ORIGINAL ARTICLE

Introducing a Technique in Sustaining Adult Worker Bees of *Heterotrigona itama* (Cockerell, 1918) in Laboratory to Facilitate Future Health Research on Kelulut Honey

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ABSTRACT

Introduction: The study is introducing a laboratory technique to sustain the longevity of *Heterotrigona itama* stingless bees collected from the farm in order to facilitate future health research on Kelulut honey. **Methods:** The worker bees were held in laboratory at 26 ± 2 ^{o}C, 57 ± 8 % relative humidity (RH) and 12:12 hours (light:dark period) in a cup covered with meshed cloth and installed with an inverted-wick system consists of a drinking straw with the bottom end loosely plugged with cotton wool. The artificial diet was pipetted into the straw to wet the cotton wool. The bees were divided into five diet groups, namely a) unfed - control, b) distilled water, c) purified tap water, d) non-carbonated isotonic drink or e) 5% honey solution. Feeding activity and survival of worker bees were observed daily. **Results:** The worker bees are seen to frequent and lick the cotton wool wetted with artificial diets. Comparison between the artificial diets, Kaplan-Meier statistical analysis showed that the 5% honey solution and non-carbonated isotonic drink have significantly ($P < 0.05$) extended the longevity of the worker bees with 50% survival probability at least 8 days. When the similar holding and feeding technique used for the bees from commercial farms, the 50% survival probability was extended to 14 days. **Conclusion:** The inverted-wick system with the use of 5% honey solution or isotonic drink as the artificial diet is capable to hold the *H. itama* worker bees at least for a week with survival above 50% for laboratory experiments.

Keywords: Artificial diet, *Heterotrigona itama*, Longevity, Stingless bees, Survival

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INTRODUCTION

Stingless bee honey has become part of the traditional health supplement with various benefits been reported. The common species of stingless bees being farmed in Malaysia is *Heterotrigona itama* (1, 2) which is in the family Apidae and tribe of Meliponini. The honey collected from *H. itama* hives has been reported to generate favourable income leading to growing stingless bee farming in Malaysia (2, 3). The production of these farm breed stingless bee is important to be protected from hazardous chemicals, as experienced by honey bee farming in Europe (4). There is a lack of information on how farm collected stingless bees are sustained in the laboratory for mass breeding and controlled experimentation.

The longevity of an insect is highly dependent on dietary intake associated with its nutritional value (5, 6), health, physical fitness and ambient conditions, i.e. temperature and humidity (7, 8, 9). The longevity of an insect in the actual field environment is usually shorter than those held in laboratories, which are given optimal condition for better survival (10). The shorter life span in the field is caused by the ecological factors that exist in natural habitats such as predation, dehydration, disease, interspecific and intraspecific competitions (5). Stingless bees are known as a chewing and lapping-sucking type of insects which enable them to forage on nectar and pollen from blooming plants of the flight range as their natural diet (11). Adult bees may be forced to defence and to forage for their colony, thus resulting in high mortality rates (12). The physical and metabolic activity will also increase once the worker bees start their foraging tasks, thus declining their longevity and survival rate (5).

There are studies conducted on the effects of artificial diets on insect's life start at the larval stage due their longevity is shorter compared to those given optimal diet (8, 13). Artificial diets to maintain insect colonies is a regular practice in modern laboratories associated with entomological research (8, 14-19). At the adult stage, nutrition influences their strength, importance in maximizing a female's lifespan and to achieve the optimal female reproductive performance (13). Artificial diets were primarily developed to study basic nutritional needs for successful growth and development of insects under laboratory conditions, especially for purposes of insect mass rearing (13, 14, 18, 20). Subsequently benefiting studies of bioassay of insecticides, toxicology, insect pathogens, attractants, hormones and pheromones where mass-reared homogenous insects are required (15, 21).

The study on the life span of adults *H. itama* in laboratories fed with artificial diets are poorly understood. Holding of adult insects under laboratory conditions requires appropriate holding cages to protect from natural predators, good hygiene to avoid infestation of parasites, right feeding for optimal supply of nutrients, correct handling to prevent injuries and regulation of both room air temperature and relative humidity to prevent dehydration (16). Study on the longevity of worker bees in the laboratory is important in understanding their tolerance to the controlled temperature and relative humidity in the laboratory conditions (5). The nutrition of artificial feeding is one of the key factors that influence longevity (22-25), whereby it determines the physiological fitness of the worker bee. The success of holding *H. itama* worker bees under laboratory condition is essential to conduct further health research on stingless bee honey under controlled environment. Therefore, the study aims to determine the capability of the developed inverted-wick feeding technique and identify most suitable artificial diets to sustain the life of the *H. itama* worker bees under laboratory condition, while the technique should be practical and economical.

MATERIALS AND METHODS

Source of stingless bee species

The *H. itama* worker bees were collected from the stingless bee farm located at the pollinator house of the Tropical Rare Fruits Genebank (2°58'51.5"N 101°41'20.6"E), Malaysian Agricultural Research and Development Institute (MARDI) in Serdang, Selangor. The bee farm area, under MARDI management, is located on a hill and surrounded by orchards.

After completing the initial phase of selecting the most suitable artificial diet by using the MARDI controlled colony, the subsequent phase of the study was continued to compare longevity of worker bees from three different farms, namely Gombak, Selangor (3°15'28.9"N 101°45'03.6"E), Kuala Kangsar, Perak (4°53'58.6"N 100°53'46.9"E) and Merbok, Kedah (5°44'09.8"N 100°24'52.1"E) that were fed with the best artificial diet.

Collection & transportation of *H. itama* worker bee

The worker bees which actively fly to forage food were collected by using empty food grade polypropylene (PP) water bottles of 350 ml as shown at Figure 1. One collection bottle contains not exceeding 20 bees at a time to avoid over-crowding. The origin of *H. itama* from the specific hive was assured by collecting the worker bees directly upon exiting the hive entrance as shown at Figure 1. The surrounding temperature and relative humidity of the stingless bee farm are 28 – 33 °C and 63 – 85 % RH, respectively. The farm experiences the typically hot and humid weather of tropical climate but is shaded by the fruit trees.

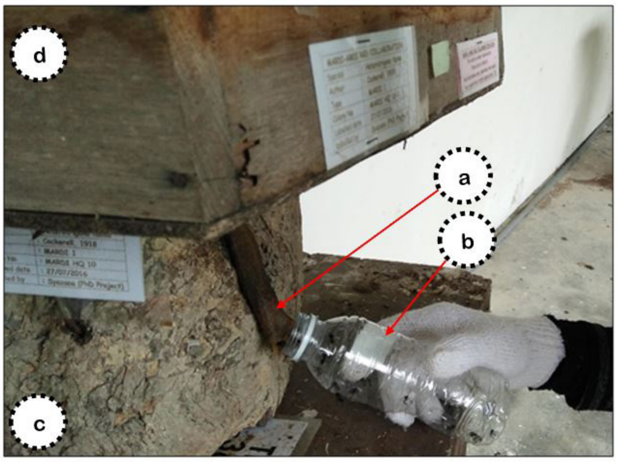

Figure 1: Collection of *H. itama* worker bees directly from the hive entrance (a) by using empty food grade polypropylene (PP) water bottles of 350 ml (b). The hive consists of timber log at the bottom (c) and wooden box on the top (d).

For the initial phase, the bees which were collected from the MARDI controlled farm were transported to Entomology Laboratory at the building of National Council for Biological Control, MARDI for sorting and acclimatization. The distance between the bee farm and the laboratory was approximately 3 km with travel time less than 10 minutes. During transportation, the bees were kept inside a corrugated cardboard box to shield from direct sunlight and also to maintain the temperature and relative humidity within the range of 27 – 33 °C and 65 – 75 % RH, respectively.

Selection of artificial diet

For the purpose of study, the stingless bees were divided to five experimental feeding groups; with either a) unfed - control (UF), b) distilled water (DW), c) purified tap water (PW), d) non-carbonated isotonic drink (ID) or e) 5% honey solution (H5). The artificial diets were selected on the following basis:

a) DW; represents as a control for this experiment because DW does not contains any nutrients. Water is basic for survival of insects (7, 26, 27).

b) PW; the water purified by reverse osmosis is relatively cheaper than distillation. The water may contain a trace level of minerals which could be harmful to bees. Therefore, PW which was free from chlorine, heavy metals and microbes were used in this study. It is more palatable than distilled water (28).

c) ID; the commercial isotonic drink (F&N 100 Plus Edge) consists of purified water that is enriched with approximately 6% w/w sugar (sucrose, glucose, fructose), electrolytes (sodium, potassium, chloride, calcium, phosphate) and B complex vitamin (B3, B6, B12) that are essential to prolong survival of living tissue (29).

d) H5; the commercial common bee honey was diluted in purified water at the ratio 1:20. Honey, in general, contains a high level of sugar (sucrose and fructose) at approximately 75% (w/w) without balanced electrolyte. However, it represents the typical nectar that is collected by the bees. The 5% (w/w) is the lowest concentration of honey that commonly found in the rearing of nectar-sucking insects (30, 31).

The reason of selecting PW and ID are due to its ease to source and instantly available compared to traditionally used DW and H5. The general comparison of nutrient composition found in the aforementioned artificial diets was shown in Table I, which shows some differences. Thus, the reason to compare the PW with DW and ID with H5 is to identify any possible differences in sustaining the worker bee longevity.

Preparation of the artificial diet

The DW was obtained from the distillation system brand Favorit Water Stills model WCM4L, whereas the PW was taken from the reverse osmosis water purifier machine brand COWAY model CHP-671 L. Both facilities were installed at the Entomology Laboratory, National Council for Biological Control, MARDI. The non-carbonated isotonic drink (F&N 100 Plus Edge) and bee honey (CED Pure Honey) were purchased from the local hypermarket. The H5 was prepared by dissolving the 50 ml commercial bee honey in 950 ml of purified tap water from the COWAY water purifier machine. The 5% (w/w) honey solution was freshly prepared prior the experiment or it was refrigerated until further use but not exceeding period of one week.

Table I: General comparison of nutrients among the four artificial diets namely distilled water (DW), purified tap water (PW), non-carbonated isotonic drink (ID) and honey solution of 5% w/w (H5)

		Type of artificial diet			
		Distilled Water*	Purified Water*	Isotonic Drink**	Honey 5% solution***
	Unit	Percentage of content (% w/w)			
Micronutrients					
Carbohydrate	g	nil	nil	6.2	3.77
Sugar	g	nil	nil	6.2	3.75
Energy – Calories	kcal	nil	nil	25	15.4
Energy – Kilojoules	kj	nil	nil	310	64.35
Protein	g	nil	nil	nil	0.02
Fat	g	nil	nil	nil	nil
Electrolytes					
Sodium	mg	nil	nil	45	0.35
Potassium	mg	nil	nil	present	2.5
Calcium	mg	nil	nil	present	0.25
Phosphorus	mg	nil	nil	present	1
Vitamins					
Vitamin C	mg	nil	nil	nil	0.16
Vitamin B3	mg	nil	nil	1.4	nil
Vitamin B6	mg	nil	nil	0.2	0.0025
Vitamin B12	mcg	nil	nil	0.2	nil

* Based on information gathered from Kozisek (28) and COWAY (47).
** Based on information of the products label and promotion material.
***Based on information provided on product label and supported by Bogdanov et al (23).

Transfer, holding and feeding the worker bees

Upon reaching the laboratory, the worker bees were released from the water bottles into a wooden-meshed cage (20 x 20 x 20 cm). Following this, the cage containing the worker bees was placed on the shelving unit. A wire mesh was secured on one side of the cage, and each cage had an operator/observer hole to provide food for bees. Temperature and relative humidity of the laboratory was maintained at 24 – 28 °C and 48 – 65 % RH, respectively. The photoperiod of 12:12 hours (light:dark period) was controlled by using the laboratory lighting system. Worker bees that were accidentally injured during collection, transfer or handling were removed and excluded from the study.

After transfer into the cage, within an hour starvation procedure, 5 of worker bees were aspirated out from the cage and placed into each food-grade polypropylene (PP) cup of 236 ml covered with polyester netting (10 x 10 cm) and fastened with cup cover. A small-diameter circular hole was created in the middle of cup cover in order to insert a straw that has been loosely plugged with cotton wool at the bottom, namely inverted-wick system as displayed at Figure 2. Polyester netting was used to confine the stingless bee while allowing the exchange of air. A volume of 0.5 ml of a selected artificial diet: DW, PW, ID or H5 was injected into the straw to directly wet the inserted cotton wool at the bottom of the straw. The artificial diets were replenished ad libitum on a daily basis while the inverted-wick system was replaced on every two to three days to prevent mold. In total, 125 worker bees were utilized for the 5 experimental regimes in 5 replicates.

The PP cup was selected because it is made for food grade use and has transparent wall that facilitates the observation of feeding activity (licking behaviour) and survival (life, knockdown, moribund or mortality) of the stingless bee as shown at Figure 2. Furthermore, the engraved microwaveable symbol also indicates the durability of PP cup to withstand for high temperature above 100 °C. This may also reflect the strong bonding of plastic material with less likelihood of leaching of hazardous plastic substance from the plastic cup (32). The PP cup is a versatile container as it can be modified by cutting for various laboratory experimental use meeting the need of future health research e.g. toxicity

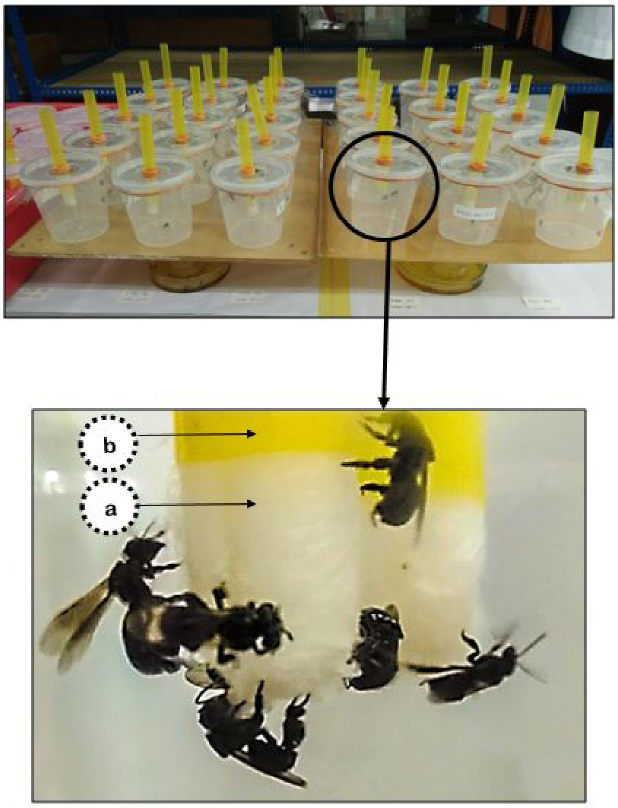

Figure 2: The inverted-wick feeding of *H. itama* worker bees confined in 236 ml food grade polypropylene cup. *H. itama* worker bees licking on the cotton plug (a) inserted into the food grade drinking straw (b) that is treated with the artificial diet, namely distilled water (DW), purified tap water (PW), non-carbonated isotonic drink (ID) or honey solution of 5% (H5).

study of agricultural and industrial chemicals against *H. itama* worker bee. Overall, the advantages of the PP cup include a) convenient size, b) transparency for easy inspection of the worker bees, c) diapause avoidance, d) space-saving, e) easy to dismantle and f) ready access for simple cleaning and sterilizing using detergent solution.

Initial Phase: Observation of longevity for comparison between diets

Feeding activity (licking behaviour) was observed, while the knockdown and mortality of stingless bee were recorded on daily basis to determine the effect of artificial diets on the longevity during holding which directly reflects the physiological fitness of the stingless bee for future toxicity testing purpose.

A total of 125 worker bees that were physically fit i.e. active feed foraging flight were equally divided into five groups, i.e. 25 worker bees per group. The knockdown behaviour and mortality show the poor performance of the artificial diet on worker bee physiological fitness. The knockdown behaviour was characterised by the inability of the stingless bee to coordinate its normal movement, i.e. inability to stand, walk and fly. Knockdown condition prolonged above 24 hours was considered moribund and counted as mortality (33, 48). The observation was performed on a daily basis until all worker bees found dead. The experiment was performed at Entomology Laboratory, MARDI, Serdang, Selangor.

Subsequent Phase: Observation of longevity for comparison between worker bees from different farms

Upon completing the initial experiment to select the most suitable diet, the study at subsequent phase explored the performance of the feeding technique and best diet among worker bees from different commercial farms namely Gombak (Selangor), Kuala Kangsar (Perak) and Merbok (Kedah) at Peninsular Malaysia. The collection, transfer and holding procedures of the 25 worker bees from each farm were the same as conducted at the initial phase of the study. The worker stingless bees that were collected at the three farms were transferred into the wooden cage (20 x 20 x 20) then separated into the individual cups at the respective farm. All the worker bees were fed with H5 artificial diet. The observation parameters i.e. knockdown, moribund and mortality were the same as the initial phase on daily basis.

Assumption and limitation

This study is unable to determine the age of the worker bee. Therefore, this study assumes that the worker bee which perform food foraging flight is mature worker bee that is healthy and physically fit. Hence, this study only selects the worker bees that fly out to forage food.

Statistical analysis

Initial Phase: Observation of longevity for comparison between diets

This study intended to establish a laboratory technique that provides an adequate condition for sustaining the life of stingless bees collected from the farm. Secondly, this study aimed to investigate the effects of artificial diets containing nutrients such as ID and H5 in comparison to plain water (DW or PW) on the survival period of the worker bees. However, the exact survival period of the worker bees outside its hive was uncertain. It is also uncertain whether the H5 is better than the ID. Thus, the alternate hypothesis (HA) suggest a significant difference in longevity among the worker bees fed with different diets. The effects of diet on the longevity of *H. itama* worker bees were evaluated by measuring survival rates (%). The significance difference between the number of surviving *H. itama* worker bees at each day that were fed with selected artificial diets were compared and determined by statistical analysis using IBM SPSS Statistics Campus Edition (SPSS 22.0.0.0) software package for Windows (34). The normally distributed data and mean mortality of the worker bees between the groups were statistically analysed using one-way analysis of variance (ANOVA) followed by post hoc comparisons using the Tukey HSD test to establish the significant difference of variance among the artificial diets. The survival time was analysed using the Kaplan-Meier test (35). Kaplan-Meier has been used to estimate a sample population survival curve. An analysis of Kaplan-Meier allows estimating survival over time, even if worker bees are studied for various lengths of time. The $P < 0.05$ is considered to indicate a statistically significant difference (36).

Subsequent Phase: Observation of longevity for comparison between worker bees from different farms

The mean mortality of the worker bees fed by H5 from the four farms (including MARDI farm) were statistically analysed using one-way ANOVA, while post hoc comparisons using the Tukey HSD test was used to compare the longevity of *H. itama* worker bees among the farms, being fed on H5. Differences were considered significant at $P < 0.05$. All analyses were conducted using statistical Software IBM SPSS Statistics Campus Edition (SPSS 22.0.0.0) package for Windows (34).

RESULTS

The finding of the study with regard to feeding activities, i.e. the behaviour of *H. itama* worker bee of licking on the surface of the cotton wool of the inverted-wick system as shown in Figure 2. This observation demonstrated that the worker bees were attracted and capable to feed with the artificial diets.

Initial Phase: Observation of longevity for comparison between diets

One-way ANOVA shows that there was a significant difference ($P < 0.05$) between artificial diets on the longevity of the worker bees. Subsequent analysis of mean comparisons using Tukey HSD test found that the worker bees unfed and fed with purified water or distilled water have a very poor survival with 0% survival at day-1 and day-4, respectively. The result of the Kaplan-Meier survival analysis is shown at Figure 3. The *H. itama* worker bees fed on 5% honey solution and isotonic drink survived significantly ($P < 0.05$) longer compared with the worker bees fed with plain water (DW or PW). Both artificial diets have significantly ($P < 0.05$) extended the longevity of the *H. itama* worker bees with 50% survival probability up to 8 days.

Subsequent Phase: Observation of longevity for comparison between worker bees from different farms

One-way ANOVA shows that there was no significant difference ($P > 0.05$) between stingless bee from the three farms. The result of the Kaplan-Meier survival analysis is shown at Figure 4. The Kaplan-Meier survival analysis showed that the inverted-wick system feeding with the 5% (v/v) honey solution was also capable to sustain the life of the *H. itama* worker bees from three commercial farms, namely Merbok, Gombak and Kuala Kangsar stingless bee farms, respectively. Whereby the life of the worker bees from all three commercial farms was prolonged above 14 days with 50% survival.

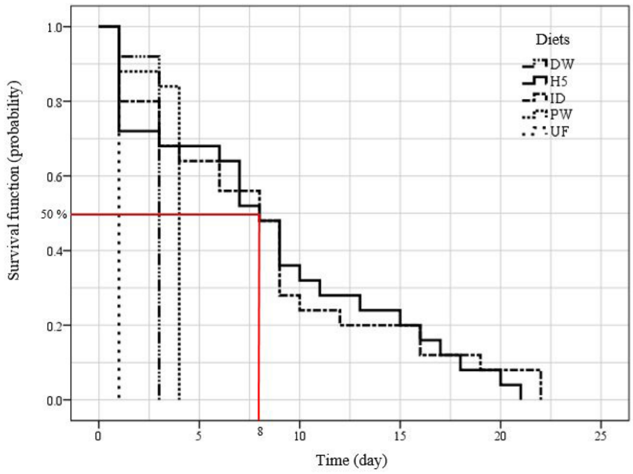

Figure 3: Kaplan-Meier plot of the farm collected *H. itama* stingless worker bee survival function on different artificial diets under laboratory conditions (24-28 °C, 48-65% RH, and 12:12 hours light period). These artificial diets are unfed (UF) as control, distilled water (DW), reverse osmosis purified tap water (PW), non-carbonated isotonic drink (ID) and 5% honey solution (H5). Total worker bees employed in the study is 125, whereby 25 worker bees are for each artificial diet.

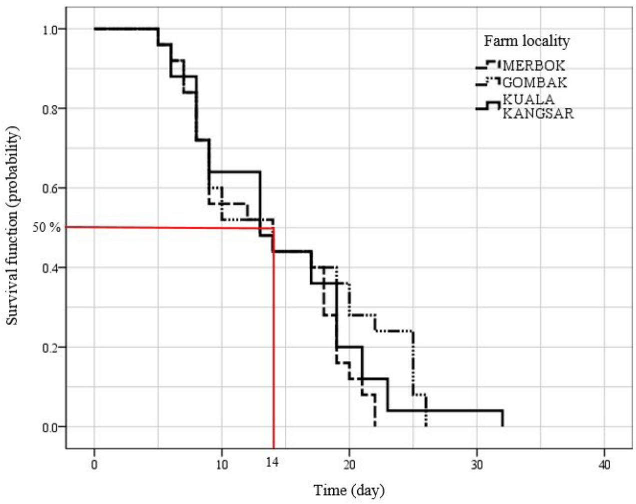

Figure 4: Kaplan-Meier plot of the farm collected *H. itama* stingless worker bee survival function on 5% honey solution (H5) under laboratory conditions (24-28 °C, 48-65% RH, and 12:12 hours light period). Total worker bees employed in the study is 100, whereby 25 worker bees are from each farms, namely Gombak, Selangor (3°15'28.9"N 101°45'03.6"E), Kuala Kangsar, Perak (4°53'58.6"N 100°53'46.9"E) and Merbok, Kedah (5°44'09.8"N 100°24'52.1"E).

DISCUSSION

The study intends to provide experimental evidence for a link between the type of diet and longevity of farm breed stingless bees. Our results suggest that the source of nutrition given to stingless bees can have a direct impact on their longevity. The diet that is available or provided to the worker bees has a great impact on their physical characteristics, robustness and behaviour. This is because each type of food has its own unique nutritional value.

According to Manning (24), information on nutrient requirements is essential for measuring the response of variables such as growth rate, reproductive performance or longevity of honey bee. This is also supported by Amadou et al (18), in which a diet that is given must be able to provide important nutrition sufficiently to ensure that the worker bees are well nourished. Similarly, Munir et al (20) stated that parasitoids receiving enough food are likely to be effective biocontrol agents. Meanwhile, Manning (24) described a substitute for pollen in terms of ingredient and nutrient specifications, including the need of an artificial diet to be attractive and palatable to foraging honey bee.

The initial observation at day-1 showed that all the UF worker bees died within 24 hours. It is shorter than the honey bees survival as reported by Haydak (37), whereby without water, the adult honey bees could survive for 3-4 days under laboratory conditions. This occurrence may be due to different physiological fitness between stingless bees and honey bees. In natural conditions, DeGrandi-Hoffman et al (26) reported that the presence of water is important for honey bees as a form of food, to dilute honey, for growth and development, for metabolism, cooling and to regulate the temperature in the hives. A study conducted by Williams et al (7) found that water availability is important in maintaining adult honey bees in cages under laboratory conditions. The visual observation on the feeding activity by the *H. itama* worker bees reaffirms the accessibility of the worker bees to lick the cotton wool wetted by the artificial diets that are loosely inserted into the drinking straw. However, for *H. itama* worker bees, the probability of survival upon feeding with water (DW/PW) did not exceed 20% as been reported for the honey bee. Furthermore, the survival probability dropped to zero in less than five days. The shorter longevity of *H. itama* without sugar (UF, PW and DW) is similar to that observed for *Cleruchoides noackae* by de Souza et al (10). Thus, the result indicates that *H. itama* stingless bee is more vulnerable compared to honey bees, in the absence of water. In comparison between DW and PW, there is no significant difference ($P > 0.05$).

The results of the survival analysis indicated that sugar has markable effect to prolong the life of stingless bee compared to water. As been reported for honey bee, the longevity of *Apanteles metesae* (Nixon) was longer when adults were fed 50% honey and 20% sucrose compared when fed 50% sucrose or only distilled water (38). Compare to unfed, females of *Diaeretiella rapae* showed higher longevity when supplied with either 20% honey and water, or when supplied with water and honeydew (39). Nutrients like sugars, proteins, enzymes, amino acids, minerals and vitamins are provided by these food sources (39). In order to maintain the longevity of *H. itama* in the laboratory, it is important to provide food (sugar solutions) to *H. itama* worker bees as their key source of energy (20), presumably because these pollinators only have the energy accumulated during their immature stage and during their foraging activity, which can limit their longevity in the laboratory (40). In addition, many researchers have described the importance of sugar feeding for energy and survival in many hymenopteran species (6, 20), in which Rivero & Casas (25) demonstrated that sugar will be stored as glycogen in the fat body or as trehalose in the hemolymph. For instance, *Diadegma insulare* parasitoids feed on carbohydrate-rich food to satisfy their energy requirements, which can increase their lifespan both under laboratory and field conditions (6).

Statistical analysis of one-way ANOVA shows that there was a significant difference between artificial diets ($P < 0.05$) on the longevity of the worker bees. Statistical analysis using Kaplan-Meier test showed that the H5 and ID had significantly ($P < 0.05$) extended the longevity of the *H. itama* worker bees with 50% survival probability up to 8 days. The last worker bee died on day-22 and day-21 for those fed with ID and H5, respectively. The results proposed that the artificial diets that contained micronutrients, electrolytes and vitamins are essential for the stingless bee survival. Several studies on honey bee feeding of macronutrients have reported similar findings on the importance of nutrient in the diet (17, 41). H5 used in the study contains electrolyte (minerals), i.e. sodium, potassium, calcium and phosphorus, while ID used in the study contains vitamin B complex (B3, B6, and B12). The study by Costa & Venturieri (19) reported that diets replacement with vitamins and minerals have a significant impact on the daily intake of stingless bee, on the size of their hypopharyngeal gland acini and on the size of oocytes. According to Haydak (37), adult honey bees have utilized vitamin B complex for muscle development, whereas Tan et al (31) reported that presence of vitamin B extended the vitality and longevity in stimulating ovarian development of adult mosquito.

Marginal nourishment prolongs longevity of worker bees compared to those unfed. The *H. itama* worker bees showed the greatest longevity when fed with H5 and ID compared to PW and DW sustained at 24 – 28 °C. Food that is provided at a suitable temperature is important for the longevity of *H. itama* worker bees in the laboratory. This finding is in line with the study by de Souza et al (10) in rearing parasitoid *C. noackae*. The results also consistent with Munir et al (20) study, in which, compared to water treatment, all sources of sugar (nectar and 10% honey) increased the longevity of *Plutella xylostella*. According to Salmah et al (38), honey fed at a concentration of 50% increased the longevity of *Apanteles metesae* (Nixon), however honey fed at higher than 50% concentrations may decrease their longevity due to the excess energy or low water content. This is supported by de Souza et al (10) who demonstrated that honey, at low concentration, containing less energy, whereas high concentrations of honey containing more energy. In addition, a total of 15 – 20% sugar solution is known to encourage bacteria formation, whereby the development of non-pathogenic bacterial strains is supported when honey is diluted with water. Solution of less than 50% honey in water sustained bacterial life for longer periods but never exceeded 40 days (42). Study by Winkler et al (43) showed that carbohydrate-rich food during the adult stage is a vital energy source for many parasitoids. Meanwhile, according to Singh (21), the honey provides instant insect food which is convenience, requires no preparation expertise, thereby saving considerable time and money. Therefore, in this study, H5 and ID are the best artificial diets for stingless bee and both are simple, practical and economical for general laboratory rearing. The diets are recommended for educational purposes to rear insects in view of its suitability for pathogens, hormones, plant-resistant chemicals and other toxins bioassays. It can be conveniently prepared for various types of tests and stingless bee species. This finding was also supported by Munir et al (20), where honey can be a good substitute for insect rearing in the laboratory in the lack of floral nectar.

Temperature is one of the main factors in the acclimatization of insects (10). Meanwhile, according to WHO (49), the relative humidity may affect the survival of worker bees during the holding period. In general, the study shows that the laboratory temperature of the 24 – 28 °C and relative humidity of 48 – 65 % RH is effective in sustaining the *H. itama* stingless bee but depends on the diet provided. Similarly, Singh (21) also recommended the same laboratory conditions at an average temperature of 21°C or constant temperatures of 25 ± 1 °C and relative humidity of 50 – 60 % RH. The result is also in accordance to study by Abou-Shaara et al (9) who found that high temperature had a negative effect on survival while high humidity is better for enhancing survival of the stingless bee. It is also consistent with findings of the study by Sheikh et al (44) in which high temperature and low humidity have been found to cause insects inability to move and shorten the life span. Khoso et al (45) also demonstrated the same parameters of 25 ± 2 °C and at 60 to 70 % RH in studying the feeding preferences of larvae and adults of zigzag beetle under laboratory conditions. According to de Souza et al (10), at lower temperatures, reduced activity and metabolism may result in increased longevity. Suboptimal temperatures can reduce lifespan, reduce body size and reserves of energy, and increase the development period (46).

Overall, the findings of the study suggest that H5 and ID are effective artificial diets for the purpose of holding farm bred stingless bee *H. itama* in the laboratory for an 8-day duration for the MARDI farm, while up to 14 days for the commercial farms, i.e. Merbok, Gombak and Kuala Kangsar stingless bee farms. For a longer longevity of the worker bees, it may take more honey to increase the nutrient concentration of honey. As recommended by Bhatkar and Whitcomb (14) who studied on artificial diet rearing various species of ants, they found that it was useful to vary the consistency of the diet. In order to determine the optimum concentration for the feeding of bees under laboratory conditions, a further study on the effect of honey solution at serial concentrations of 5 – 15 % on the longevity of worker stingless bee is required. In addition, information about either the higher concentration of honey may be obstructive or too thick for *H. itama* worker bees to lick is still unknown. Meanwhile, even though ID is easily obtained at a low price, the content of the micronutrient in a commercial non-carbonated isotonic drink cannot be increased, nevertheless, a separate study using lab prepared an isotonic solution containing micronutrients and vitamins at higher concentration would further clarify the benefit of micronutrient to prolong the survival of the stingless bee. In addition, it may have been that the feed supply provided was inadequate in nutrients for the bees and this may have led to fatigue with resulting limited longevity and flight control responses and floating.

In the subsequent phase, for the comparison on the longevity of *H. itama* worker bees fed on H5 from different stingless bee farms was conducted. Statistical analysis of one-way ANOVA shows that there was no significant difference ($P > 0.05$) between stingless bee farms on the longevity of the worker bees fed on H5. Statistical analysis using Kaplan-Meier test also predicted the similar 50% survival at 14 to 15 days for the *H. itama* worker bees from the three farms. In overall comparison, the last worker bee fed with H5 that died is on day-21, day-22, day-26 and day-32 for MARDI, Merbok, Gombak and Kuala Kangsar farms, respectively. The results proposed that the H5 could sustained the longevity of *H. itama* worker bees collected from the commercialized farms longer than MARDI stingless bee farms, most probably due to the inherent genetic differences because the MARDI colonies were controlled colonies whereby it is in-breed to protect from genetic drift.

CONCLUSION

From the outcome, the findings suggest that the inverted-wick system with the use of 5% honey solution or non-carbonated isotonic drink is suitable feeding system to sustain the physical and physiological needs of *H. itama* worker bees. Hence, allowing the *H. itama* worker bees to be kept in laboratory between 8 to 14 days for controlled experiment. The results of this study could be useful for future studies, especially on Kelulut honey and toxicological studies.

ACKNOWLEDGEMENTS

Appreciation to the staff of the Agrobiodiversity Resources Utilization Program, Agrobiodiversity and Environment Research Centre, MARDI Serdang for stingless bee supply and holding facilities. Special thanks to the stingless bee farmers, Mr. Syafiq Syazwan, Mr Firdaus and Mr. Nizam from stingless bee farms namely Merbok, (Kedah), Kuala Kangsar (Perak) and Gombak (Selangor) respectively, for providing a comprehensive support and for letting us to collect the stingless bees for research purposes. We appreciate the technical support and research facility provided by the Animal Research Section of the Advanced Medical and Dental Institute, Universiti Sains Malaysia. We are also thankful to OROTROPICOS Sdn. Bhd. for their contribution of research consumables.

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