exists a gap in understanding its specific impact on the colony multiplication of *L. terminata.* The inadequacy of research on the application of the MORY technique to this particular bee species hinders a comprehensive assessment of its efficacy in promoting colony growth and sustainability.

This study is motivated by the need to evaluate the influence of the MORY technique on *L. terminata* colony multiplication. The lack of detailed insights into how this technique interacts with the unique characteristics and behaviors of *L. terminata* limits our understanding of its potential contributions to sustainable beekeeping practices. Therefore, the problem emphasizes the necessity of investigating the effects of the MORY technique specifically on the colony multiplication of *L. terminata*. Addressing this knowledge gap is crucial for advancing our understanding of sustainable beekeeping practices and optimizing the management of this particular bee species forecological and economic benefits.

1.3 Objectives

The objectives of the study are as follows:

1.3.1 To document the MORY techniques in transplanting the *Lepidotrigona terminata.*

1.3.2 To examine the environmental data parameters such as light intensity, relativehumidity, and temperature of the study area.

1.3.3 To assess the growth of Colony, pollen pots, and yield of new Colony.

1.4 Scope of the study

The scope of this study aims to evaluate and analyze the effect of the implementation of the MORY Technique on the multiplication of colonies of *L. terminata* species. By conducting a comprehensive evaluation, this study aims to

investigate the potential benefits, drawbacks, and overall impact of using the MORY Technique in promoting the growth and expansion of *L. terminata* Colonies. Throughcareful observation, data collection, and analysis, this research aims to provide valuable insight into the effectiveness and practicality of the MORY Technique in increasing colony size, pollen pots, and yield of new colonies of *L. terminata.* In this study as well, environmental parameters involving temperature, light intensity, and relative humidity (Rh) were taken.

1.5 Significant of study

I. Beekeepers engage - in the cultivation of stingless bees, such as *L. terminata*, not only for the production of honey but also for various other valuable products like bee pollen and propolis. The research focused on Colony multiplication.

II. Ecosystem Health - Bees are indicators of environmental health. Studying *L. terminata* colonies can provide insights into the health of the ecosystems in which they are found and any potential environmental changes affecting them.

III. Scientific Understanding - The study can add to our broader understanding of bee behavior, reproduction, and social organization, which can have implications beyond just *L. terminta*.

CHAPTER 2

LITERATUR REVIEW

2.1 Classification of *Lepidotrigona terminata*

The class of insects within Arthropoda boasts the highest number of members, comprising approximately 675,000 species that are found across the globe, as reportedby Freitas and Paxton, (1996). This vast diversity includes the genus Lepidotrigona, which belongs to the Meliponini subfamily. Stingless bees, a subset of Hymenoptera in the Apidae family, are remarkable eusocial insects that coexist within hive communities, as highlighted by Michener, (2007). These Colonies of stingless bees, as further discussed by Michener, (1974), exhibit complex eusocial behaviors (Inoue et al., 1985).

2.2 Morphology of *Lepidotrigona terminata*

The external morphology of stingless bees can be divided into three main body parts: the head, thorax, and abdomen. Notable features present on the head include the antennae, compound and simple eyes (ocelli), and the lower jaw. The thorax, on the other hand, houses two pairs of wings and three pairs of legs, serving as essential appendages for flight and movement (Bąk-Badowska et al.,2019).

A distinct characteristic of many stingless worker bees is the hind legs, which are equipped with specialized corbicular structures often referred to as "pollen baskets" (Bąk-Badowska et al., 2019). These structures are designed for efficient collection and transportation of pollen and other essential materials for the hive.

The third body part, the abdomen, is home to a unique feature in the stingless bee, which is the "sting" apparatus. Unlike some other bee species, the stingless bee's sting apparatus is non-functional (Kwapong et al., 2010). It is worth noting that a significant portion of the body of these non-stinging bees is covered in fine hair, whichserves various purposes in their daily activities.

2.3 Colony of *Lepidotrigona terminata*

Lepidotrigona terminata is a species of stingless bee commonly found in various parts of Southeast Asia, with its habitat spanning countries like Malaysia, Indonesia, and Thailand. These bees are highly valued by beekeepers for their production of honey and their crucial role in pollination services.

These colonies of *L. terminata* are active and bustling every day, as evidenced by research from Roubik (1989) and Hansell (1993). Typically, a colony of *L. terminata* comprises several thousand worker bees, along with a queen and several male bees. The worker bees carry out a multitude of tasks, including foraging for foodresources, nurturing the young, and safeguarding the colony. On the other hand, the primary role of the queen is to lay eggs and

oversee the reproductive cycle of the colony, while the male bees are responsible for mating with the queen, thus contributing to the colony's genetic diversity.

2.4 Nest structures

The nest of stingless bees forms a captivating and diverse structure, mirroring theunique needs of their colonies. The entrance pathway, serving as the link between the outside world and colony life, plays a vital role in the nest's dynamics. This pathway, not just an entry point, acts as a strategic area where bees store natural resources like resin, adding versatility to the nest (Kofi et al., 2010).

Digging into the nest, we see the distinctive Brood Section. Using cerumen, a blend of wax and plant resin, to build brood cells and storage pots introduces diversity in material use. This section, coated with cerumen membranes (involucrum), provides both a robust structure and temperature control within the nest (Roubik, 2020).

In the Storage Section, carefully crafted oval-shaped pots for honey and bee bread are made using cerumen. These serve as storage and contribute an artistic touch to the nest. Open Spaces within the nest accommodate resin, propolis, and essential materials, strategically organized to give the Colony freedom in resource management (Roubik, 2020).

Nest Volume, integral to the ecosystem, adapts dynamically to the colony's size. When a cavity exceeds the colony's needs, the nest intelligently seals both ends temporarily with membranes resin, mud, and wax mix. The nest structure of stingless bees not only meets biological needs but also represents a marvelous and adaptable natural art (Siqueira et al., 2012).

2.5 Hive of *Lepidotrigona terminata* species

Stingless bees, particularly those found on Java, tend to construct hives significantly smaller than those created by domesticated honeybees. In many instances,the hives of stingless bees are discovered nestled within bamboo culms, wooden structures, or even the recesses of stone or brick walls. Essentially, these remarkable creatures make use of any available small hollow space to establish their hives. Notably, the individual cells within these hives often feature darker hues, a common characteristic observed in the majority of stingless bee species. This dark pigmentationtypically indicates the presence of younger larvae within the cells, while cells of a pristine white color suggest that the larvae contained within are on the verge of undergoing pupation (Fisher, 2021).

Stingless bees employ a variety of natural materials in nest construction. Upon locating a suitable cavity, colony workers meticulously utilize gums, resins, and wax to craft a robust nest structure. Interestingly, in some species of stingless bees, they augment propolis and wax with sand and mud to enhance the strength and durability of their nests (Kofi et al, 2010)

2.6 Traditional and modern techniques

Normally the stingless bees built their nest on the trunks of trees, logs, wall crevices, and under the roof of dwellings. Stingless bees mix the plant resin with wax to construct the entrance of the nest and also coat the resins over the hive to protect it from their enemies like ants and wasps. The majority of these bees prefer teak trees for building their hives. Teak trees maintain temperature and humidity at an optimum level, So. that it prefers teak trees. In the natural settlement, the nest arrangement of this bee is very peculiar. This nest is entirely different from the other hive honeybees. The unique feature of this nest is its multilayer arrangement. In the nest different chambers are arranged in the following order, viz. pollen storage chamber (food chamber), honey storage chamber, and brood rearing chamber. The combs are built in a horizontal or vertical pattern in the trunk of the tree.

Commercial stingless bee farming has the potential for better economic growth (Soh et al., 2021). These bees produce honey and pollen that are beneficial for human health while aiding in plant pollination, thereby enhancing fruit agricultural yields (Windra et al., 2020). Entrepreneurs in stingless bee farming have developed various modern techniques, such as the Invents Rapid Split Technique, cooling technique hive, invitro queen bee production method, Artificial Propagation Technique, and grafting method.

The Invents Rapid Split Technique, introduced in 2017, enables rapid colony reproduction, generating new colonies in less than a minute and a quick splitting process in just 20 seconds. This approach aims to ensure the

sustainability ofthe bee ecosystem (Mahani, 2020). The nest box is coated with a material containing bee pheromones to make the bees comfortable in their new habitat (Mahani, 2020).

The cooling technique involves fitting the hive with a green roof, known as the MUSTAFA hive, which demonstrates impressive cooling performance (Halim et al., 2017). The MUSTAFA hive, developed by the University of Malaysia Pahang, comprises three main components, the brood cell compartment, the honey cassette, and the roof (Halim et al., 2017).

The Artificial Propagation Technique involved three methods, splitting, narrowing, and grafting connection conducted over eight consecutive weeks, recording honey pot and pollen pot quantities each week (Shilan et al., 2022). This study successfully explored different artificial propagation techniques for stingless bees (Shilan et al., 2022).

The invitro queen bee production method was employed in the mass rearing of Scaptotrigona depilis in 2013 and Plebeia droryana in 2015. Success in rearing stingless bee queens was found to depend on humidity levels in the incubator, which was stabilized using potassium chloride (KCl) and sodium chloride (NaCl) (Fahimee, 2020). The study was conducted at the Insect Laboratory in MARDI Serdang, Selangor, and the Pollinator House in the GeneBank of Rare Fruits at MARDI (Fahimee, 2020).

Grafting involves integrating natural nests of stingless bees into artificial hives. This method, which takes 4 to 6 months for a new Colony to occupy the artificial hive, includes matching the hole diameters of natural and artificial hives. Once there are young cells in the box, it can be transferred to storage (Windra et al., 2020).

CHAPTER 3

MATERIAL AND METHOD

3.1 Description of study area

This study area is located at Kg Paloh, Tendong, Pasir Mas, Kelantan (Figure 1). This area is laying between longitude and latitude, 6°04'11.8"N and 102°12'38.9"E. The area is characterized by a dense thicket of trees, creating unique research opportunities. In addition to the mentioned plant species, many more plant varieties such as *Psidium guajava* (Common guava), *Syzygium malaccense* (Malay apple), *Antigonon leptopus* (Mexican creeper), and *Citrus aurantiifolia* (Key lime), serve as primary food sources for *L. terminata*. The flat topographical conditions and access to a diverse range of plants make this area highly intriguing for understanding the ecology and activities of these stingless bees.

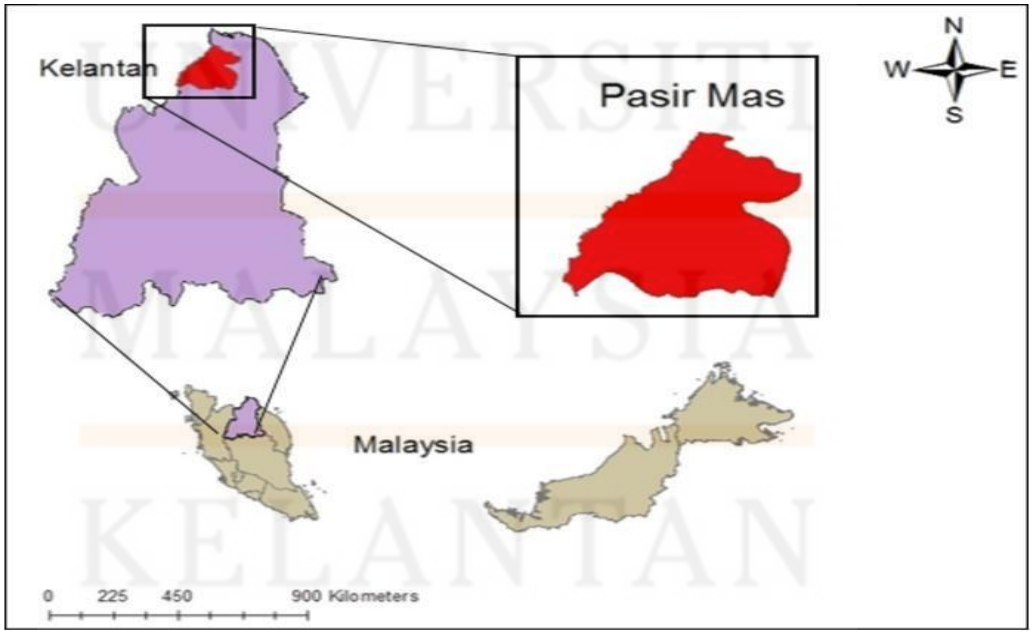


Figure 1: Location of Study Area at Kg Paloh, Tendong, Pasir Mas, Kelantan

3.2 Apparatus and material

Table 1 below shows the equipment and their functions used in this study.

Table 1: Equipment and functions

No.	Equipment	Function
1.	Lux meter	The lux meter is employed to measure the light intensityin the environment where the *L. terminata* Colony is situated. This aids researchers in ensuring suitable lighting conditions for the growth and well-being of thecolony.
2.	Meteorology data	Get temperature data and relative humidity data from the center of the meteorological department.
3.	Image J application	The Image J application is used to measure the centimetre-square growth of a colony. This allows to analysis and measure Colony development over time.
4.	Topping	The topping is used for separating or isolating the Colony, possibly for research purposes or to control colony size. It provides flexibility in managing the colony's structure.
5.	Tree trunk	The tree trunk serves as the natural habitat and entry point for the *L. terminata* colony. It may also be modified for research or observational purposes.

6.	Chisel	The chisel, wielded with precision, finds its purpose in the careful modification of the tree trunk. Through its actions, it artfully creates openings and entrances, ensuring that the Colony's integration with its environment remains seamless.
7.	Torch fire gun	The torch fire gun is used because propolis, a substance used by bees to seal openings in the hive, may need to be melted for accessing or modifying the colony structure.
8.	Transparent plastic cover	The transparent plastic cover provides a clear view for researchers to observe the activities and growth of the *L. terminata* colony.
9.	Nail	Nails are used to secure equipment or structures in place, such as attaching the topping or securing the transparent plastic cover.
10.	Hammer	The hammer is used in conjunction with the nail to secure equipment and make modifications to the tree trunk.
11.	Notebook and pen	notebook and pen emerge as the chroniclers of the Colony's story. Through meticulous data collection, researchers create a tangible record of the Colony's journey, an indispensable resource for ongoing analysisand understanding.

12.	Digital scale (SF400)	The yield from the new colony was accurately measured by carefully weighing the collected honey on a digital scale to ensure precise data collection.
13.	Stingless bee honey pump	The honey was carefully extracted from the pots using a honey pump.
14	Plastic strainer	After the honey was extracted, it was filtered using a strainer. This method ensures that the honey is purified from any impurities during the filtration process.
15.	Plastic jug	The collected honey was carefully poured into a clean plastic jug.

3.3 Methods

Flowchart below shows the evaluating the Colony development of *Lepidotrigona terminata* using MORY technique.

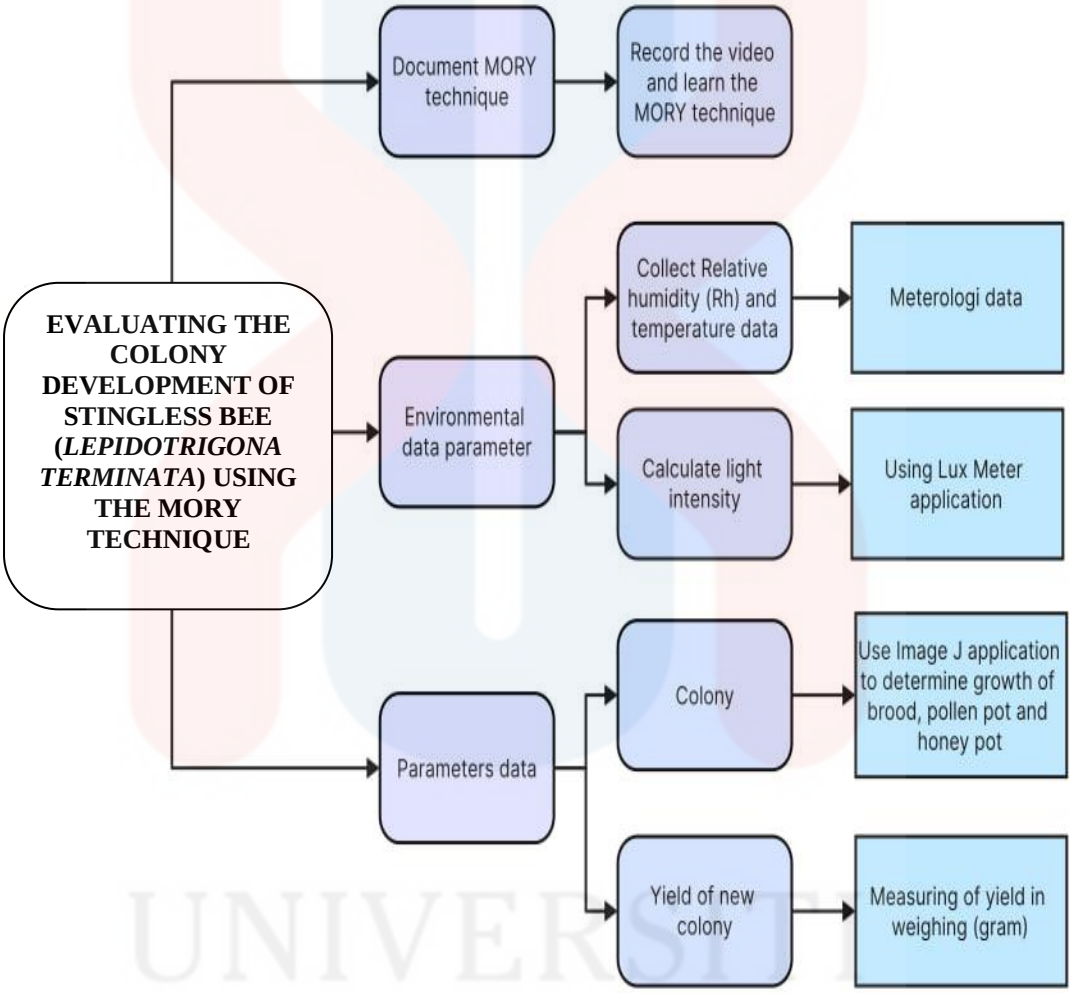


Figure 2: Flow diagram of the study

3.3.1 Documenting the MORY technique in transplanting the *Lepidotrigona terminata*

The MORY methodology, developed by Cikgu Mohd Rosli Bin Yaakub based on his five years of experience in stingless beekeeping, outlines a precise procedure for transplanting *L. terminata.* This innovative method involves using a log as the entrance for the stingless bees, along with topping to initiate the formation of a new Colony. The MORY techniques successfully established three new colonies of *L. terminata.* For Colony 1, the process of creating a new log began on October 21, 2023, and continued until January 27, 2024, spanning 14 weeks. Meanwhile, for Colonies 2 and 3, the process started on March 5, 2024, and concluded on May 7, 2024, covering 10 weeks each. Using nails, the log and topping were attached.

Figures 3 and 4 show that the log was measured at a length of 0.61 meters, and a hole was created at its center, with a diameter of 0.02 meters and a depth of 0.03 meters. The log was positioned at 0.79 radians from the hole. Subsequently, an entrance measuring 0.01 meters in length, 0.01meters in height, and 0.02 meters in width was carved on the top of the log. The topping, which already had a central hole measuring 0.05 meters in diameter for *L. terminata,* and measured 0.36 meters in length, 0.36 meters in width, and 0.08 meters in height, was then obtained.

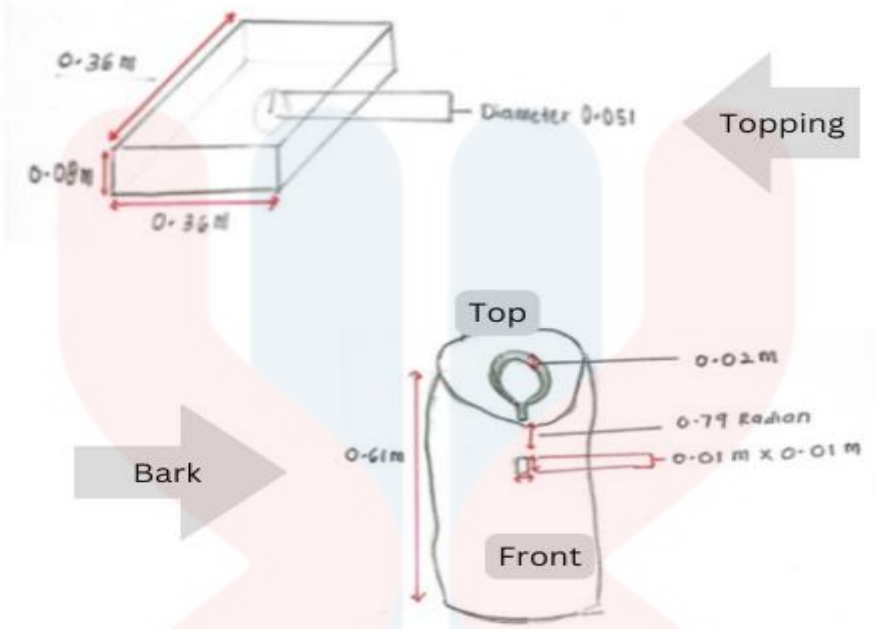
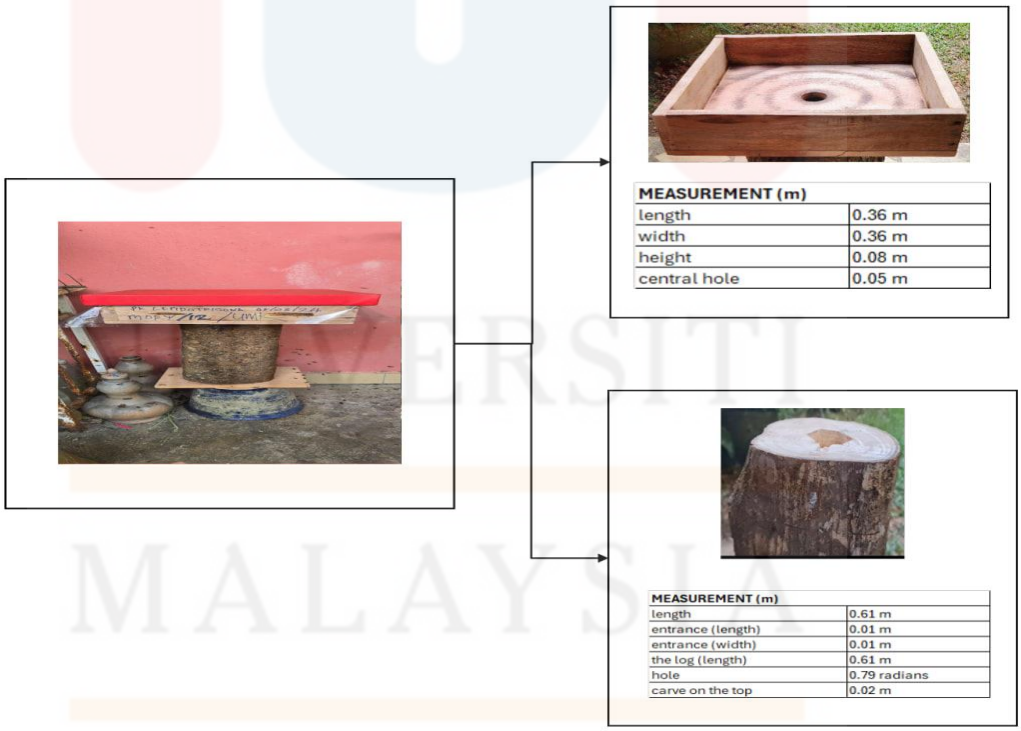


Figure 3: Sketch measurement of topping and bark



MEASUREMENT (m)	
length	0.36 m
width	0.36 m
height	0.08 m
central hole	0.05 m

MEASUREMENT (m)	
length	0.61 m
entrance (length)	0.01 m
entrance (width)	0.01 m
the log (length)	0.61 m
hole	0.79 radians
carve on the top	0.02 m

Figure 4: The new hive of *Lepidotrigona terminata*

During the transfer process, a new topping was crafted by procuring brood, beebread, honey pots, and queen or immature queen stages from various *L. terminata* Colonies, and carefully situating them within the topping. Colony 1, put the queen while Colonies 2 and 3 put the immature queen. The pivotal role of the queen bee within the colony was acknowledged, emphasizing the importance of placing queen bee eggs and selecting queens from the old topping onto the new one. The meticulous selection of mature queen bees became crucial in enhancing reproductive capabilities. The documentation process was elevated by capturing the intricacies of the MORY Technique through compelling photo and video recordings.

3.3.2 Measure the environmental parameters of the study area.

Three environmental data parameters were measured in this study, relative humidity, temperature, and light intensity. The relative humidity (RH) and temperature data were recorded from the Malaysian Meteorological Department Centre (MET Malaysia). Relative humidity (Rh) and temperature values are recorded weekly. Data collection allows us to create a comprehensive data set that tracks fluctuations in humidity levels over time.

Furthermore, detailed measurements of light intensity were conducted using the Lux Meter application at specific locations within the species' nest. These measurements were taken at five distinct points, marked as A, B, C, D, and E (each point was taken on the surface of the topping) as shown in Figure 5. Following the light measurements, the average amount of light was calculatedto

obtain the true light quantity within the nest. These points were carefully chosen to represent different areas of the nest, where light conditions could vary. By recording light levels at each of these points weekly, it aimed to gain a comprehensive understanding of how light availability changed within the hive environment.

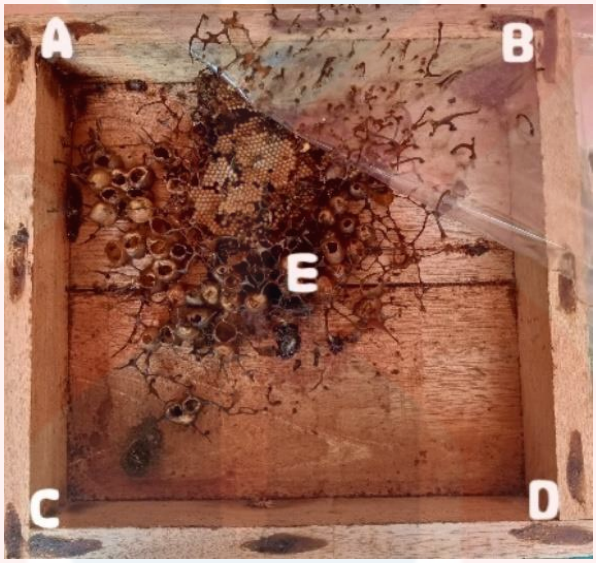


Figure 5: The different points taken for the light measurement

3.3.3 Assess the growth of the Colony, pollen pots, and yield of the new Colony

In this study, the main data parameters measured are Colony growth (pollen pot, honey pot, and brood) and the yield of new Colonies as shown in Figure 6. To assess colony growth, images were captured from the initial construction of the nest until it reached the peak of activity and development. Colony growth measurement using the Image J application shown in Figure 7, which is capable of measuring Colony growth in centimeters squares. These measurements are done consistently every week to get an in-depth picture of the dynamics of Colony development.

Figure 6: The Colony of brood, beebread, and honey pots

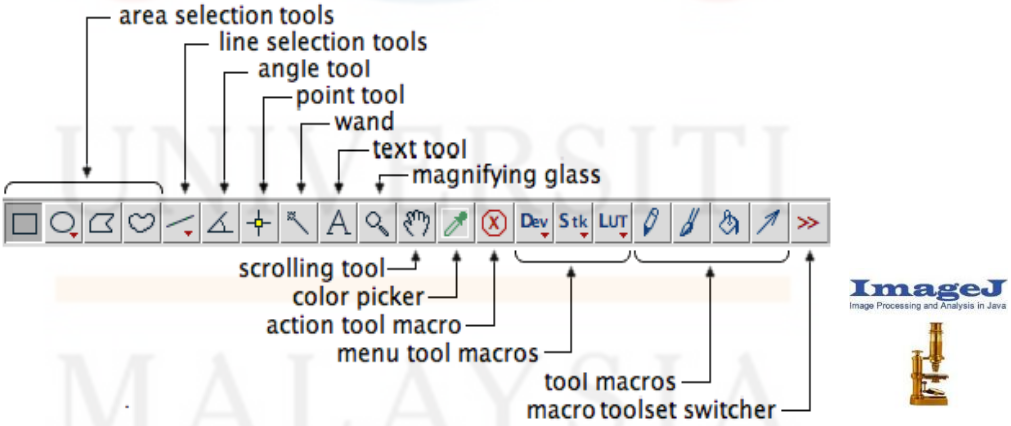


Figure 7: The tool functions in Image J software

For measuring honey, a specific amount was collected and placed in a jug after being carefully extracted from the pots using a honey pump. The jug was then positioned on a Digital Scale (SF400) to accurately measure the

honey's weight in grams as shown in Figure 8. This meticulous process ensures precise measurement of the honey's weight. Essential tools such as a digital scale and a container were utilized for this purpose, with the scale providing accurate weight readings and the container facilitating efficient measurement of the honey (Saraswathy, 2019). Additionally, the honey was collected on May 11, 2024, for all Colonies 1, 2, and 3.

Figure 8: The digital scale and Stingless bee honey pump

3.4 Data analysis

In this study, One-way ANOVA was employed to examine the effectiveness of the MORY techniques across different components, specifically beebread, honeypots, and brood. This statistical method allowed to determine whether there were any significant differences in the outcomes among these three components. To further investigate these differences, Tukey's test was subsequently applied, providing a detailed comparison, and identifying which specific groups differed significantly from each other.

Besides, to assess the impact of environmental variables on the effectiveness of the MORY techniques, an Independent T-test was utilized. This test compared the significance of differences in relative humidity, temperature, and lux meter readings.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Evaluation of the MORY Technique

This study emphasizes the latest innovations in stingless bee breeding, particularly using the MORY technique. Compared to conventional approaches documented in previous journals, MORY techniques revolutionized the development of new *Lepidotrigona terminata* Colonies. This technique focuses on breeding queen bees and immature queens, making the breeding process easier and more efficient.

The MORY technique offers several advantages over commercial hive breeding techniques, such as those used in MUSTAFA hives. As described in the literature, MUSTAFA hives focus on commercialized hives with elements like APA roofs, honeycassettles, split-able thrones, and air-jacketed palaces. Then, the honey pot and bee bread are in the honeycassettles while the brood cell compartment is in the split-able thrones and air-jacketed palaces (Ramli et al., 2017). In contrast, the MORY technique is its systematic approach using a log and topping system to facilitate the establishment and growth of new *L. terminata* Colonies. Referring to Figure 9, this process begins with the preparation of a log that serves as a bee entrance. It integrates colony eggs, pollen pots, and queen or immature queen stages from various *L. terminata* Colony into a topping. The selection of queens and immature queens accelerates the development of stingless bees. The careful placement of stingless bees and the strategic selection of queens

from strong toppings to new ones are crucial for enhancing the reproductive capacity and stability of new Colonies. However, this study advances beyond these methods by introducing MORY techniques, which combine breeding elements with a new systematic and structured approach.

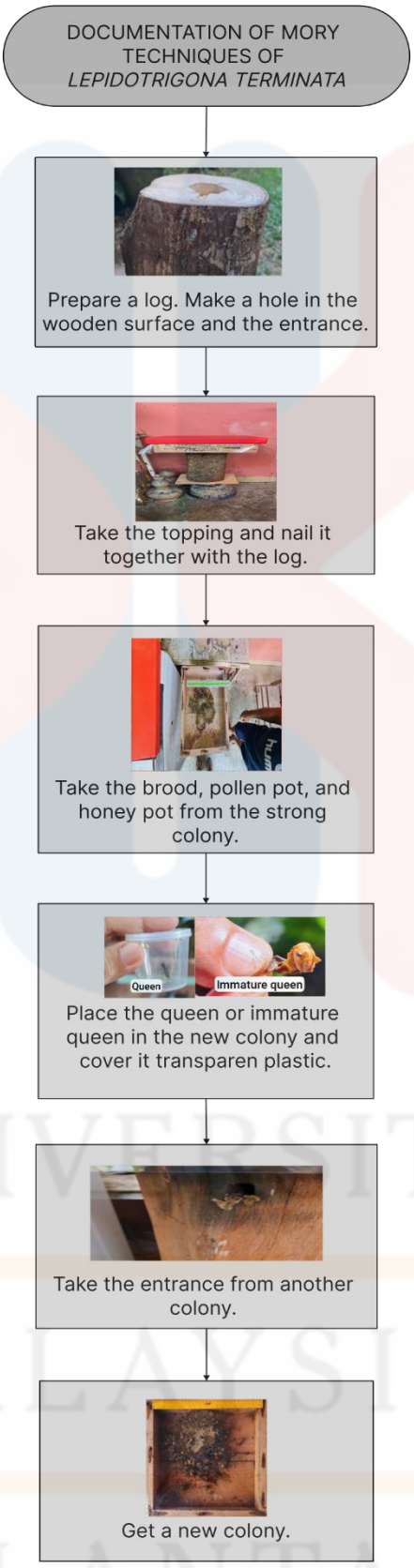


Figure 9: Documentation of MORY technique

Data collection for the MORY technique is carried out in two different stages. Based on Figure 10, the first phase, involving Colony 1, lasted from October 21, 2023, to January 27, 2024, for 14 weeks. This period allows for comprehensive data collection, observing the progress of the Colony from its initial migration to its large growth. The second phase, involving Colonies 2 and 3, runs from March 5, 2024, to May 7, 2024, over 10 weeks. The period was shorter for Colonies 2 and 3, but valuable insights have been obtained from the early stages of the formation and growth of the colony. From the observations made during the study, it was noted that *L. terminata* was very difficult to cultivate compared to other species of bees. The small body size and *L. terminata* brood cells contribute to slow colonial development and decreased reproductive rates. The difficulty that exists in this spread requires a long period of observation and data collection.

Despite the shorter periods for Colonies 2 and 3, the data collected is crucial in assessing the early success of the MORY technique. Observations included the adaptation of bees to new environments, queen breeding activities, and the development of the entire Colony. Comprehensive documentation through images and videos provides a rich visual record of the process and its results. In particular, the MORY technique achieves a 100% survival rate during the breeding process, demonstrating its effectiveness in ensuring the eligibility and growth of new Colonies. The MORY technique offers several advantages, making it the preferred method for transferring *L. terminata.* First, the use of masks and leather facilitates easy movement and monitoring of Colonies. Second, this method supports rapid colonial formation, allowing efficient duplication of brood, honey pots, and beebread.

Figure 10: The survival breeding Colonies 1, 2, and 3

4.2 The environmental data parameters

This study examined the effectiveness of the MORY technique in measuring the number of light intensity (lux meter) in a sample. A one-sample t-test was conducted to determine whether the lux values obtained from the sample differed significantly from the test value of 700 lux.

The sample consisted of 14 observations (N = 14). The mean lux value (M = 705.64, SD = 459.41). The one-sample t-test showed that the difference between the sample mean and the test value of 700 lux was not significant. The obtained t statistic was $t(13) = 0.046$, $p = 0.964$. The 95% confidence interval for the difference between the sample mean and the test value ranged from -259.61 to 270.90 lux, indicating considerable uncertainty about the true difference as shown in Figure 11.

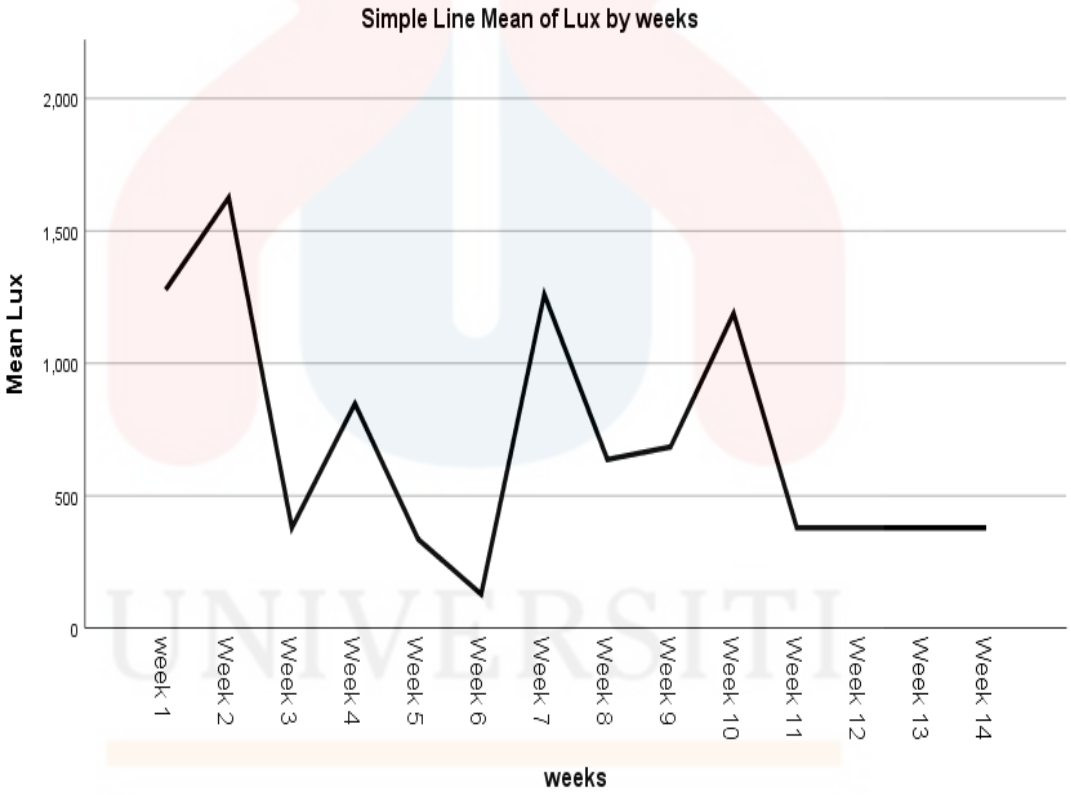


Figure 11: The mean light intensity

In the context of this temperature measurement study, the analysis results indicate a significant difference between the observed sample mean (M = 26.44, SD = 1.13) and the standard value set at 25. This difference is striking, with a $t(13) = 4.78$, p <.001. P value is a very low level of significance, highlighting the

importance of this difference both practically and statistically.

These findings affirm that the observed temperature significantly exceeds the expected standard, with a mean difference of M = 1.44. Theonally, with a 95% confidence interval encompassing a difference of 0.79 to 2.09 units, confidence in the accuracy of this estimation is further reinforced as shown in Figure 12.

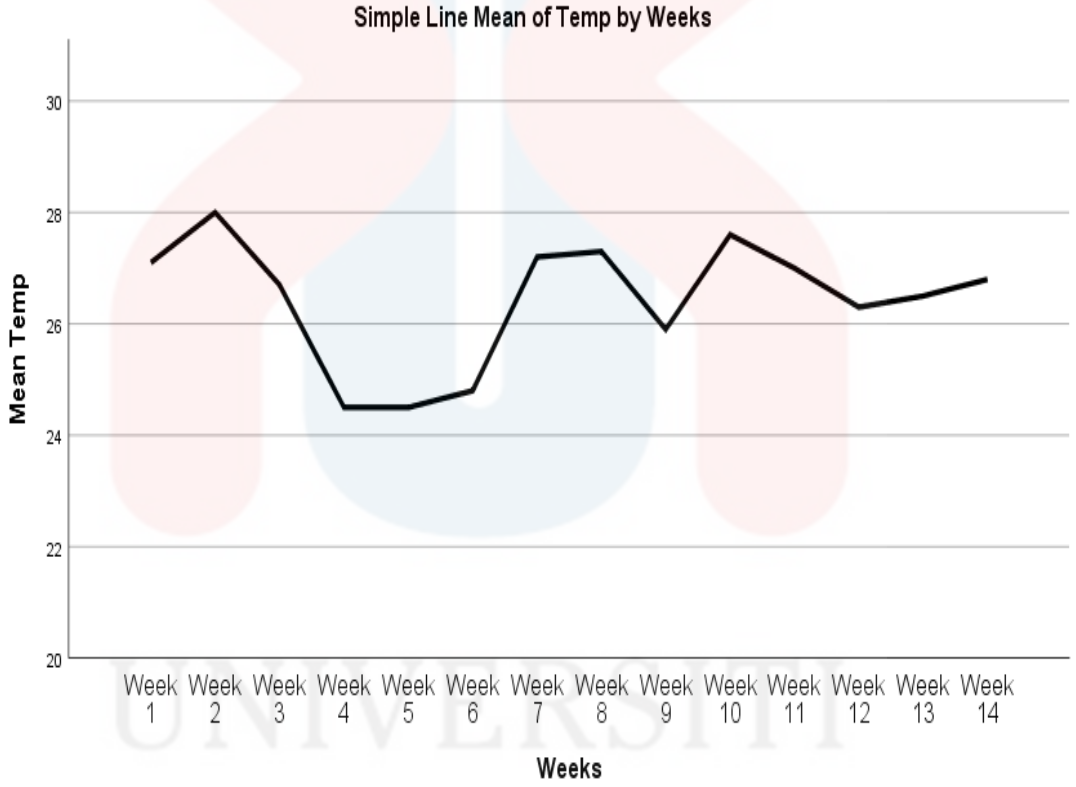


Figure 12: The mean of temperatures

In the study measuring relative humidity (Rh), statistical analysis revealed a significant difference between the observed sample mean (M = 73.23, SD = 1.79) and the test value set at 70. The results showed a $t(13) = 6.764$, p = <.001 with a very low level of significance, indicating that this difference is highly significant from a statistical standpoint as shown in Figure 13.

The mean difference obtained was 3.23 units, with a 95% confidence interval ranging from 2.20 to 4.26 units. This suggests that the measured mean relative humidity consistently exceeds the expected value, and there is high confidence in the accuracy of this estimate.

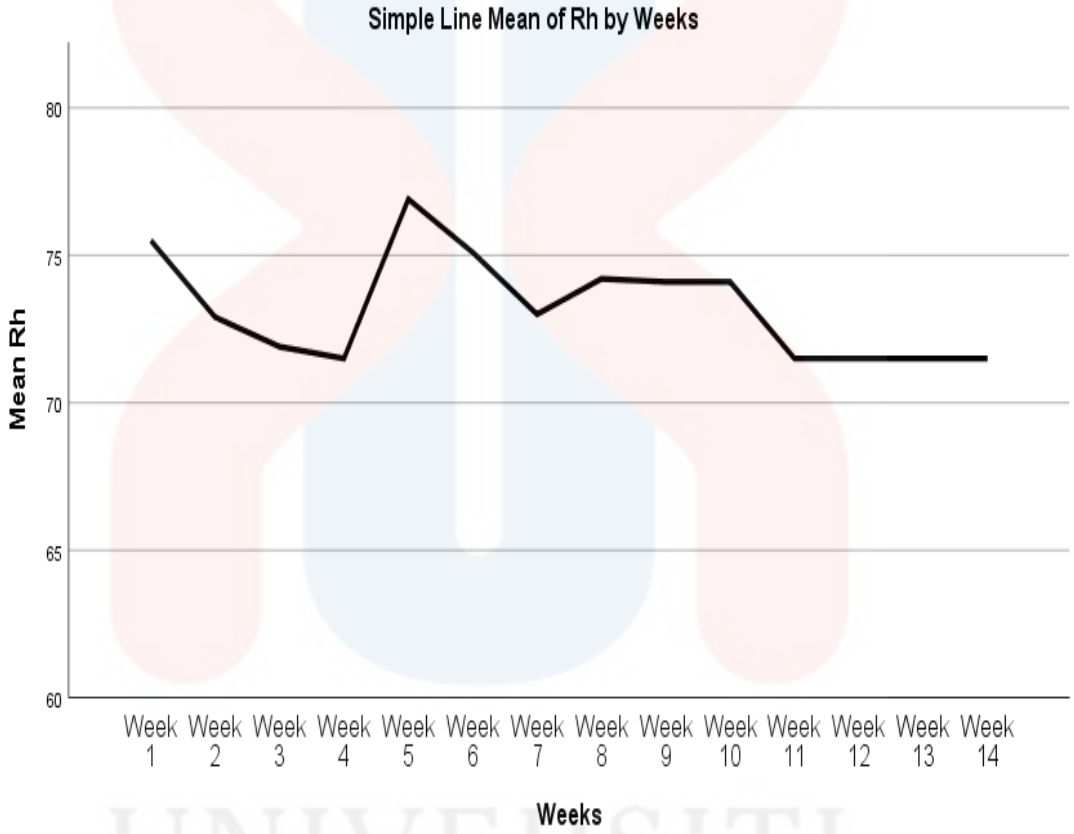


Figure 13: The mean relative humidity

4.3 Assess the growth of the Colony, pollen pots, and yield of the new Colony.

In this study, an analysis of variance (ANOVA) was conducted to examine the effect of Colony treatments on the growth of brood and pots (honeypot and beebread pot). The results indicated a significant difference in brood growth across the different log treatments, with an F value of $F(2, 31) = 17.057$ and a $p = < .001$. This means that the different Colony treatments had a

significant impact on brood growth.

The detailed data show that Colony 1 had slower brood growth based on Figure 14 starting from 0 cm^2 and reaching 25.74 cm^2 over approximately three months. In contrast, Colony 2 showed a rapid increase from 0 cm^2 to 47.416 cm^2 within two months based on Figure 15. Meanwhile, Colony 3 exhibited a less stable growth pattern but still reached 43.258 cm^2 within the same period based on Figure 16. These differences are significant, indicating that Colonies 2 and 3 had a more positive effect on brood growth compared to Colony 1.

Post hoc analysis using Tukey HSD criteria confirmed these differences. There were significant differences between Colonies 1 and 2, as well as between Colonies 1 and 3. The mean difference between Colonies 1 and 2 was -15.03 with a standard error *(SE)* of 2.97, significant at $p = < .001$. Similarly, the mean difference between Colonies 1 and 3 was -14.14 with an $SE = 2.97$, also significant at $p = < .001$. However, no significant difference was found between Colonies 2 and 3, suggesting that both treatments had a similar effect on brood growth.

For the growth of honeypot and beebread pots, the ANOVA results showed no significant effect of Colony treatments, with $F(2, 31) = 2.519$ and a $p = .097$. This indicates that the Colony treatments did not have a significant impact on the growth of honeypot and beebread pots. The data show that Colony 1 exhibited steady growth from 0 cm^2 to 148.93 cm^2 over approximately 14 weeks based on Figure 14, while Colony 2 showed rapid growth from 0 cm^2 to

155.093 cm² within 10 weeks based on Figure 15. Meanwhile, Colony 3 showed variable growth, reaching 123.791 cm² within the same period based on Figure 16. Although there were variations in growth, these differences were not statistically significant.

Post hoc tests (Tukey HSD) confirmed these findings, as no significant differences were found between any of the logs for honeypot and beebread pot growth. The mean differences between Colonies 1 and 2, as well as between Colonies 1 and 3, were not significant, indicating that the variations in honeypot and beebread pot growth were due to random variation rather than the log treatments.

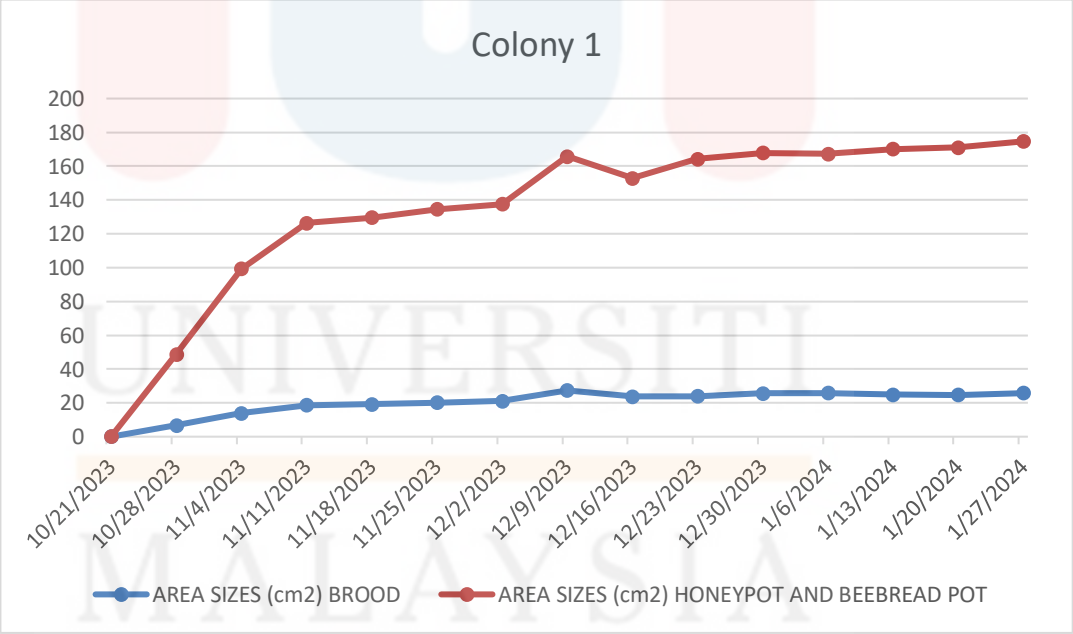


Figure 14: Growth of Colony 1

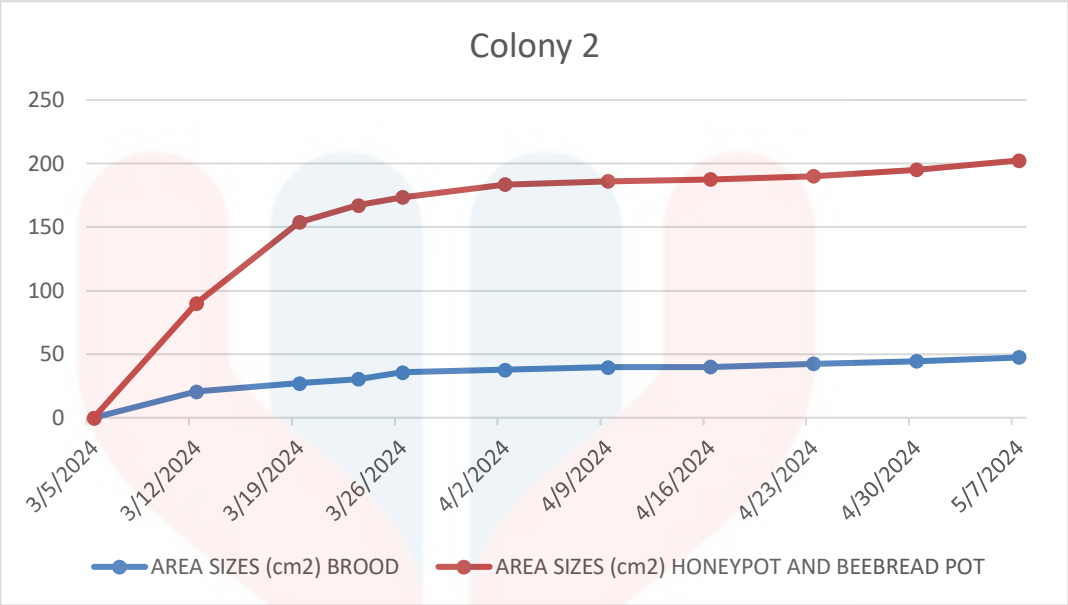


Figure 15: Growth of Colony 2

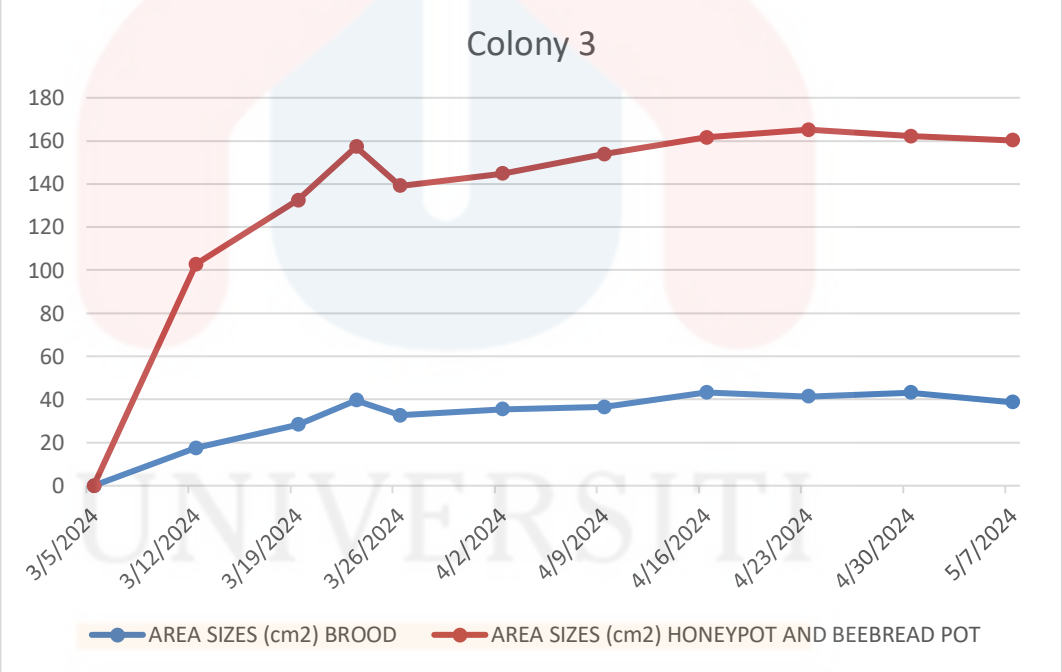


Figure 16: Growth of colony 3

The study investigated the honey yield from a new colony that started using the MORY technique in three different Colonies. Colony 1, starting with a new colony on 21 October 2023, produced 76 grams or 37% of honey, while Colonies 2 and 3, both starting on 5 March 2024, produced 71 grams or 35% and

56 grams or 28%, respectively. Based on Table 2 and Figure 17, the honey collection was carried out on May 11, 2024, using a Stingless bee honey pump and a digital scale, ensuring accurate measurement. These results suggest that the time of colonization can affect honey production, with longer periods of establishment of Colony 1 may contribute to higher results than Colonies 2 and 3.

Table 2: The yield of a new Colony

Colony	Yield of new Colony (gram)
1	76
2	71
3	56

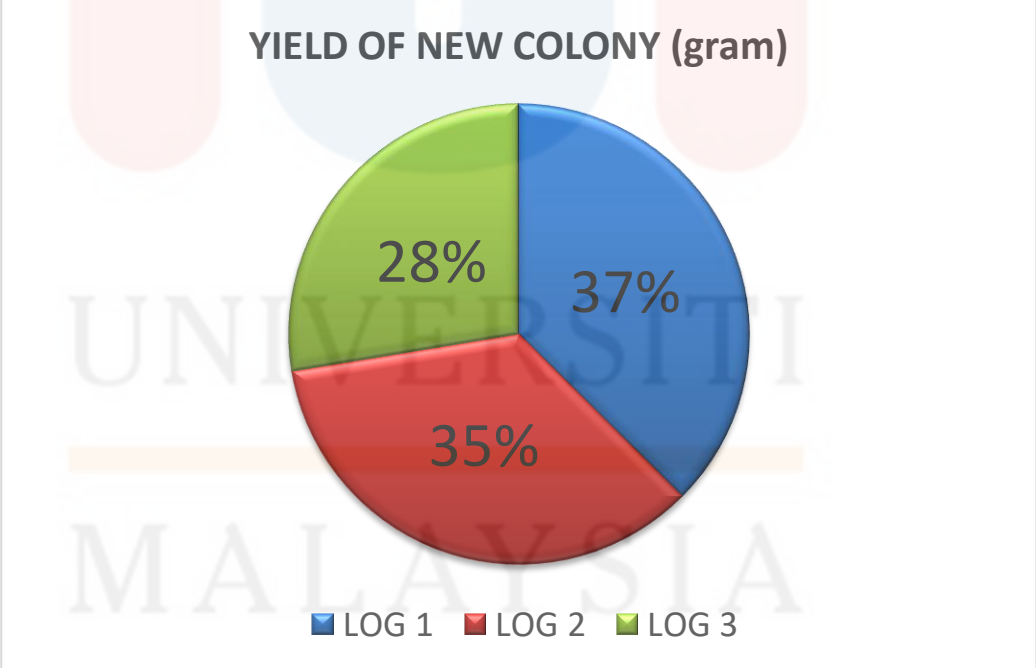


Figure 17: The Percentage yield of a new Colony

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The MORY technique, innovated by Mohd Rosli bin Yaakub, has been rigorously evaluated for its effectiveness in promoting the growth and productivity of the *Lepidotrigona terminata* colony. Through experimentation and data analysis, this study has explained the implications of various aspects of the MORY technique in stingless bees.

The findings underline the importance of MORY techniques in improving various aspects of colonial development. From the beginning of a new colony to the harvesting of honey, MORY techniques have demonstrated the potential to optimize bee practices and maximize yields. The formation of new colonies using the MORY technique reveals promising results. Colonies started at different periods show different levels of productivity, indicating that the time of colony establishment plays an important role in honey production. Colony 1, which began in October 2023, showed the highest results, indicating the importance of providing Colonies 2 and 3 with enough time to establish and gather resources.

Besides, the honey collection process, which is carried out using a Stingless bee honey pump and a digital scale, ensures accurate measurement and reliable data collection. The consistency of this methodology emphasizes the credibility

of the study findings and reinforces the effectiveness of MORY techniques in facilitating precise evaluation of results.

5.2 Recommendations

Several recommendations can be proposed to optimize the implementation of the MORY technique in stingless beekeeping practices. Firstly, it is advisable to conduct further research to refine and enhance the efficacy of the MORY technique, exploring factors such as optimal colony establishment timings and environmental conditions conducive to colony growth. Additionally, beekeepers should prioritize continuous monitoring and management of colonies, implementing proactive measures to address any issues promptly and maintain colony health. Education and training programs should be developed to educate beekeepers and the wider community about the importance of stingless bees and sustainable beekeeping practices, empowering stakeholders to effectively contribute to bee conservation efforts. Lastly, collaboration between beekeepers, researchers, policymakers, and conservation organizations is essential for promoting the widespread adoption of the MORY technique and implementing evidence-based conservation strategies, ultimately driving positive outcomes for stingless bee populations.

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APPENDIX A

Lux meter T-test (Colonies 1, 2, 3)

One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
Lux	14	705.6429	459.41396	122.78354

One-Sample Test

Test Value = 700

	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
Lux	.046	13	.964	5.64286	-259.6149	270.9006

Temperature T-test (Colonies 1, 2, 3)

One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
Temp	14	26.4429	1.12913	.30177

One-Sample Test

Test Value = 25

	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
Temp	4.781	13	.000	1.44286	.7909	2.0948

Relative humidity T-test (Colony 1, 2, 3)

One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
Rh	14	73.2286	1.78603	.47734

One-Sample Test

Test Value = 70

	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
Rh	6.764	13	.000	3.22857	2.1974	4.2598

Growth of colony (One Way Anovs) (Colony 1, 2, 3)

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Brood	Between Groups	1755.609	2	877.805	17.057	.000
	Within Groups	1595.314	31	51.462		
	Total	3350.923	33			
Pots	Between Groups	2962.664	2	1481.332	2.519	.097
	Within Groups	18229.104	31	588.036		
	Total	21191.769	33			

Post hoct test

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) Logs	(J) Logs	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
Brood	Log 1	Log 2	-15.026143*	2.970187	.000	-22.33633	-7.71595
		Log 3	-14.142843*	2.970187	.000	-21.45303	-6.83265
	Log 2	Log 1	15.026143*	2.970187	.000	7.71595	22.33633
		Log 3	.883300	3.208169	.959	-7.01261	8.77921
	Log 3	Log 1	14.142843*	2.970187	.000	6.83265	21.45303
		Log 2	-.883300	3.208169	.959	-8.77921	7.01261

Pots	Log 1	Log 2	-14.383329	10.040224	.337	-39.09422	10.32756
		Log 3	9.778671	10.040224	.599	-14.93222	34.48956
	Log 2	Log 1	14.383329	10.040224	.337	-10.32756	39.09422
		Log 3	24.162000	10.844682	.082	-2.52881	50.85281
	Log 3	Log 1	-9.778671	10.040224	.599	-34.48956	14.93222
		Log 2	-24.162000	10.844682	.082	-50.85281	2.52881

*. The mean difference is significant at the 0.05 level.

Homogeneous subsets

Brood

Tukey HSD[a,b]

		Subset for alpha = 0.05	
Logs	N	1	2
Log 1	14	21.51936	
Log 3	10		35.66220
Log 2	10		36.54550
Sig.		1.000	.955

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 11.053.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

Pots

Tukey HSD[a,b]

Logs	N	Subset for alpha = 0.05 1
Log 3	10	112.26690
Log 1	14	122.04557
Log 2	10	136.42890
Sig.		.065

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 11.053.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

Raw data growth of a colony

Colony 1

DATES	AREA SIZES (cm2)	
	BROOD	HONEYPOT AND BEEBREAD POT
21/10/2023	0	0
28/10/2023	6.64	42.086
4/11/2023	13.981	85.305
11/11/2023	18.609	107.78
18/11/2023	19.136	110.38
25/11/2023	20.15	114.379
2/12/2023	21.072	116.39
9/12/2023	27.338	138.457
16/12/2023	23.825	129.103
23/12/2023	23.9	140.334
30/12/2023	25.55	142.334
6/1/2024	25.8	141.5
13/1/2024	24.8	145.33
20/1/2024	24.73	146.33
27/1/2024	25.74	148.93

Colony 2

DATES	AREA SIZES (cm2)	
	BROOD	HONEYPOT AND BEEBREAD POT
5/3/2024	0	0
12/3/2024	20.66	69.505
19/3/2024	27.146	126.783
23/3/2024	30.364	136.793
26/3/2024	35.624	137.793
2/4/2024	37.721	145.959

9/4/2024	39.82	146.257
16/4/2024	39.9	147.643
23/4/2024	42.416	147.727
30/4/2024	44.388	150.736
7/5/2024	47.416	155.093

Colony 3

DATES	AREA SIZES (cm2)	
	BROOD	HONEYPOT AND BEEBREAD POT
5/3/2024	0	0
12/3/2024	17.489	85.326
19/3/2024	28.34	104.084
23/3/2024	39.573	117.613
26/3/2024	32.648	106.502
2/4/2024	35.501	109.209
9/4/2024	36.526	117.362
16/4/2024	43.258	118.304
23/4/2024	41.406	123.791
30/4/2024	43.163	119.012
7/5/2024	38.718	121.466

Raw environmental data

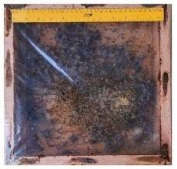
week	Min lux meter	Min daily temperatures (°C)	Min Relative humidity (Rh)
1	1277.6	27.1	75.5
2	1625.8	28	72.9
3	378.8	26.7	71.9
4	847.8	24.5	71.5
5	336	24.5	76.9
6	128.4	24.8	75.1
7	1260	27.2	73
8	636.6	27.3	74.2
9	684.2	25.9	74.1
10	1188.6	27.6	74.1
11	378.8	27	71.5
12	378.8	26.3	71.5
13	378.8	26.5	71.5
14	378.8	26.8	71.5

Colony 1 (Started 28/10/2023 – 27/01/2024)

1. BROOD SIZE: 0
POTS SIZE: 0

5. BROOD SIZE: 19.136
POTS SIZE:110.38

9. BROOD SIZE: 23.825
POTS SIZE: 129.103



13. BROOD SIZE: 24.80
POTS SIZE: 145.330

2. BROOD SIZE: 6.64
POTS SIZE: 42.086

6. BROOD SIZE: 20.15
POTS SIZE: 114.379

10. BROOD SIZE:23.90
POTS SIZE: 140.334

14. BROOD SIZE: 24.730
POTS SIZE: 146.330

3. BROOD SIZE:13.981
POTS SIZE: 85.305

7. BROOD SIZE: 21.072
POTS SIZE: 116.39

11. BROOD SIZE: 25.55
POTS SIZE: 142.334

15. BROOD SIZE: 25.74
POTS SIZE: 148.93

4. BROOD SIZE: 18.609
POTS SIZE: 107.78

8. BROOD SIZE: 27.338
POTS SIZE: 138.457

12. BROOD SIZE: 25.80
POTS SIZE: 141.50

Colony 2 (Started 12/03/2024 – 07/05/2024)

1. BROOD SIZE: 0
POTS SIZE: 0

5. BROOD SIZE: 35.624
POTS SIZE: 137.793

9. BROOD SIZE: 42.416
POTS SIZE: 147.727

2. BROOD SIZE: 20.66
POTS SIZE: 69.505

6. BROOD SIZE: 37.721
POTS SIZE: 145.959

10. BROOD SIZE: 44.388
POTS SIZE: 150.736

3. BROOD SIZE: 27.146
POTS SIZE: 126.783

7. BROOD SIZE: 39.82
POTS SIZE: 146.257

11. BROOD SIZE: 47.416
POTS SIZE: 155.093

4. BROOD SIZE: 30.364
POTS SIZE: 136.793

8. BROOD SIZE: 39.90
POTS SIZE: 147.643

Colony 3 (Started 12/03/2024 – 07/05/2024)

1. BROOD SIZE: 0
POTS SIZE: 0

5. BROOD SIZE: 32.648
POTS SIZE: 106.502

9. BROOD SIZE: 41.406
POTS SIZE: 123.791

2. BROOD SIZE: 17.489
POTS SIZE: 85.326

6. BROOD SIZE: 35.501
POTS SIZE: 109.209

10. BROOD SIZE: 43.163
POTS SIZE: 119.012

3. BROOD SIZE: 28.34
POTS SIZE: 104.084

7. BROOD SIZE: 36.526
POTS SIZE: 117.362

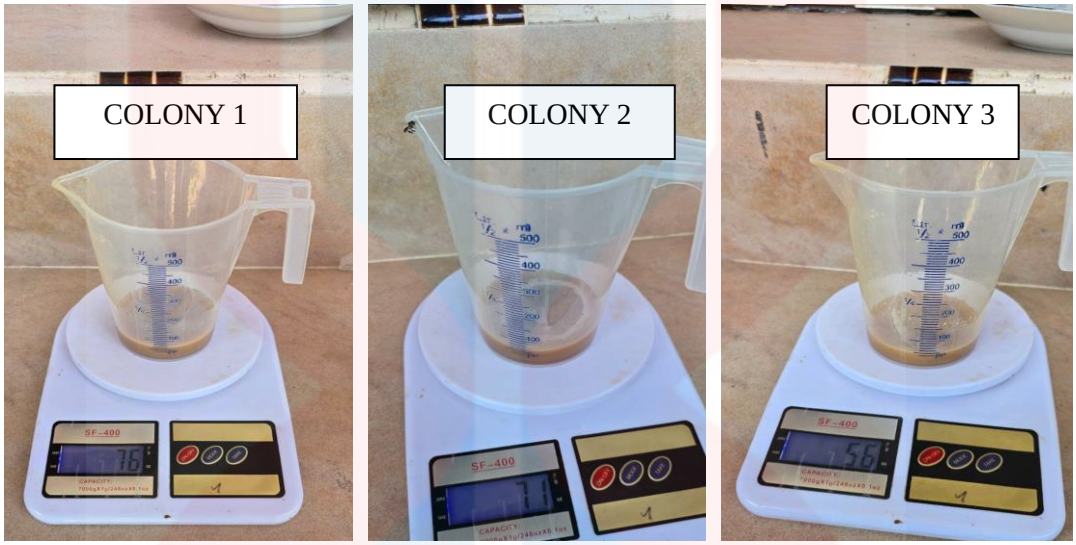
11. BROOD SIZE: 38.718
POTS SIZE: 121.466

4. BROOD SIZE: 39.573
POTS SIZE: 117.613

8. BROOD SIZE: 43.258
POTS SIZE: 118.304

APPENDIX B

Collected yield of a new colony from Colony 1, Colony 2, and Colony 3



Pictures below show the honey carefully extracted from the pots using a honey pump, and the honey collected was filtered using a strainer.