Pollen Analysis of Kelulut's Honey Collected from Different Location in Selangor

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DECLARATION

I hereby declare that the work embodied in here is the result of my own research except for the excerpt as cited in the references.

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Analisis Debunga Madu Kelulut Yang Dikumpul dari Lokasi Berbeza di Selangor

ABSTRAK

Madu yang dihasilkan di negara membangun pada umumnya lebih murah daripada madu yang dihasilkan di negara maju. Ini menyebabkan sesetengah penternak lebah memberi makan secara tidak sah kepada lebah dengan menggunakan pemanis tiruan yang menyebabkan penghasilan madu tidak asli dan seterusnya tidak dapat menentukan asal usul bunga atau geografi madu tersebut. Kajian ini dijalankan dengan menganalisis debunga ke atas 20 sampel madu Kelulut yang dikumpul dari lokasi berbeza di Selangor termasuk Hulu Langat, Shah Alam, Rawang, Batang Berjuntai, Petaling Jaya dan Bangi. Matlamat kajian ini memfokuskan kepada menentukan sumber botani madu yang dikumpul. Objektif kajian ini adalah untuk mengkaji jumlah debunga madu yang dikumpul dan mengenal pasti jenis debunga dengan mengkaji morfologinya. Sampel madu telah menjalankan proses acetolisis dan telah dikenal pasti di bawah mikroskop. Hasilnya, sebanyak 55 jenis debunga telah dikenal pasti daripada 30 keluarga tumbuhan. Daripada analisis dijalankan, 10 sampel madu dikelaskan sebagai unifloral dan selebihnya adalah multifloral. Analisis debunga atau melissopalinologi adalah kaedah yang berguna untuk menyelesaikan sumber botani dan asal sampel madu selain itu dapat mengetahui spesies bunga dimana lebah madu mengumpul nektar.

Kata kunci: Analisis debunga madu Malaysia, jenis debunga, pengenalpastian debunga, asetolisis, morfologi debunga

Pollen Analysis of Kelulut's Honey Collected from Different Location in Selangor

ABSTRACT

Honey produced in developing countries is generally less expensive than honey produced in developed countries. This cause some beekeepers illegally feeding the bee with artificial sweetener which led the adulteration of honey and purposely mislabeling the floral or geographical origin of honey. This study conducting pollen analysis of 20 Kelulut honey sample that collected from different location in Selangor include Hulu Langat, Shah Alam, Rawang, Batang Berjuntai, Petaling Jaya and Bangi. This present study focused to determine the botanical source of collected honey. Hence, the objective was to study the pollen amount of collected honey and identify type of pollen by studying their morphology and characteristics. The honey samples were acetolyzed and identified under microscope. 55 types of pollen were identified from 30 plant families. From the analysis, 10 honey samples were classified as unifloral and the rest were multifloral. Pollen analysis or melissopalynology is a useful method for settling the botanical and origin sources of honey samples besides it acts on to reveal the species of flower from which the honeybees gathered nectar.

Keywords: Pollen analysis of Malaysian honey, Pollen types, Pollen identification, acetolysis, pollen morphology

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r.p.m	revolutions per minute
g	gram
mL	milliliter
%	percentage
°C	Degree celcius

CHAPTER 1

INTRODUCTION

1.1 Research background

Honey is a natural sweet with a viscous liquid food that is dark golden in color that is created by honeybees in their honey sacs from flower nectar that is stored in a honeycomb and beehive nest. Honey is one of the most commonly used sweeteners (Bagameri et al., 2021; Srećković et al., 2019) The nectar's flavor and color are derived from the flowers from which it is obtained. Bees produce this substance, which has great implications for human health and beyond (Abdiniyazova et al., 2016; Nguyen et al., 2018 ; Bagameri et al., 2021). Honey is fundamentally having a very concentrated solution of monosaccharide texture in water. It contains several elements of nutrient include carbohydrate, amino acids, vitamin (Fechner et al., 2016). In addition, it contains other elements like organic acids, esters, volatile components, hydroxymethylfurfural, enzymes and phytochemicals (Siddiqui et al., 2017). The composition of honey is highly dependent on the flowers consumed by the honey bees and weather factors (Hamid et al., 2015). Honey is characterized by its physical

state (liquid) and color (light and darkness) and is divided into honeybees (*Apis mellifera*) and honeybees (*Meliponini*) (Silva et al., 2013; Bagameri et al., 2021). The produced amount and taste are the major difference between honeybee and stingless bee (Imtiazah et al., 2021 ; Bagameri et al., 2021). Pollen is collected by honeybees in addition to nectar from flowers to supplement the diet of bee larvae. Pollen is a fine powder secreted by the anther. Several analytical techniques and parameters are needed in combination with statistical methods to determine the composition and geographic origin of honey(Council Directive 2001/110/EC) (Bagameri et al., 2021). Honey's botanical origin is determined by the presence of pollen. Moreover, honey pollen plays main tool in analysis of honey because it points out the major and minor plant taxa employed by honeybees. Hence, it is made up of pollen grains composed by honeybees (Shubharani et al., 2012) and melissopalynological will be able to point out of plant species. Melissopalynology is the main analysis used to demonstrate the presence of pollen grains. Microscopic analysis of honey is possible because it contains pollen particles that are concentrated by centrifuging diluted honey (Bagameri et al., 2021). The identification of abundant plant species is vital for composition of honey (Sodré et al., 2007) and assist in the authentication of honey. In this study, the pollen analysis was carried out from Kelulut's honey that collected from different location in Selangor. The pollen analysis was identified by using microscope. Yet, pollen analysis aid in determining the floral origin and detect whether the honey is contaminated or not.

1.2 Problem statement

Malaysia is a diverse country of where 45 type of stingless bees have been recorded. *Heterotrigona itama*, *Geniotrigona thoracica*, *Lepidotrigona terminata*, *Tetrigona apicalis* and *Homotrigona alicae* are one of the species distributed in Malaysia (Jaapar et al., 2016; Ismail et al., 2021). However, to date, very little attention has been made to study the melissopalynology and foraging activity of the stingless bees, specifically in Malaysia region. Despite this, local apiculture industry is not developing like other southeast Asia countries (Ismail, 2016). In addition, Malaysia's beekeepers are still far behind compared to neighbor countries such as Thailand and Vietnam. The problem involved the beekeepers lead to low honey production and inconsistent quality of existing honey production has resulted in the production of adulterated honey, which is less expensive and available all year. In addition, majority of Malaysian honey is imported, and a study by Mardan and Osman found that honey adulteration is popular in the Malaysian market (Fakhlaei et al., 2020). Since Selangor is played main role as one of the largest area of honey production, it has been chosen to do pollen analysis on Kelulut's honey on developing relationship between plant sources and honey in order to aid in the development of honey production in this region as well as serve as a guide for honey quality analysis. Therefore, pollen analysis is important to the beekeeping industry in the development of marketable premium honey and honey products (Azmi et al., 2015). Hence, Kelulut bee is one of stingless bee that produces honey with a higher medicinal value than other bee species, as claimed by traditional medical practitioners (Yaacob et al., 2018).

1.3 Scope of the study

This study is aiming to perform the pollen analysis via using the acetolysis technique on Kelulut honey that collected from different location in Selangor. Furthermore, this research aims to determine the origin of botanical sources and categorized honey sample into two categories which is unifloral and multifloral honey. There is a few research was carried out on Kelulut honey sample. Then, the honey sample will be analysed by using microscope in order to identify type of pollen, morphology as well as pollen count after conducting acetolysis procedure.

1.4 Significance of the study

Pollen analysis or melissopalynology used to assess the geographical and floral sources of honey (Rodopoulou et al., 2018). Pollen analysis of honey able to identify the origin of plant species and properties of honey that collected from different places in Selangor. This study is focused on the analyzing the kelulut's honey pollen. The honey was collected from different location in Selangor. Each location of honey collected determine the pollen print used to decide geographical origin of analysed honey sample.

1.5 Research objectives

a) To analyse the pollen amount of collected honey

b) To identify the type of pollen by studying their morphology and characteristics

c) To determine the type of honey (Unifloral or Multifloral) based on their botanical source

CHAPTER 2

LITERATURE REVIEW

2.1 Pollen analysis

Pollen analysis is a scientific method that forward the evidence of past ecological and climate change where it combines the principles of stratigraphy with observations of actual pollen-vegetation relationships in order to reconstruct the terrestrial vegetation of the past. Honey is made up of pollen grains gathered by honeybees, so pollen analysis of honey can help recognize plant species (Shubharani et al., 2012). Thus, the pollen analysis or melissopalynology is very important to determine and control the origin of botanical and geographic of honey (Rodopoulou et al., 2018 ; Selvaraju et al., 2019). So far, melissopalynology is comprises with the basic instruments in determining the botanical of honey. Although the sensory and physicochemical analyzes are also needed for a proper

diagnosis of the botanical origin (Rodopoulou et al., 2018). Past research of pollen analysis proves can help to determine the condition of honey whether the honey has been contaminated due to poisonous pollen or degraded (Rosdi et al., 2016) . Using pollen analysis, honey was determined as monofloral or polyfloral (Rašić et al., 2018). These studies able to differentiate whether the honey sample is unifloral or multifloral which classified as high commercial value of honey. Additionally, the pollen spectra prints will provide data of flowering plants used by the bees in the research area. Consequently, the pollen analysis of Malaysian honey lays the groundwork for determining the honey's sources in terms of location and floral source. Pollen contained in honey can be used to determine which plants honeybees visit to obtain nectar (Hamid et al., 2015).

2.2 Types of Malaysian honey

Malaysian's common types are Tualang honey, Gelam honey and Kelulut honey. They are tropical rainforest honeys that mostly originated from Malaysia. These types of honey are contributed by floral source. Honey might also influence by several factors include climate and its geographical origins mainly in tropical countries (Selvaraju et al., 2019). Malaysia has roughly estimated contain 100 species of bees (Saludin et al., 2019), and majority of Malaysian honey is produced in Sarawak, Sabah, Johor and Melaka. It includes *Apis cerana* (local bee), *A. mellifera* (imported bee from Australia), *A. dorsata* (forest bee or

giant bee and the meliponines (stingless bee). There are around 750 to 1,000 beekeepers that produce approximately 30 metric tonnes per annum (Saludin et al., 2019). In Malaysia, 35 species of stingless bees were documented and bee farming is a brand new capability enterprise in agriculture (Majid et al., 2020). According to the Malaysian Agricultural Research and Development Institute (MARDI) in 2013, 30 species of stingless bee have been identified in Malaysia. These stingless bees are very active and safe to human compared to other bees. Stingless bees are known to be important pollinators in the rainforest (Eltz & Bru, 2003; Azmi et al., 2015). Honey bees is a good candidate for providing pollination services in agroecosystems such as starfruit, mango, durian, watermelon, guava and coconut (Slaa et al., 2006; Azmi et al., 2015). Besides, stingless bee become favorite for local consumption as it attracts major attraction among the beekeepers due to increased demand for producing high honey production. There are two most popular species of stingless bees which broadly reared namely *Heterotrigona itama* and *Geniotrigona thoracica*. These type of stingless bee is prevalent for the commercialization of honey production (Majid et al., 2020).

2.3 Melissopalynology of Malaysian honey

Bees, the world's most important pollen maters, play an important role in wild and cultivated plants, especially in the tropics where entomophily is important (Ollerton et al., 2011; Ponnuchamy et al., 2014). Studies of Melissopalynology, or pollen, are well established and have been used to determine the origin of flowers, their geographical origin and the genus of plants visited by honeybees (Ponnuchamy et al., 2014; Azmi et al., 2015). Next, melissopalynology is the study of pollen they deliberately and accidentally collect, which eventually becomes honey (Ponnuchamy et al., 2014). Melissopalynology has been widely used to determine the purity, geography and origin of flowers of honey (Ponnuchamy et al., 2014). However, some melisopalinic studies have been reported, especially on Malaysian stingless bee species. Among the various species of stingless bees, only two are bred on a large scale for commercial honey production, *Heterotrigona itama* and *Geniotrigona thoracica*. Of these, *H. itama* in southern Malaysia is an easily found species in the forest and is very popular with beekeepers (Majid et al., 2020).

2.4 Classification of different floral types of honey

Pollen grain classification is a qualitative process that involves observing and identifying properties (Ticay-Rivas et al., 2011). Pollen types are categorized by percentage and evaluated in standard class. The classes are (a) predominant pollen class (> 45% of total pollen), (b) secondary pollen class (15% – 45% of total pollen), and (c) major pollen class (3% –15%) of total pollen. Qualitative method described the types of pollen present in honey are compared with reference leaf samples stored in pollen vouchers (Palynology Institute, Faculty of Botanical Sciences, Institute of Biology, and Reference Pollen Slide Collection of the Federal University of Rio de Janeiro) and literature. Each type of pollen found in the sample was cataloged and photographed. Meanwhile, quantitative method defined after identifying pollen types, more than 300 pollen grains were counted (Haidamus et al., 2019). Honey samples were categorized according to the predominance of flower sources, based on the frequency class of pollen species which single flower if one pollen species predominates and multi-pollen (so-called secondary pollen and isolated pollen) when there are few types of pollen. Bifloral honey was also included in this diagnosis, and there were two types of pollen as a secondary pollen class. Certain honey samples with underestimated pollen grains, such as Croton, Hyptis, Citrus, Vernonia, and Anadenatella had to apply the coefficients. Due to the lack of knowledge about the types of honey, the term "pollen and nectar plants" was assigned. In the case of Bath, it is necessary to consider the characteristics of the previously

presented nectar or pollinated species (nectar and pollen plants) with respect to under- or over-expression in pollen grains (Haidamus et al., 2019)

2.5 Pollen identification

The identification of plant species that contribute to the composition of honey nectar requires knowledge of the pollen morphology of honey plants. This pollen found in honey is used to determine the honey's botanical origin. These studies aid in distinguishing multifloral honeys from unifloral honeys or honeys of a particular type that have a high commercial value. Color sorting is one of method to identify the pollen because it saves time and easy process that may not have acceptable accuracy for certain pollination research studies since different plant taxa may have similar pollen pellet colors. Additionally, the number of distinct colour groups pollen pellets can be divided into has a logistical limit. As a result, pollination studies may not always be able to separate each individual plant taxon pollen into its own distinct pellet colour group. By distinguishing pollen from two or more taxa in pellets of the same colour group, morphological characterization of pollen grains through light microscopy also complements colour separation of pellets. Morphological characterization of pollen grain through light microscopy can aid in distinguishing pollen from two or more taxa in pellets of the same colour group pollen grains also complements colour separation of pellets

(Topitzhofer et al., 2021). The most popular approach for taxonomic identification is to examine the morphological characteristics of acetolyzed pollen grains. The pollen grain's protoplasm is removed during the acetolysis process, exposing diagnostic characteristics that can be seen under light microscopy. Researchers may report different taxa, the frequency of taxa found in specific cropping systems, and the predominant taxa of pellet colors using this process. Acetolysis is the most effective method for determining pollen morphology (Topitzhofer et al., 2021).

2.6 Pollen count and characterization

Pollen count need to take into account in order to determine the pollen types and density (Selvaraju et al., 2019). According to Rosdi et al., (2016) the total amount of pollen present in the honey samples was recorded. Pollen is measured in groups of 100 grains, with parallel equidistant lines evenly spread from one side of the cover slip to the other. Using the dilution factor and the amount of pollen in the sample, the total number of pollen is determined. The pollen characterization was focused on percentages of each pollen form, which will aid in the development of honey production in the region and serve as a basis for honey quality analysis. Pollen is divided into four categories include primary pollen, secondary pollen, essential minor pollen, and minor pollen. This pollen classification is based

on the percentage of the calculation's result. In addition, there is a current research that has successfully collected over 1000 pollen grains in their analysis.

2.7 Pollen identification and morphology

Early studies to take advantage of pollen morphological diversity were directly related to plant classification and used a larger set of pollen letters, such as shape, decoration, number of openings and others to define the range of pollen types (El-Amier., 2015). Pollen morphology is referred to as the study of different forms of features present in pollen. The study of morphological features in pollen is essential for identifying pollen types and assisting researchers in learning about the vegetation during a particular geological period. Pollen morphology is involved in solving some taxonomic problems at the family, general, or specific level and part of an interdisciplinary and collaborative approach to plant taxonomy and evolution. The engraving of the integument and certain features make the pollen grain a distinct feature that can identify the genus or even the species of the parent (El-Amier., 2015). This pollen morphology can be used to classify, recognize, and interpret plants at various levels of plant taxonomy (Selvaraju et al., 2019). Previously, numerous experiments on pollen morphology were conducted around the world, and palynological investigations of forest trees were conducted in relation to forest history and natural mixtures of tree species based on pollen profiles. Several taxonomist classifies plant species based on the phenotypic

characteristics of the plant. Besides, pollen morphological research can be utilized to identify type of plant. The complete application of pollen morphology in systematics, paleobotany, and allergy has been recognized. The study of pollen has important implications for identifying bee plants (Roopa et al., 2013). Pollen morphology includes a variety of characteristics such as apertures, alignment, ornamentation or groove, size and form, exine stratification, and pollen wall (Autoridad Nacional del Servicio Civil., 2021). Pollen grains are categorised based on their physical appearance and other characteristics, such as their shapes, forms of apices, and modes of pollen wall contours. Pollen is classified into two types: symmetric and asymmetric pollen. Asymmetric pollen does not have radial or bilateral planes of symmetry, while symmetric pollen does (Selvaraju et al., 2019).

CHAPTER 3

METHODOLOGY

3.1 Materials and apparatus

Table 3.1 List of chemical and its amount for acetolysis process

Chemical	Amount (ml)
Glacial acetic acid	1
Alcohol (70%)	10
Acetolysis mixture	1

Table 3.2 List of apparatus and its quantity

Apparatus	**Quantity**
Compound microscope with camera Leica DM 2500	1 set
Microspatula	2 set
Hot plate	1 set
Water bath	1 set
vortex	1 set
micropipette	1 set
Laboratory centrifuge	1 set
Microscope slide	20 pieces
Cover slip	20 pieces
Falcon tube (15ml)	20 pieces
Microcentrifuging tubes	20 pieces
Petri dish	20 pieces
Beaker	3 container
Measuring cylinder	1 set
water bath rack	1 set
Microcentrifuge tube rack	1 set

3.2 Honey samples

Twenty honey samples (50g) were collected from different locations in Selangor such as Hulu Langat, Shah Alam, Petaling Jaya, Batang Berjuntai, Rawang and Bangi. All the honey samples were subjected to pollen analysis.

Figure 3.1: Honey Sample 1

Place: Hulu Langat, Selangor

Collection date: 8/9/2021

Bee species: *Geniotrigona thoracica*

Honey colour: Dark Brown

Figure 3.2: Honey Sample 2

Place: Hulu Langat, Selangor

Collection date: 7/9/2021

Bee species: *Heterotrigona itama*

Honey colour: Dark Brown

Figure 3.3: Honey Sample 3

Place: Petaling Jaya, Selangor

Collection date: 17/9/2021

Bee species: *Heterotrigona itama*

Honey colour: Dark Brown

Figure 3.4: Honey Sample 4

Place: Bangi, Selangor

Collection date: 7/9/2021

Bee species: *Heterotrigona itama*

Honey colour: Brown

Figure 3.5: Honey Sample 5

Place: Hulu Langat, Selangor

Collection date: 7/9/2021

Bee species: *Heterotrigona itama*

Honey colour: Brown

Figure 3.6: Honey Sample 6

Place: Petaling Jaya, Selangor

Collection date: 21/9/2021

Bee species: *Geniotrigona thoracica*

Honey colour: Light Brown

Figure 3.7: Honey Sample 7

Place: Petaling Jaya, Selangor

Collection date: 17/9/2021

Bee species: *Tetrigona Binghami*

Honey colour: Brown

Figure 3.8: Honey Sample 8

Place: Petaling Jaya, Selangor

Collection date: 21/9/2021

Bee species: *Heterotrigona itama*

Honey colour: Brown

Figure 3.9: Honey Sample 9

Place: Bangi, Selangor

Collection date: 6/9/2021

Bee species: *Heterotrigona itama*

Honey colour: Brown

Figure 3.10: Honey Sample 10

Place: Hulu Langat, Selangor

Collection date: 8/9/2021

Bee species: *Hetetrigona apicalis*

Honey colour: Brown

Figure 3.11: Honey Sample 11

Place: Shah Alam, Selangor

Collection date: 10/9/2021

Bee species: *Heterotrigona apicalis*

Honey colour: Brown

Figure 3.12: Honey Sample 12

Place: Bangi, Selangor

Collection date: 7/9/2021

Bee species: *Heterotrigona itama*

Honey colour: Brown

Figure 3.13: Honey Sample 13

Place: Hulu Langat, Selangor

Collection date: 17/9/2021

Bee species: *Heterotrigona itama*

Honey colour: Brown

Figure 3.14: Honey Sample 14

Place: Batang Berjuntai, Selangor

Collection date: 17/9/2021

Bee species: *Heterotrigona itama*

Honey colour: Brown

Figure 3.15: Honey Sample 15

Place: Petaling Jaya, Selangor

Collection date: 17/9/2021

Bee species: *Geniotrigona thoracica*

Honey colour: Dark Brown

Figure 3.16: Honey Sample 16

Place: Bangi, Selangor

Collection date: 17/9/2021

Bee species: *Heterotrigona itama*

Honey colour: Brown

Figure 3.17: Honey Sample 17

Place: Hulu Langat, Selangor

Collection date: 7/8/2021

Bee species: *Heterotrigona itama*

Honey colour: Brown

Figure 3.18: Honey Sample 18

Place: Shah Alam, Selangor

Collection date: 9/9/2021

Bee species: *Heterotrigona apicalis*

Honey colour: Brown

Figure 3.19: Honey Sample 19

Place: Rawang, Selangor

Collection date: 17/9/2021

Bee species: *Heterotrigona itama*

Honey colour: Brown

Figure 3.20: Honey Sample 20

Place: Hulu Langat, Selangor

Collection date: 7/9/2021

Bee species: *Heterotrigona itama*

Honey colour: Brown

3.3 Location of study

3.3.1 Honey samples collected from different places in Selangor

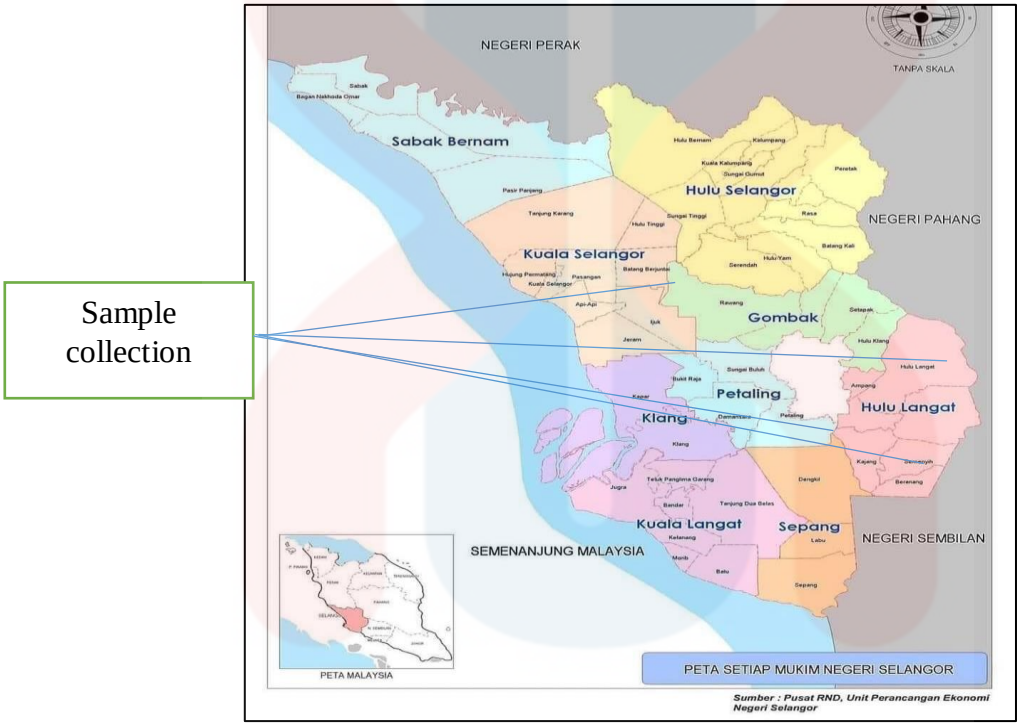

Figure 3.21 Selangor's map indicated the location of kelulut honey collected

(Source: Pusat RND, Unit Perancangan Ekonomi Negeri Selangor)

Hulu Langat
Petaling Jaya
Rawang
Bangi
Shah Alam
Batang Berjuntai

3.4 Method

3.4.1 Pollen acetolysis

5 mL of honey samples was measured carefully and mixed with 10 mL of 70% alcohol in a 15ml falcon tube . The falcon tube was placed on a vortex and agitated manually so that pure honey mixed with 70% alcohol forming homogeneous mixture. The falcon tube was placed in a centrifuging machine and centrifuged at 5 000 r.p.m (revolutions per minute) for 10 minutes. After centrifugation, a clear separation of the supernatant and pellet were obtained. The pellet in the falcon tube was retained and the supernatant was discarded. Then, 1 mL of glacial acetic acid was added to the pellet for acidic hydrolysis process to occur in order to remove the protoplasmic content of the pollen grains. The residue was transferred into microcentrifuging tube. Then, centrifugation was processed again for the mixture at 5000 r.p.m for 10 minutes and the supernatant had been eradicated. The residue was mixed with 1.0 mL of Acetolysis mixture. The Acetolysis mixture was prepared by dissolving 5 mL of sulphuric acid in 45 mL of acetic anhydride. This mixture was the first class acid therefore, following the precaution steps is a must. The mixture was placed in a water bath at 80°C for 20 minutes to allow the digestion process of protoplasmic. Next, the mixture was centrifuged at 5 000 r.p.m for 10 minutes and transferred. The residue that contained pollen material was

washed with 1.0 mL of glacial acetic acid, centrifuged and decanted. Finally, the pollen material obtained was mounted into glycerine jelly and placed onto microscopic slides to be viewed under a compound microscope. In this way, the morphological structures of the pollen types were identified (Selvaraju et al., 2019)

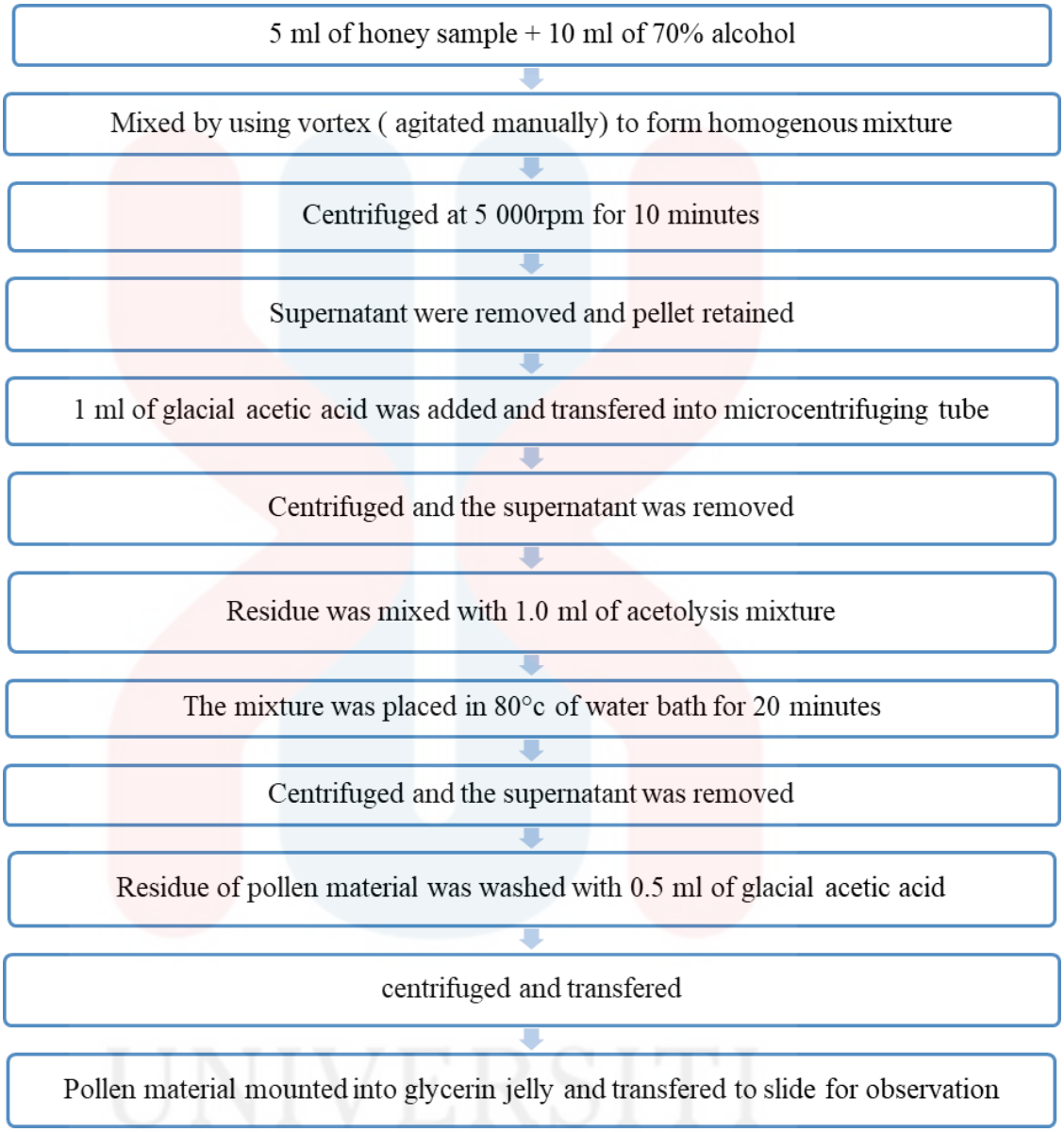

Figure 3.22 Flowchart of acetolysis method

3.4.2 Preparation of Pollen Slides for Microscopic Observation

For the pollen morphology, a small amount of glycerine jelly were taken and mixed with the pollen materials that were obtained as pellets at the bottom of centrifuging tubes. If the jelly solidifies, it was better to gently heat it so that it can spread evenly throughout the glass slides. The pollen pellet which had been mounted into glycerine jelly was placed onto the glass slide that covered with a cover slip. The slides containing pollen samples were viewed under a compound microscope with camera to examine pollen morphology (Selvaraju et al., 2019). Microscopic analysis of honey was possible because it contains pollen particles that were concentrated by centrifuging diluted honey (Bagameri et al., 2021).

3.4.3 Pollen Identification

Pollen atlases and other published floras that offered brief descriptions of the observed pollen in the samples were used to identify pollen kinds. A Leica DM 2500 compound microscope with camera was used to take photomicrographs (Abdulrahaman et al., 2013). Pollen characteristics were detailed in the sequence followed such as pollen class, apertures, exine, ornamentation, outlines and measurements. The pollen shapes are determined according to the ratio between the polar axis and equatorial diameter. Specific training and significant expertise are necessary for the identification of pollen kinds and the interpretation of pollen spectra. Pollen types found in each sample were categorised as follows as predominant pollen types (>45%), secondary pollen types (16-45%), important minor pollen types (3-15%) and minor pollen types (<3%).

3.4.4 Pollen count

In order to know the types and density of the pollen, the relative frequencies of the pollen had to know first (Zappi., 2018).The pollen should identified and counted in groups of 100 according to the 5 parallel uniform equidistant lines from one edge of the cover slip till the other edge. If the relative frequencies were not stabilized, or if 500 pollen grains were insufficient for interpretation, (complex spectrum, over-represented pollen, abundant pollen of nectarless plants or other conditions that can mask the actual nectar source of the honey), then the pollen count should continued to 1000 according to another 5 parallel lines located between the first 5 (Selvaraju et al., 2019).

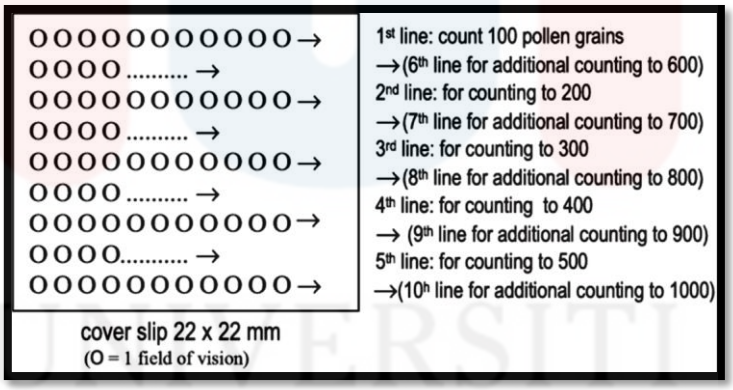

Figure 3.23 Matrix for counting pollen grains that guarantees a homogeneous examination of the slide (O = a whole microscopic field of view) Source:(Selvaraju et al., 2019)

These following formula is use to obtain the total pollen count per slide:

Square coverslip = 50 views per side

Area of coverslip = L x L

= 50 x 50

= 2 500

$$\text{Total pollen count per slide} = \frac{N \times 2500}{10}$$

Where N represents the number of pollen counted in a slide

$$\text{Percentage of abundance, \%} = \frac{\text{Total number of pollen of a particular species}}{\text{Total number of observed pollen}} \times 100$$

3.5 Experimental design

In the experiment, completely randomized design (CRD) was used to analyse data on pollen after calculating the percentage of pollen abundance and type of pollen. The CRD method was accomplished using the Microsoft Excel Data Sheet.

3.6 Overview of sample analysis

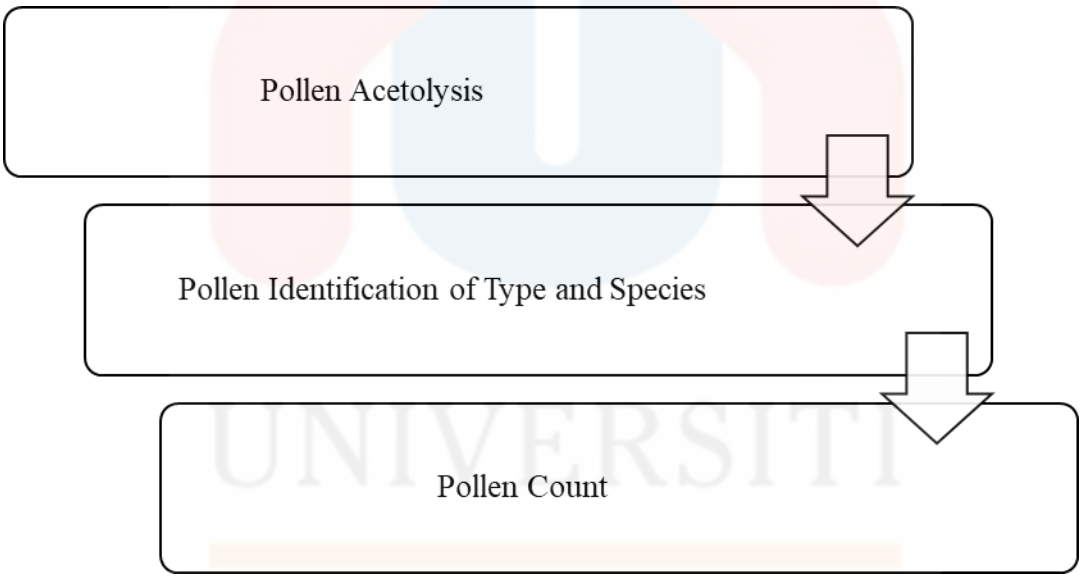

Figure 3.24 Flow of sample analysis

CHAPTER 4

RESULT AND DISCUSSION

The outcomes of the microscopic analysis of the honey from the studied areas illustrated the abundant and diversified pollen composition of the honey samples examined (Abdulrahaman et al., 2013). Acetolysis and pollen identification had been done for 20 honey samples collected from 6 different locations of Selangor such Hulu Langat, Petaling Jaya, Bangi, Shah Alam, Batang Berjuntai and Rawang.

4.1 *Total number of pollen according to their areas of origin and types of honey*

Table 4.1: Total number of pollen according to their areas of origin and types of honey

Locality (District)/ Sample	**Total No of pollen**	**Classification of Honey**
Hulu Langat 1 (HL1)	74	Multifloral
Hulu Langat 2 (HL2)	105	Unifloral
Petaling Jaya 1 (PJ1)	134	Multifloral
Bangi 1 (BG1)	112	Multifloral
Hulu Langat 3 (HL3)	48	Unifloral
Petaling Jaya 2 (PJ2)	45	Unifloral
Petaling Jaya 3 (PJ3)	38	Multifloral
Petaling Jaya 4 (PJ4)	92	Unifloral
Bangi 2 (BG2)	69	Unifloral
Hulu Langat 4 (HL4)	373	Unifloral
Shah Alam 1 (SA1)	71	Multifloral
Bangi 3 (BG3)	26	Multifloral
Hulu Langat 5 (HL5)	24	Unifloral
Batang Berjuntai (BB)	108	Multifloral
Petaling Jaya 5 (PJ5)	157	Multifloral
Bangi 4 (BG4)	1759	Unifloral
Hulu Langat 6 (HL6)	113	Multifloral
Shah Alam 2 (SA2)	222	Multifloral
Rawang (RG)	119	Unifloral
Hulu Langat 7 (HL7)	263	Unifloral

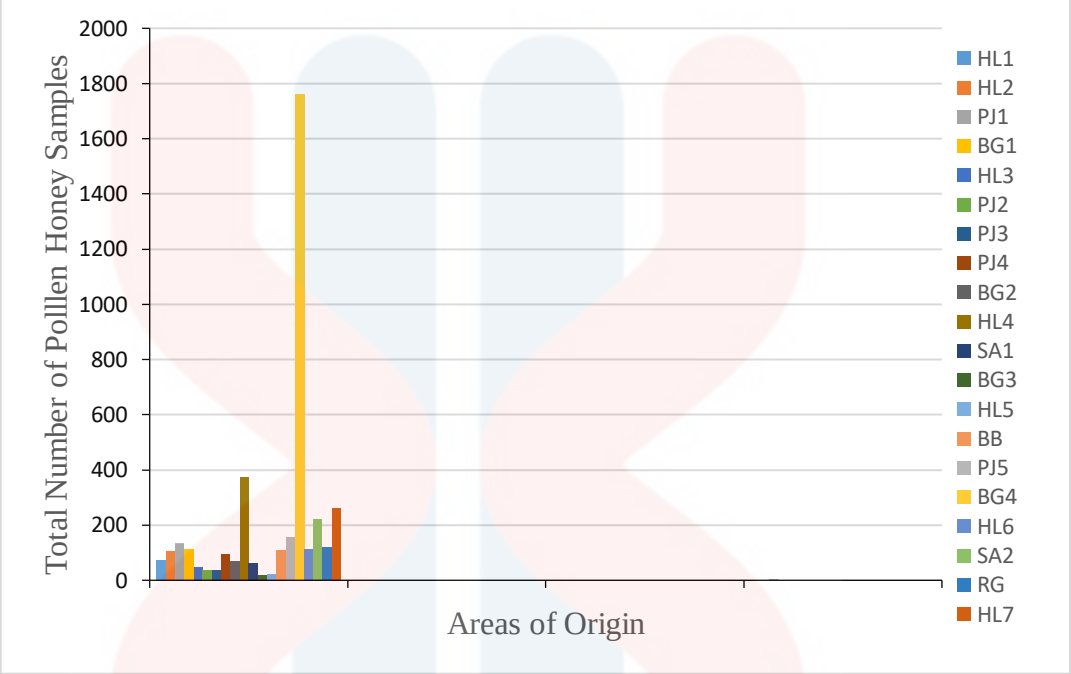

Figure 4.1: Total number of pollen honey samples according to areas of origin

Table 4.1 shown that 10 of honey samples were unifloral while the other 10 were multifloral. 10 honey samples were from Hulu Langat 2 (HL2), Hulu Langat 3 (HL3), Petaling Jaya 2 (PJ2), Petaling Jaya 4 (PJ4), Bangi 2 (BG2), Hulu Langat 4 (HL4), Hulu Langat 5 (HL5), Bangi 4 (BG4), Rawang (RG) and Hulu Langat 7 (HL7). The multifloral honey were from Hulu Langat 1 (HL1), Petaling Jaya 1 (PJ1), Bangi 1 (BG1), Petaling Jaya 3 (PJ3), Shah Alam 1 (SA1), Bangi 3 (BG3), Batang Berjuntai (BB), Petaling Jaya 5 (PJ5), Hulu Langat 6 (HL6) and Shah Alam 2 (SA2). According to Figure 4.1, the honey samples collected from BG4 had the highest number of pollen which was 1759. HL5 had the least number of pollen 24 compared with all other honey samples collected.

Table 4.2 showed that the Mangifera *indica* was the plant species that most dominant honey sample from HL1 with 37.8% of pollen abundance. The least abundance of pollens was *Eupatorium purpureum*, *Caesalpinia pulcherrima* and *Passiflora foetida* which got only 1.4%. However, in Table 4.3, *Cassia fistula* was the highest percentage of pollen from HL2 as the predominant pollen with 67.6% of pollen abundance while the least number *was Cocos nucifera* with 2.9%. One of the important indicator for pollen in Malaysian honey was found in this sample, namely *Cocos nucifera* (coconut). As for Table 4.4, shown that honey sample from PJ1 was abundant with *Solanum melongena* pollen with 35.1%. *Papaver somniferum* showed rare occurrence with 0.7%.

Table 4.5 described that the sample from BG1 has Ruta graveolens from Rutaceae family as the highest percentage of pollen abundance with 26.8%. While the least pollen grain frequencies were belonging to *Borassus flabellifer, Croton sp.* And *Acacia sp* with 0.9% respectively. Table 4.6 showed the highest percentage is 45.8% which came *from Melastoma sp.* of plant species. For Table 4.7, the most abundance was *Cassia fistula* from Myrtaceae family which only got 68.6% and this sample was classified as unifloral honey as consisting of predominant pollen (>45%). While in Table 4.8 showed *Ricinus communis* plant species has the highest percentage of pollen with 31.1% in PJ3 location.

In PJ4 (Table 4.9), *Ixora coccinea* was the most predominant pollen whereas in BG3 and HL4 (Table 4.10 & Table 4.11) were also identified as unifloral honey where *Ruta graveolens* and *Ricinus communis* percentage of abundance pollen more than 45%. It was vice versa for the honey samples of SA1 in Table 4.12, *Conium maculatum* is the most abundance pollen while the least frequency *Eupatorium purpureum, Callistemon linearis, Punica granatum* and *Tecoma stans* were shared same percentage of abundance with 1.4%. It was clearly showed that there was a variety of pollen types present due to variety of local flora that can be found around the places. There was no predominant pollen in BG3 as referred to the Table 4.13 whereas it had been stated, even though 8 different pollen species found in this sample, so, those samples can be classified as multifloral honey. Table 4.14 demonstrated samples in HL5 was an unifloral hone where *Ixora coccinea* has the most

abundance pollen with 70.8% from the overall total pollen distribution. Table 4.15 & 4.16 were revealed there was predominant for BB and PJ5 due their highest percentage of abundance of *Solanum melongena* and *Moringo oleifera* pollen. Table 4.17 showed there is no predominant pollen in BG4 as presence due to variability of local flora that can be found this their nearest places.

Table 4.18 discovered there is predominant in HL6 due to their highest percentage *Mimosa pudica* with 65.5% in honey samples. Table 4.19 which was from SA2 successfully identified 7 types of pollen and the most abundance was *Commelina diffusa* with 92.3% of overall total pollen were observed. Besides, honey sample from Rawang (RG) (Table 4.20) was a multifloral honey as absence of predominant pollen where the highest percentage of abundance was 28.6% from Fabaceae family. For the last sample from Hulu Langat 7 (HL7) showed the predominant pollen, *Solanum melongena* with 92.0% of pollen distributrion.

4.2 *Honey sample from Hulu Langat, Selangor*

Table 4.2: The number of pollen and percentage of abundance of pollen types in the honey sample from Hulu Langat (HL1)

Plant species	**Family**	**No. of pollen**	**Percentage of Abundance (%)**
Cassia Fastula	Fabaceae	6	8.1%
Mangifera indica	Anacardiaceae	28	37.8%
Syzygium jambos	Myrtaceae	2	2.7%
Eupatorium purpureum	Asteraceae	1	1.4%
Callistemon linearis	Myrtaceae	2	2.7%
Ricinus communis	Euphorbiaceae	5	6.8%
Caesalpinia pulcherrima	Fabaceae	1	1.4%
Cocos nucifera	Arecaceae	2	2.7%
Melastoma sp.	Melastomaceae	10	13.5%
Passiflora foetida	Passifloraceae	1	1.4%
Nelumbo nucifera	Nymphaceae	2	2.7%
Gliricida sepium	Fabaceae	13	17.6%

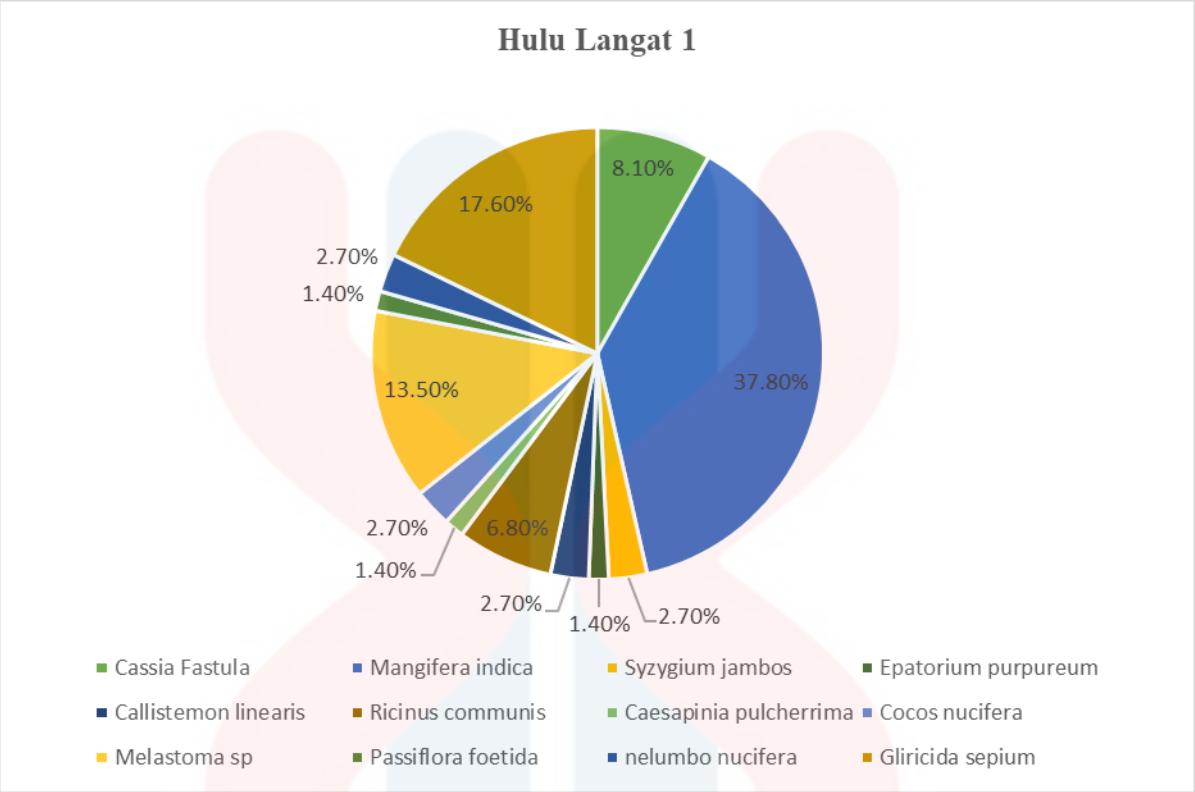

Figure 4.2: Pollen spectrum of honey sample from Hulu Langat, Selangor

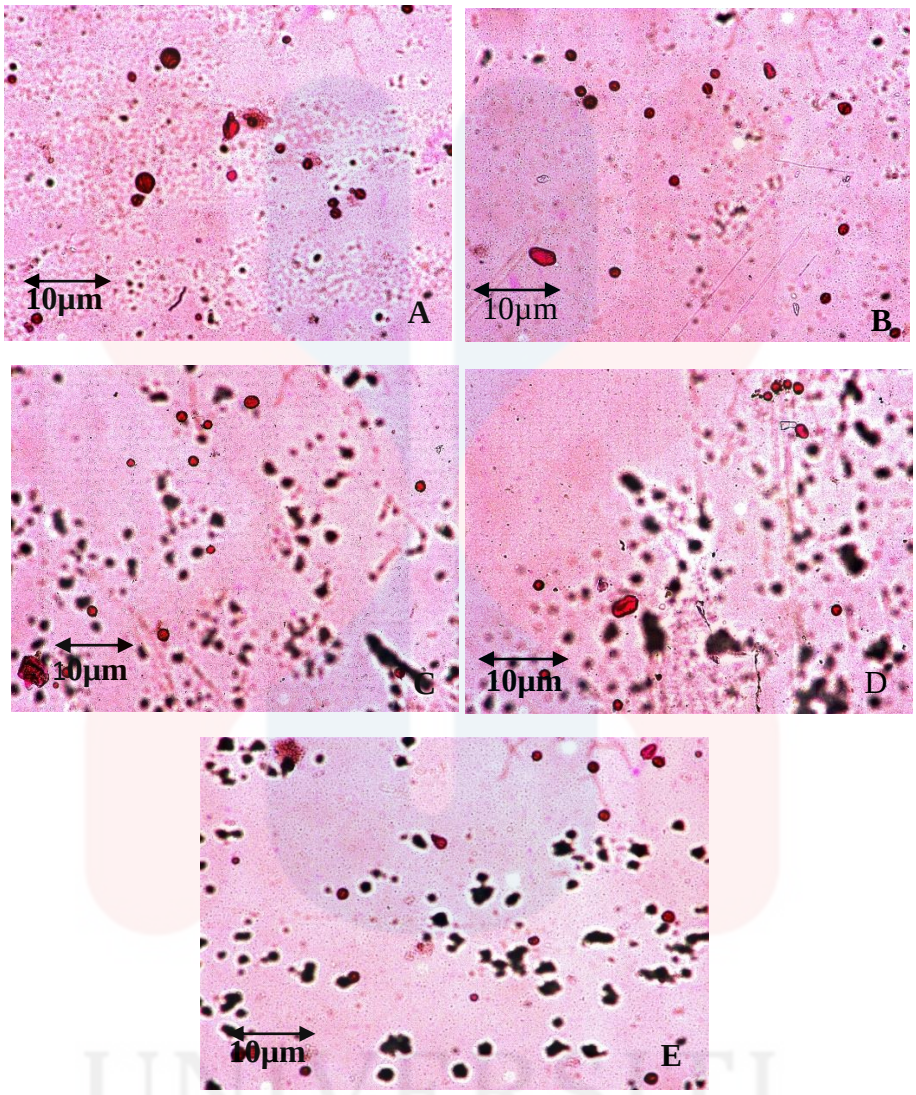

Figure 4.2 (a): Microscopic overviews of pollen density in honey sample from Hulu Langat 2 under 10x magnification. Overviews of A- Left edge of cover slip (top); B- Right edge cover slip (top); C- Left edge of cover slip (bottom); D- Right edge of cover slip (bottom) and E- center of the cover slip (middle)

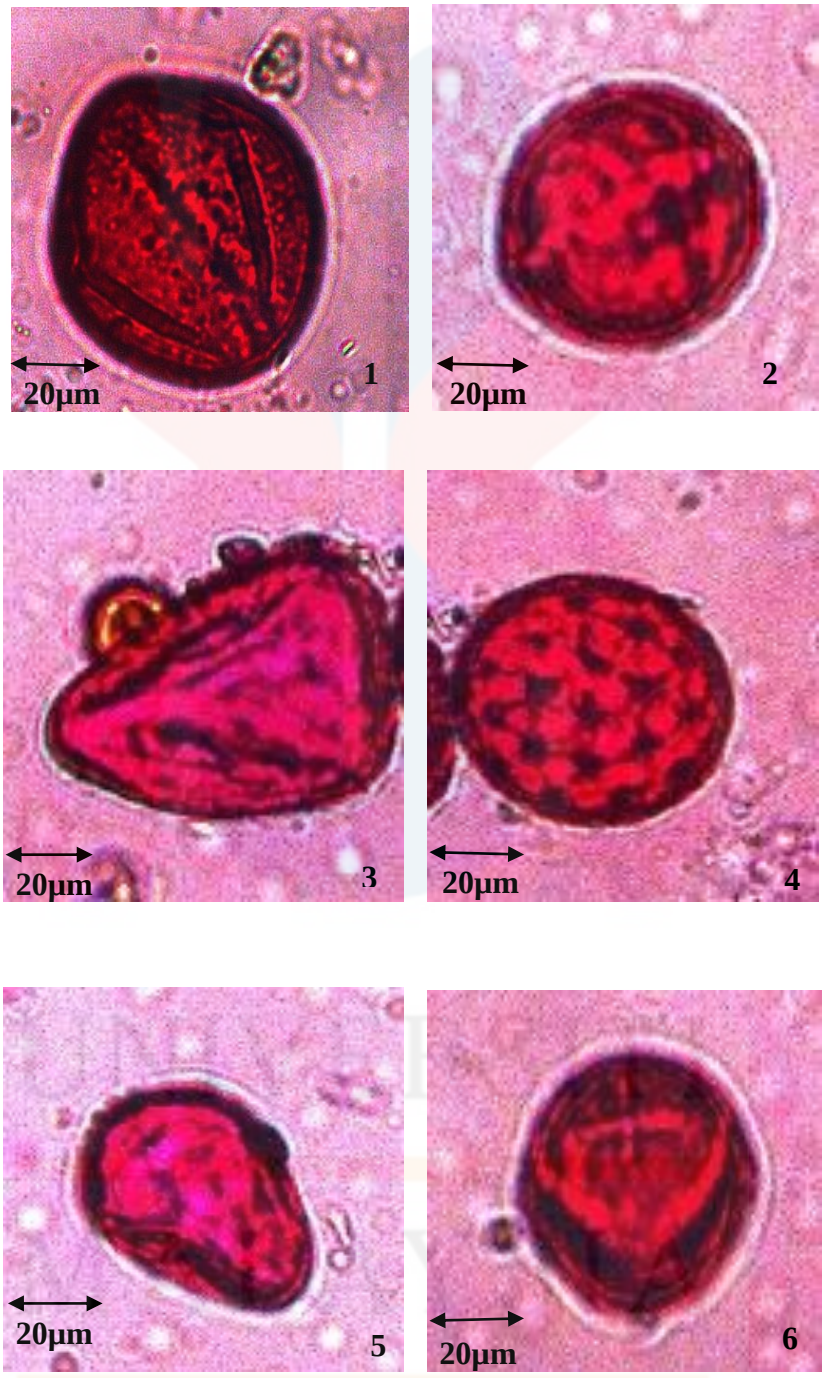

Figure 4.2 (b): Morphology of different pollen honey sample from Hulu Langat 1 under 40x magnification. 1- *Cassia fastula* (Fabaceae); 2- *Mangifera indica* (Anacardiaceae); 3- *Syzygium jambos* (Myrtaceae); 4- *Eupatorium purpureum* (Asteraceae); 5- *Callistemon linearis* (Myrtaceae) and 6- *Ricinus communis* (Euphorbiaceae)

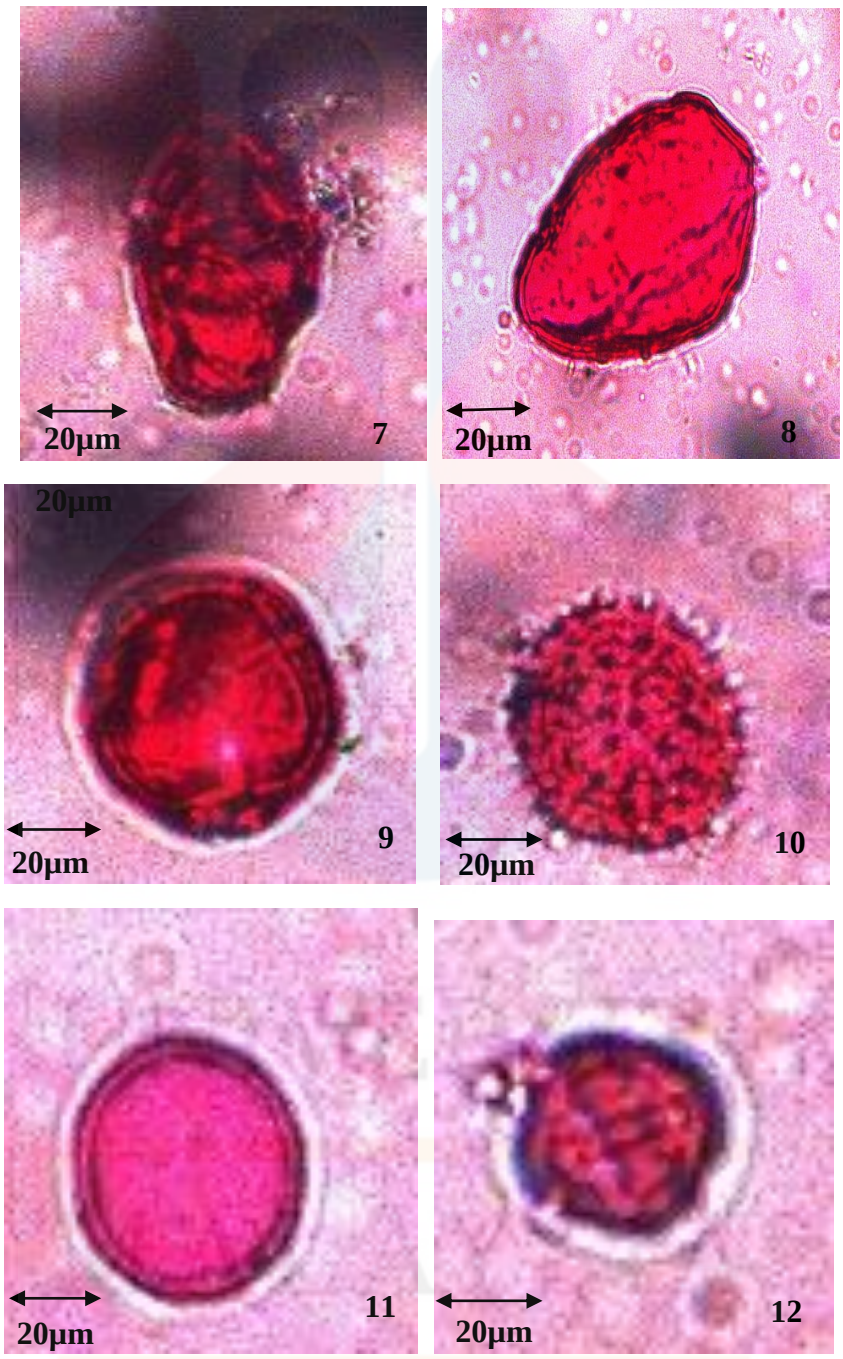

Figure 4.2 (c): Morphology of different pollen honey sample from Hulu Langat 1 under 40x magnification. 7- *Caesalpinia pulcherrima* (Fabaceae); 8- *Cocos nucifera* (Arecaceae); 9- *Melastoma sp* (Melastomaceae); 10- *Passiflora foetida* (Passifloraceae);11- *Nelumbo nucifera* (Nymphaceae) and 12- *Gliricida sepium* (Fabaceae)

Honey sample from Hulu Langat, Selangor 1 comprises of 12 different types of pollen which the most abundant pollen was from *Mangifera Indica* tree. The pollen from this tree species accounted up to 37.8% of the total pollen abundance overall. It followed by pollen grains from *Gliricida sepium* tree (17.6%), *Melastoma sp***.** (13.5%), *Cassia fistula* (8.1%) and *Ricinus communis* (6.8%). *Cocos nucifera*, *Nelumbo nucifera*, *Callistemon linearis* and *Syzygium jambos* with 2.7% each. Meanwhile, *Caesalpinia pulcherrima Passiflora foetida*, and *Eupatorium purpureum* has 1.4% pollen distribution. The extent to which a particular honey varies from other types of honey from other plant sources can be found by analysing the alteration in the pollen abundance in it (Layek et al., 2020). It can be deducted that honey sample from Hulu Langat (HL1), Selangor was multifloral. The honey was foraged by bees from nectar of a wide range of flowers and plants showed the multiflorality of the honey from this area.

Mangifera Indica was a common mango that most economically planted around the area where beehives are located. The pollen of this plant was described by (Ramírez & Davenport, 2016) that it was tricolporate, spherical in shape with pitted walls and two-celled at dispersal. *Gliricida sepium* tree species was classified as the secondary pollen based on the preference of honeybee. Secondary or accompanying pollen consist of 16-45% of pollen distribution (Abdulrahaman et al., 2013). *Melastoma sp*., *Cassia fistula*, and *Ricinus communis* were categorized as minor pollen as they got 3-15% from the overall of the pollen count. *Melastoma sp* was the scientific name of tree from Melastomaceae

family. It was 3-colporate, prolate, spheroid and radial symmetry shape. While the rest of 7 different families such as *Cocos nucifera* (Arecaceae), *Callisteman linearis* (Myrtaceae), *Sygyium jambos* (Myrtaceaeae), *Caesalpinia pulcherrima* (Fabaceae), *Passiflora foetida* ((Passifloraceae) and *Eupatorium purpureum* (Asteraceae) were identified as important minor pollen as less than 3%.

4.3 Honey sample from Hulu Langat, Selangor

Table 4.3: The number of pollen and percentage of abundance of pollen types in the honey sample from Hulu Langat (HL2)

Plant species	**Family**	**No of pollen**	**Percentage of Abundance (%)**
Pongamia pinnata	Fabaceae	4	3.8%
Cocos nucifera	Arecaceae	3	2.9%
Commelia diffusa	Commelinaceae	7	6.7%
Mimosa pundica	Fabaceae	10	9.5%
Mangifera indica	Anacardiaceae	6	5.7%
Nelumbo nucifera	Nelumbonaceae	4	3.8%
Cassia fistula	Fabaceae	71	67.6%

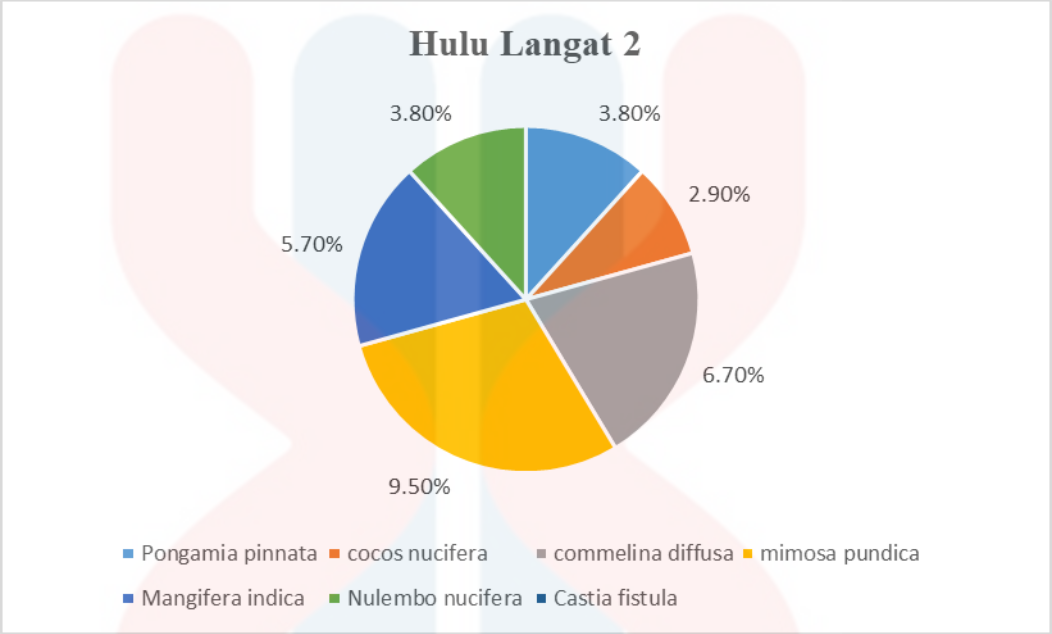

Figure 4.3: Pollen spectrum of honey sample from Hulu Langat, Selangor

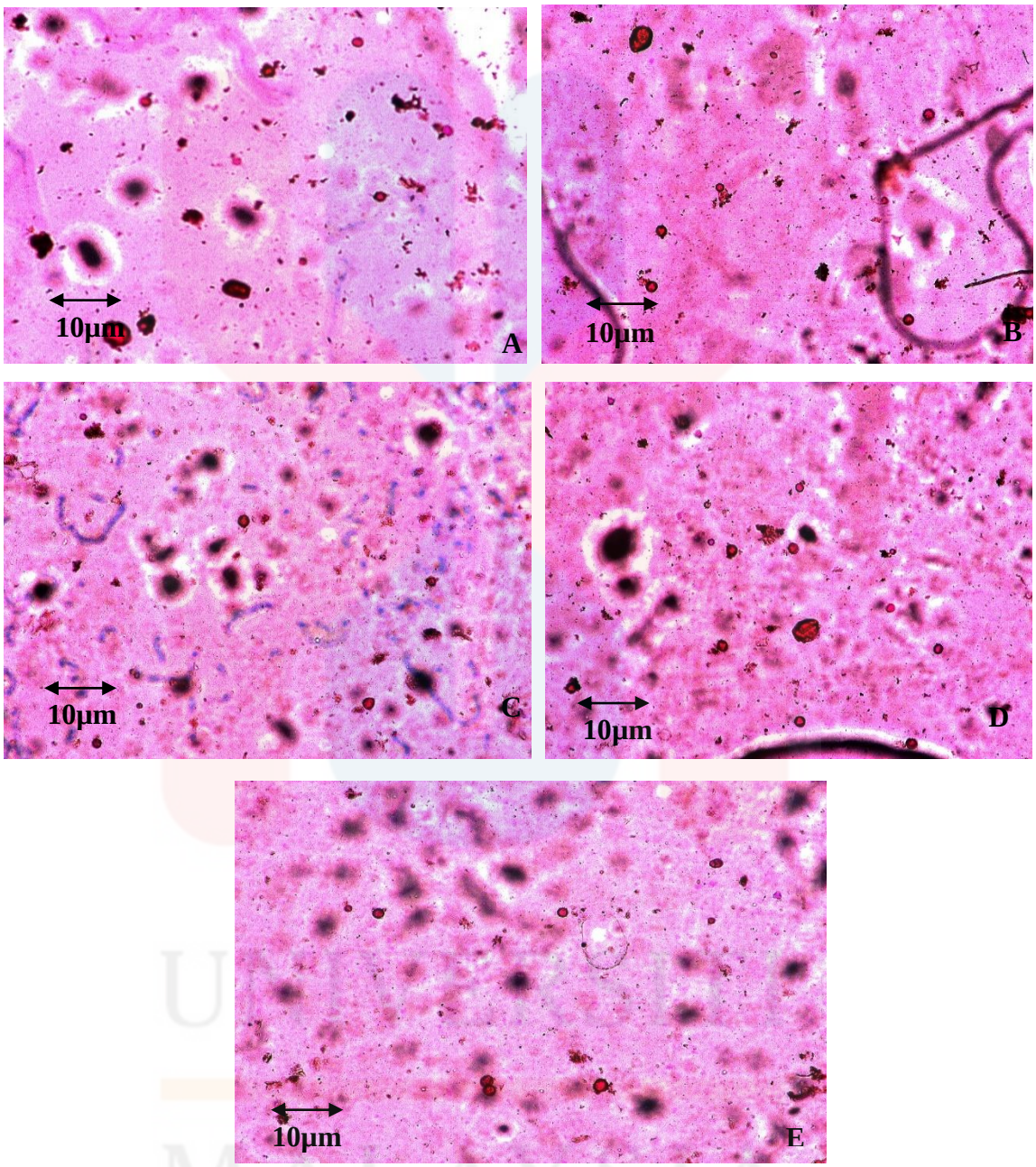

Figure 4.3 (a): Microscopic overviews of pollen density in honey sample from Hulu Langat 2 under 10x magnification. Overviews of A- Left edge of cover slip (top); B- Right edge cover slip (top); C- Left edge of cover slip (bottom); D- Right edge of cover slip (bottom) and E- center of the cover slip (middle)

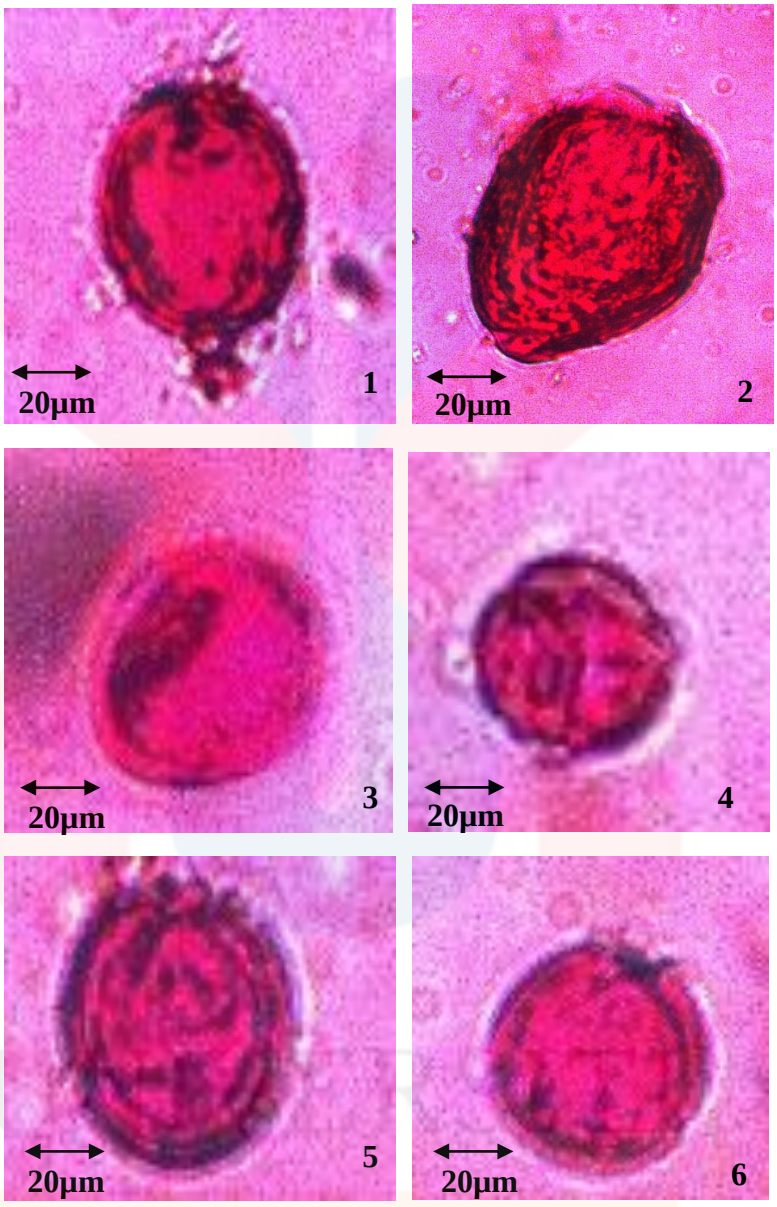

Figure 4.3 (b): Morphology of different pollen honey Hulu Langat 2 under 40x magnification.1- *Pongamia pinnata* (Fabaceae); 2- *Cocos nucifera* (Arecaeae); 3- *Commelia diffusa* (Commelinaceae); 4- *Mimosa pundica* (Fabaceae); 5- *Mangifera indica* (Anacardiaceae) and 6- *Nelumbo nucifera* (Nelumbonaceae)

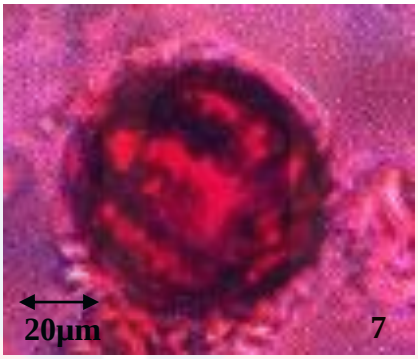

Figure 4.3 (c): Morphology of different pollen honey sample from Hulu Langat 2 under 40x magnification. 7- *Cassia fistula* (Fabaceae)

From the honey samples collected in Hulu Langat 2, there were 105 pollen grains found in 1 slide under the compound microscope with camera. 7 different types of pollen species were present in the sample in table 4.3 such as *Pongamia pinnata* (Acanthaceae), *Cocos nucifera* (Arecaceae), *Commelia diffusa* (Commelinaceae), *Mimosa pundica* (Fabaceae), *Mangifera indica* (Anacardiaceae), *Nelumbo nucifera* (Nelumbonaceae) and *Cassia fistula* (Fabaceae). *Cassia fistula* got the highest percentage abundance of pollen in this sample which was 67.6%. This pollen grain constituting more than 45% of the total pollen count which made it predominant plant species in the area. Therefore, this honey sample is unifloral honey. The pollen made up colporate, prolate, prolatespheroid, punctitegillate and bilateral symmetry morphology. *Cassia fistula* commonly named golden shower tree. It was native to India, Malaysia and Southeast Asia. Hence, this types of pollen were favourable for foraging among the honeybees. There was no secondary pollen has been

observed but there were 6 important minor pollen were identified. Thus, there was only 1 pollen species had been identified as minor pollen which was *Cocos nucifera*. *Cocos nucifera* or coconut tree was a member of the palm tree family (Arecaceae) and the merely living species of the genus Cocos. It was widely planted throughout tropics include Malaysia. The coconut is known for its great adaptability as seen in the many domestic, commercial, and industrial uses of its different parts and it played as part of daily diet of people (Victor, 2013).

4.4 *Honey sample Petaling Jaya, Selangor*

Table 4.4: The number of pollen and percentage of abundance of pollen types in the honey sample from Petaling Jaya (PJ1)

Plant species	**Family**	**No of pollen**	**Percentage of Abundance (%)**
Rosa canina	Rosaceae	14	10.4%
Cassia fistula	Fabaceae	5	3.7%
Ixora coccinea	Rubiaceae	3	2.2%
Nelumbo nucifera	Nelumbonaceae	38	28.4%
Eupatorium purpureum	Asteraceae	8	6.0%
Mangifera indica	Anacardiaceae	10	7.5%
Syzygium jambos	Myrtaceae	3	2.2%
Papaver somniferum	Papaveraceae	1	0.7%
Mimosa pudica	Fabaceae	2	1.5%
Solanum melongena	Solanaceae	47	35.1%
Caesalpinia pulcherrima	Fabaceae	3	2.2%

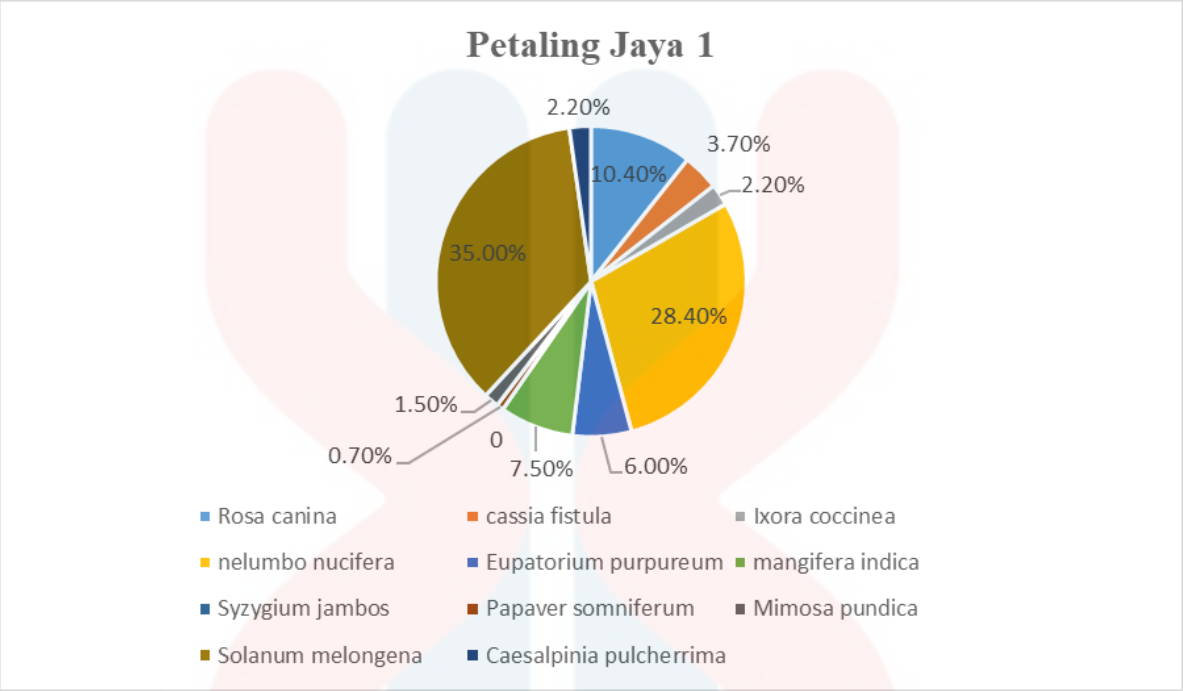

Figure 4.4: Pollen spectrum of honey sample from Petaling Jaya, Selangor

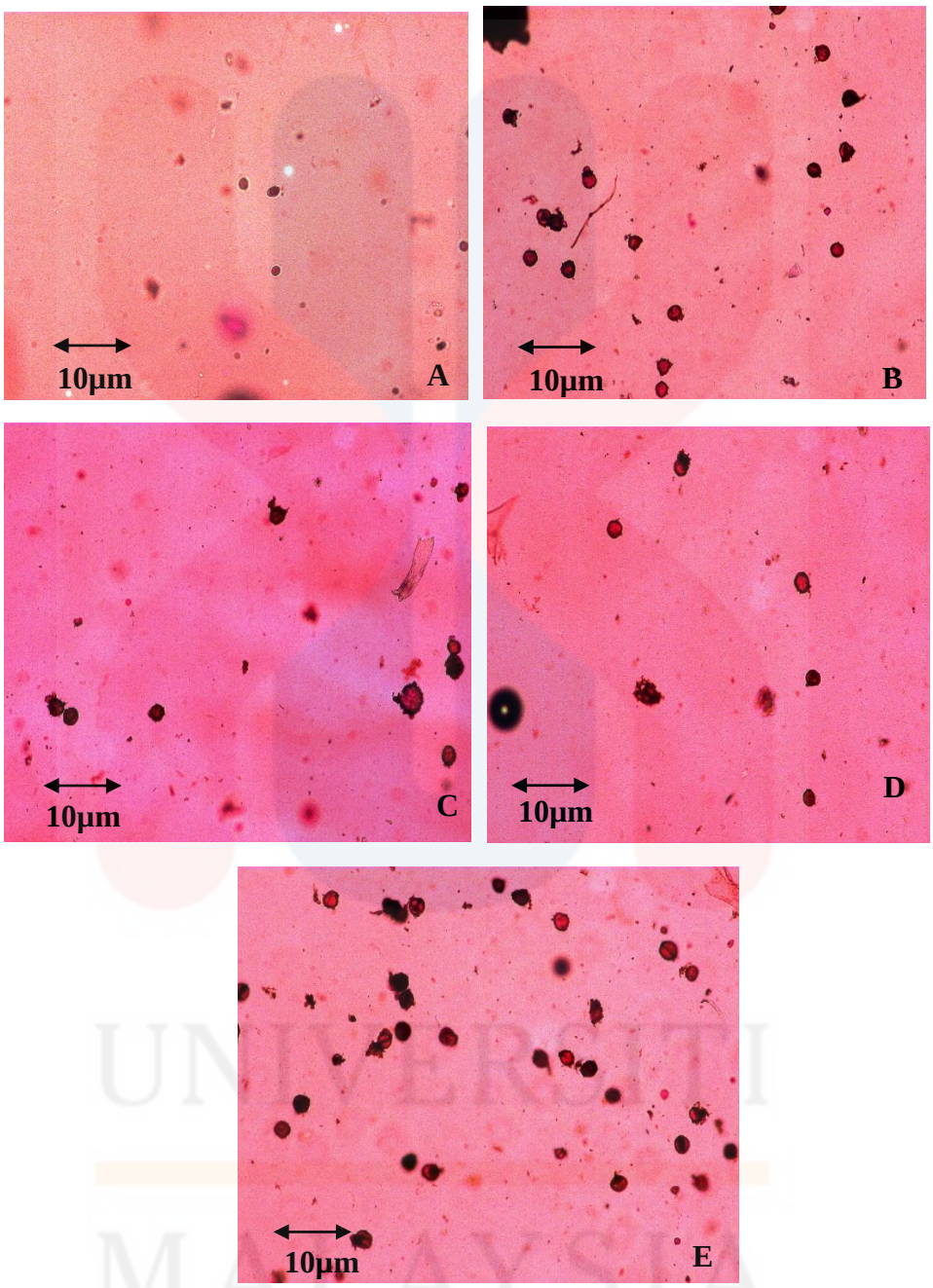

Figure 4.4 (a): Microscopic overviews of pollen density in honey sample from Petaling Jaya 1 under 10x magnification. Overviews of A- Left edge of cover slip (top); B- Right edge of cover slip (top); C- Left edge of cover slip (bottom); D- Right edge of cover slip (bottom) and E- center of the cover slip (middle)

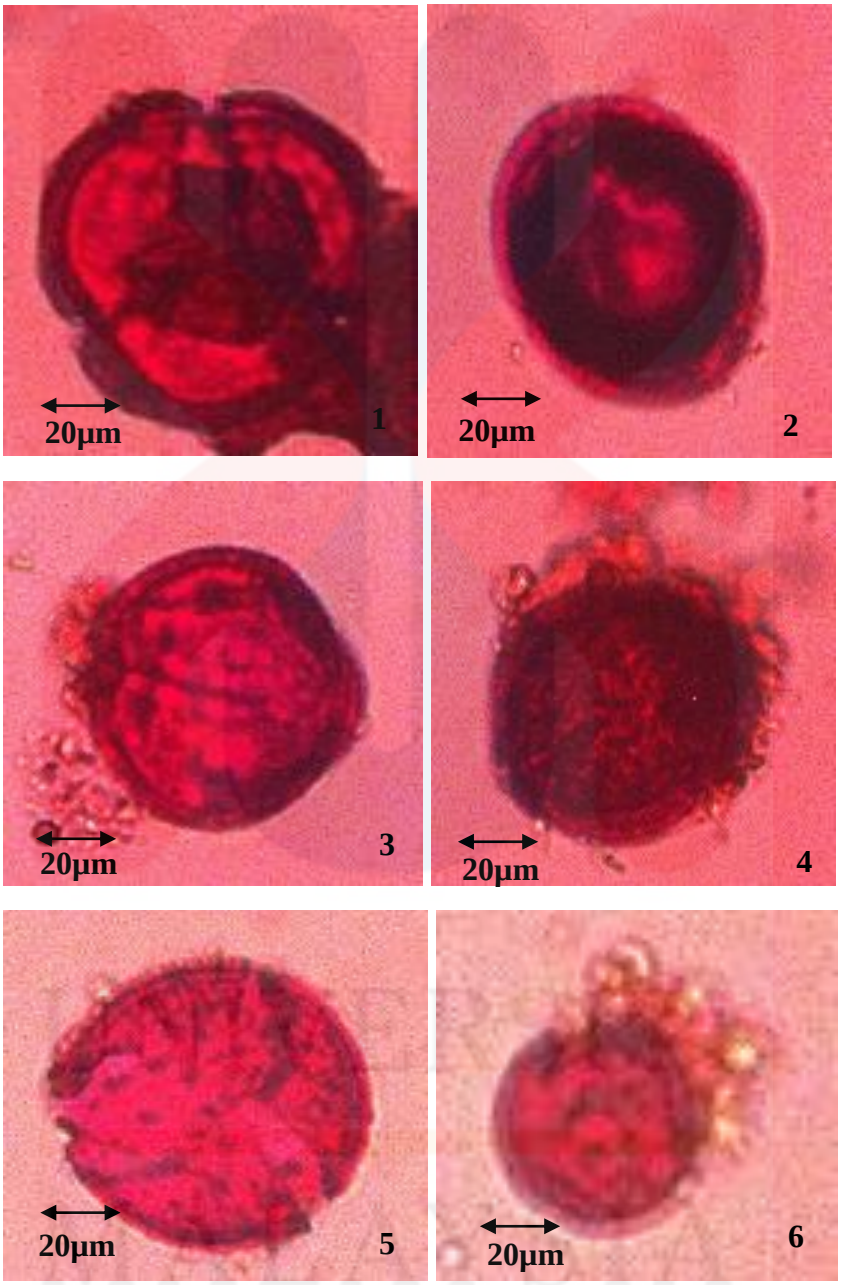

Figure 4.3 (b): Morphology of different pollen honey sample from Petaling Jaya 1 under 40x magnification. 1- *Rosa canina* (Rosaceae); 2- *cassia fistula* (Fabaceae); 3- *Ixora coccinea* (Rubiaceae); 4-*Nelumbo nucifera* (Nelumbonaceae); 5- *Eupatorium purpureum* (Asteraceae) and 6- *Mangifera indica* (Anacardiaceae)

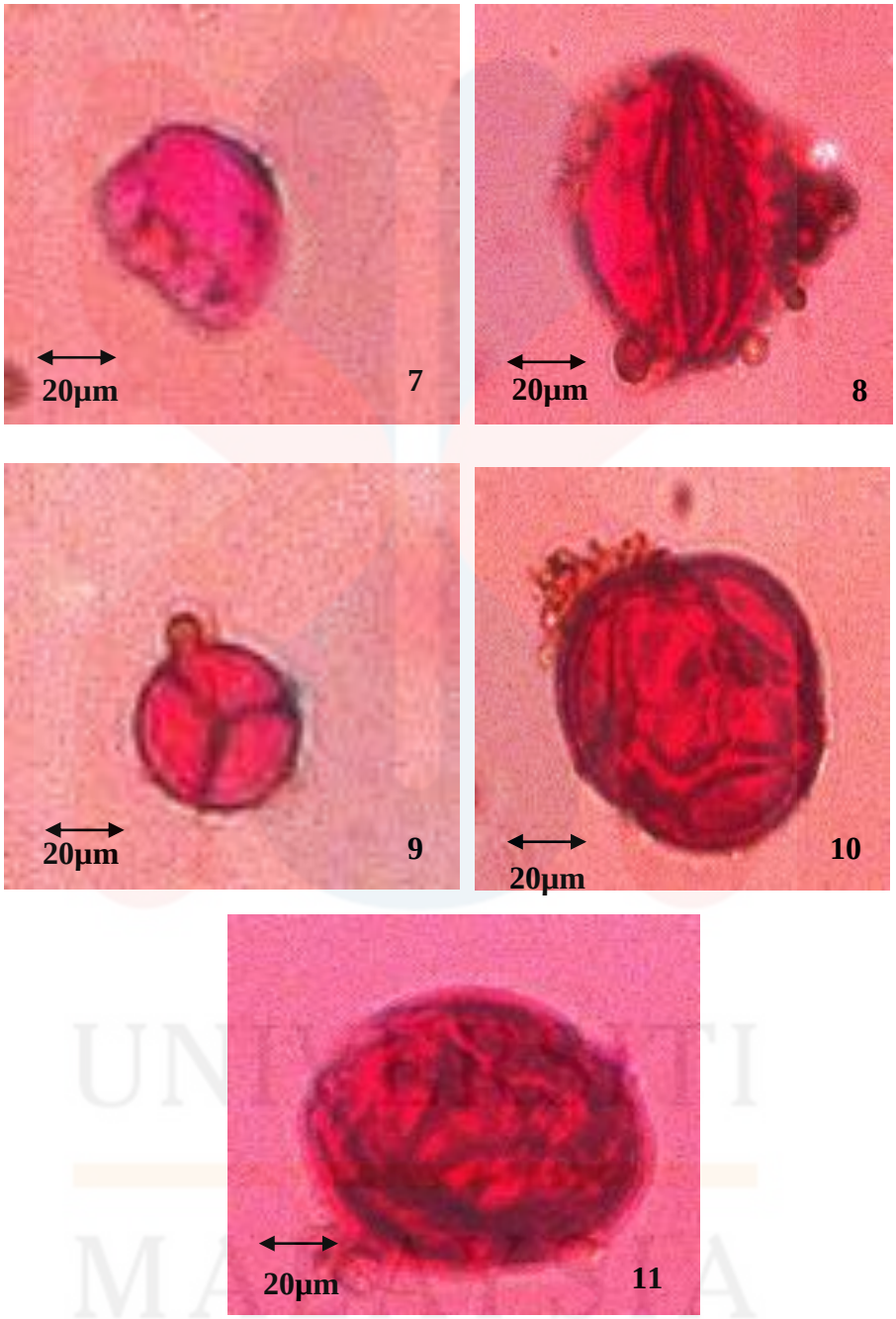

Figure 4.3 (c): Morphology of different pollen honey sample from Petaling Jaya 1 under 40x magnification. 7- *Syzygium jambos* (Myrtaceae); 8- *Papaver somniferum* (Papaveraceae); *Mimosa pudica* (Fabaceae); 10- *Solanum melongena* (Solanaceae) and 11- *Caesalpinia pulcherrima* (Fabaceae)

From the honey sample collected at Petaling Jaya 1, table 4.4 shown 11 different types of pollen species were discovered. Among the species were *Rosa canina* (Rosaceae), *Cassia fistula* (Fabaceae), *Ixora coccinea* (Rubiaceae), *Nelumbo nucifera* (Nelumbonaceae), *Eupatorium purpureum* (Asteraceae), *Mangifera indica* (Anacardiaceae), *Syzygium jambos* (Myrtaceae), *Papaver somniferum* (Papaveraceae), *Mimosa pudica* (Fabaceae), *Solanum melongena* (Solanaceae) and *Caesalpinia pulcherrima* (Fabaceae). No predominant pollen had been present in the sample. The most abundant pollen which had a percentage of 35.1% was *Solanum melongena* and it has been classified as secondary pollen. Solanum melongena was the scientific name for eggplant which belongs to the Solanaceae family. It was a popular and staple vegetable crop in Asia include Southeast Asian country. It was widely grown in Malaysia. It has Prolate, sub-spheroid, punctitegillate, furrows long, exinepsilate and thin bilateral symmetry in morphology (Abdulrahaman et al., 2013).

Futhermore, there was another secondary species of pollen had been identified such as *Nelumbo nucifera* followed by the rest which were 4 important minor pollen species came from Rosaceae (10.4%), Asteraceae (6.0%), Anacardiaceae (7.5%) and Fabaceae (3.7%). Minor pollen such as Myrtaceae (2.2%), Rubiaceae (2.2%), and Fabaceae (1.5%) (2.2%) of different each plant species and Papaveraceae (0.7%) as the lowest percentage of abundancy for overall of this sample. This pollen grain was classified as multifloral as they did not have any predominant taxa in them and they had different proportions of pollen taxon distribution.

Local floral dominates distinctive of plant associations which reveal a specific corresponding spectrum in pollen types being characterized in honey. The existence of floral pollen grains within honey assists the identification of the nectariferous plants that a honey sample was sourced from (De et al., 2012).

4.5 *Honey sample Bangi, Selangor*

Table 4.5: The number of pollen and percentage of abundance of pollen types in the honey sample from Bangi (BG1)

Plant species	**Family**	**No of pollen**	**Percentage of Abundance (%)**
Solanum melongena	Solanaceae	27	24.1%
Psidium guajava	Myrtaceae	3	2.7%
Borassus flabellifer	Arecaceae	1	0.9%
Ixora coccinea	Rubiaceae	28	25.0%
Nelumbo nucifera	Nymphaeceae	19	17.0%
Tridax procumbens	Asteraceae	2	1.8%
Ruta graveolens	Rutaceae	30	26.8%
Croton sp.	Euphorbiaceae	1	0.9%
Acacia sp	Fabaceae	1	0.9%

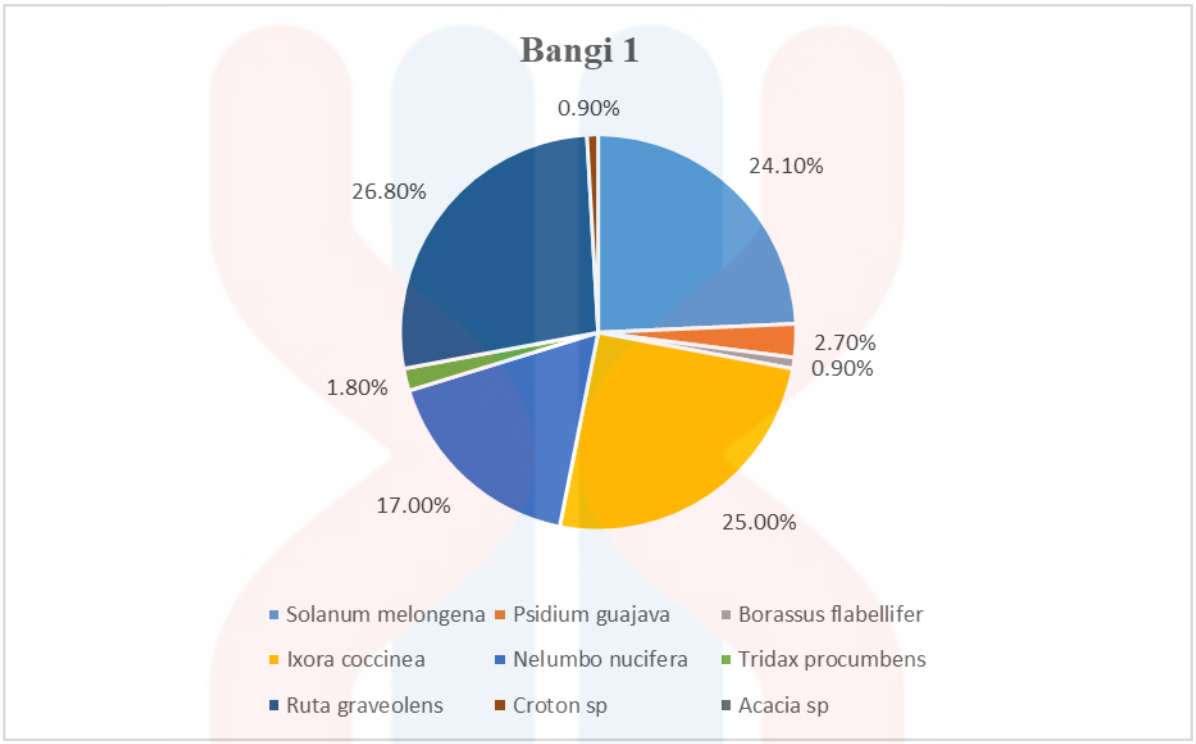

Figure 4.5: Pollen spectrum of honey sample from Bangi, Selangor

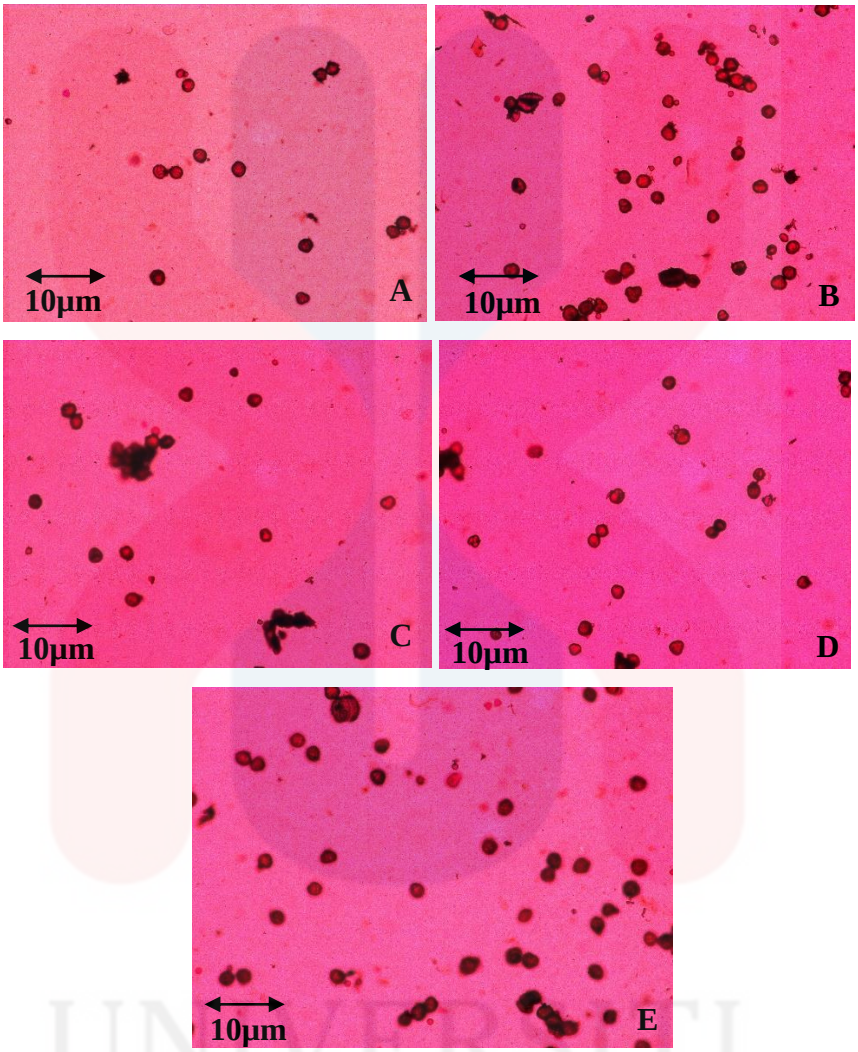

Figure 4.5 (a): Microscopic overviews of pollen density in honey sample from Bangi 1 under 10x magnification. Overviews of A- Left edge of cover slip (top); B- Right edge of cover slip (top); C- Left edge of cover slip (bottom); D- Right edge of cover slip (bottom) and E- centre of the cover slip (middle)

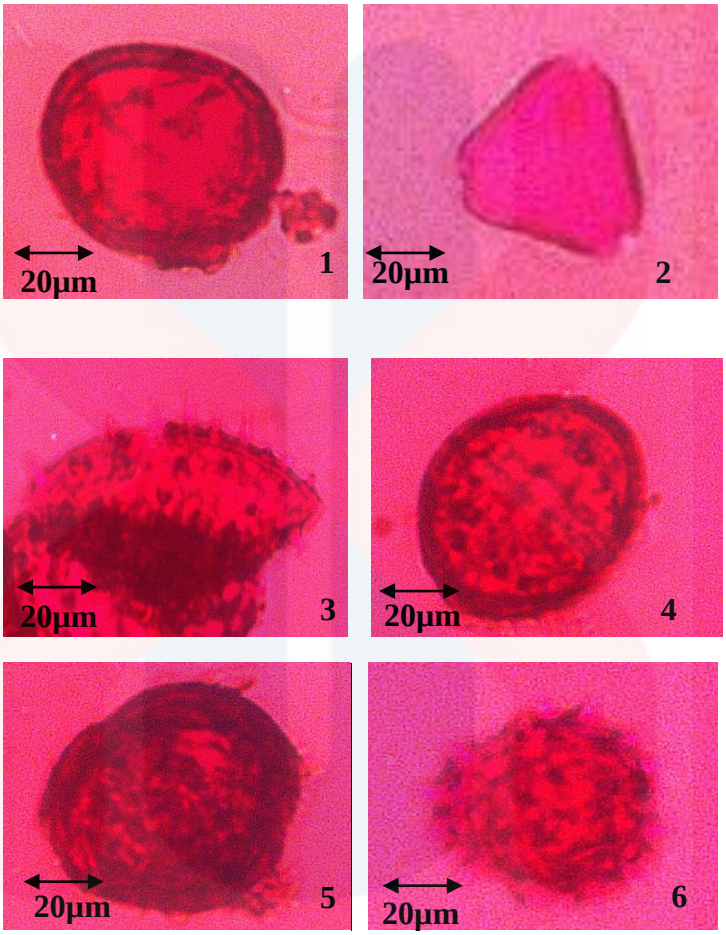

Figure 4.5 (b): Morphology of different pollen honey sample from Bangi 1 under 40x magnification. 1- *Solanum melongena* (Solanaceae); 2- *Psidium guajava* (Myrtaceae); 3- *Borassus flabellifer* (Arecaceae); 4- *Ixora coccinea* (Rubiaceae) and 5- *Nelumbo nucifera* (Nymphaeaceae) and 6 - *Tridax procumbens* (Asteraceae)

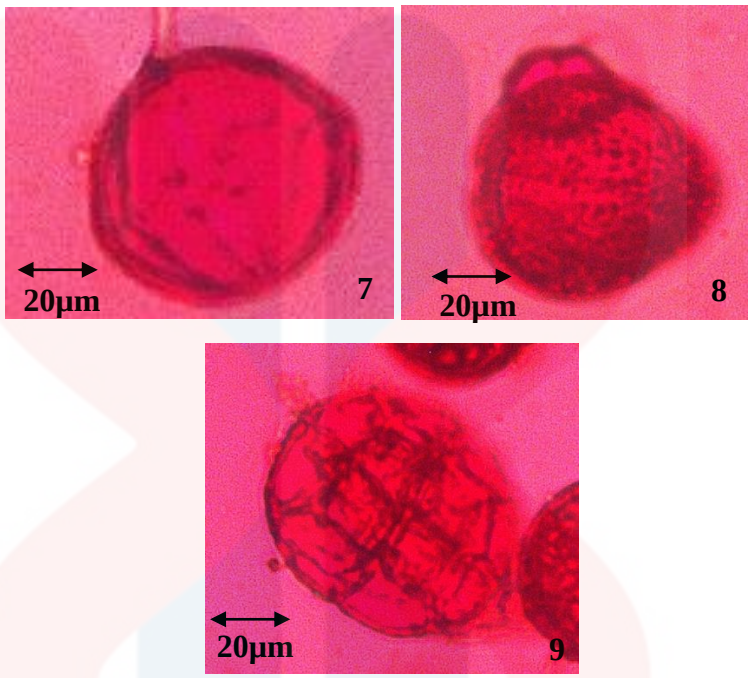

Figure 4.5 (c): Morphology of different pollen honey sample from Bangi 1 under 40x magnification. 7- *Ruta graveolens* (Rutaceae); 8- *Croton sp*. (Euphorbiaceae) and 9- *Acacia sp* (Fabaceae)

There were 9 different types of pollen identified from the honey collected in Bangi 1 as stated in table 4.5. The most abundance plant species was *Ruta graveolens* from Rutaceae with percentage abundance 26.8%. *Ruta graveolens* commonly known as rue was a species of Ruta from Rutaceae family. It was a popular ornamental plant act as a medicinal plant widely used in the Mediterranean region to treat pain, dermatitis and other inflammatory disease. Moreover, this plant has widely cultivated in Malaysia and favorable among the plant enthusiast due to the medicinal properties and its morphology Colporoidate, exinepsilate, and bilateral symmetry especially for its bluish leaves which suitable for landscaping. This clearly proved that honeybees preferred to forage this plant due to the tolerance of hot and dry soil.

Solanum melongena (24.1%), *Ixora coccinea* (25.0%) and *Nelumbo nucifera* (17.0%) can be classified as secondary pollen. The minor or isolated pollen which less than 3% were *Psidium guajava* (2.7%), *Tridax procumbens* (1.8%), *Croton sp* and *Acacia sp* with 0.9% respectively. The least amount of pollen had been found in this sample were came from Euphorbiaceae and Fabaceae family. Based on the result shown, it was understandable that honey bees in Bangi region more preferred to forage flowers from the species and families mentioned above compared to others. Honey collected in Bangi 1 was categorized as multifloral as these samples had different proportions of pollen taxon distribution and none of any samples reached the requirement of being predominant taxa.

Microscopic analysis of the pollen matters of seasonal honeys and pollen loads augmented with phenology and floral biological study deliver reliable information of floral types which serve as major or minor nectar of pollen sources for the bees (Azmi et al., 2015).

4.6 *Honey sample Hulu Langat, Selangor*

Table 4.6: The number of pollen and percentage of abundance of pollen types in the honey sample from Hulu Langat (HL3)

Plant species	**Family**	**No of pollen**	**Percentage of Abundance (%)**
Cocos nucifera	Arecacea	13	27 0%
commelina diffusa	Commelinaceae	4	8.3%
Mangifera Indica	Anacardiaceae	8	16.6%
Antigonon leptopus	Polygonaceae	1	2.1%
Melastoma sp.	Melastomataceae	22	45.8%
Clerodendrum inerme	Lamiaceae	1	2.1%

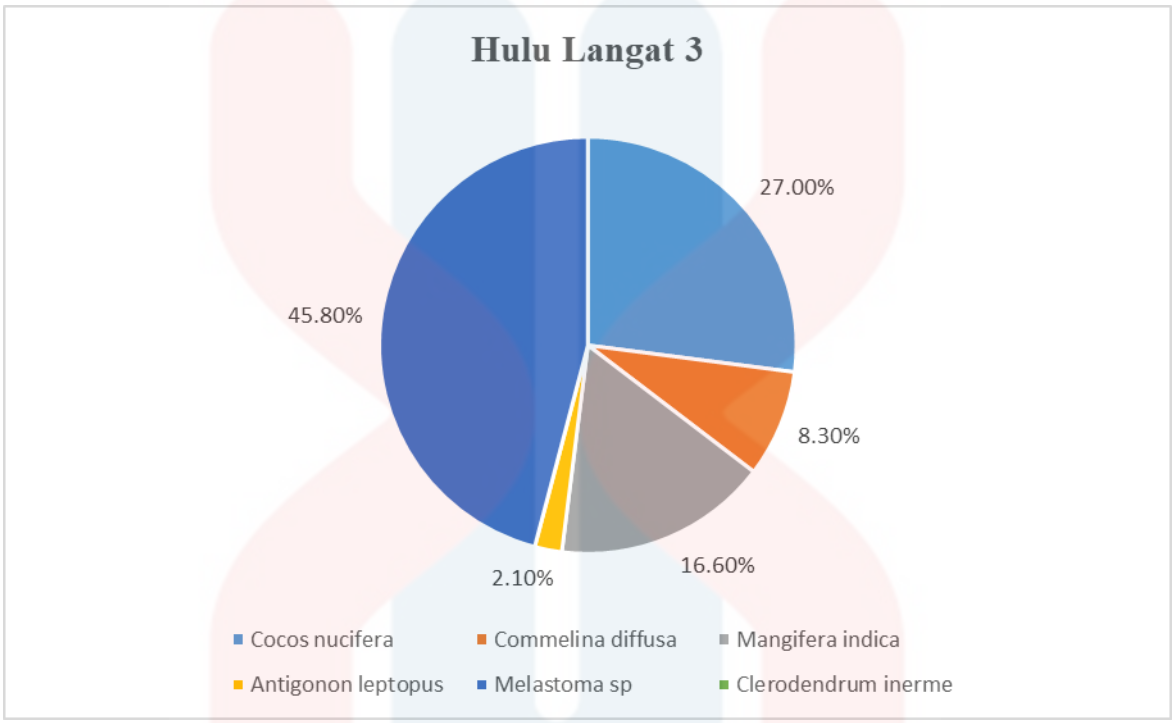

Figure 4.6: Pollen spectrum of honey sample from Hulu langat, Selangor

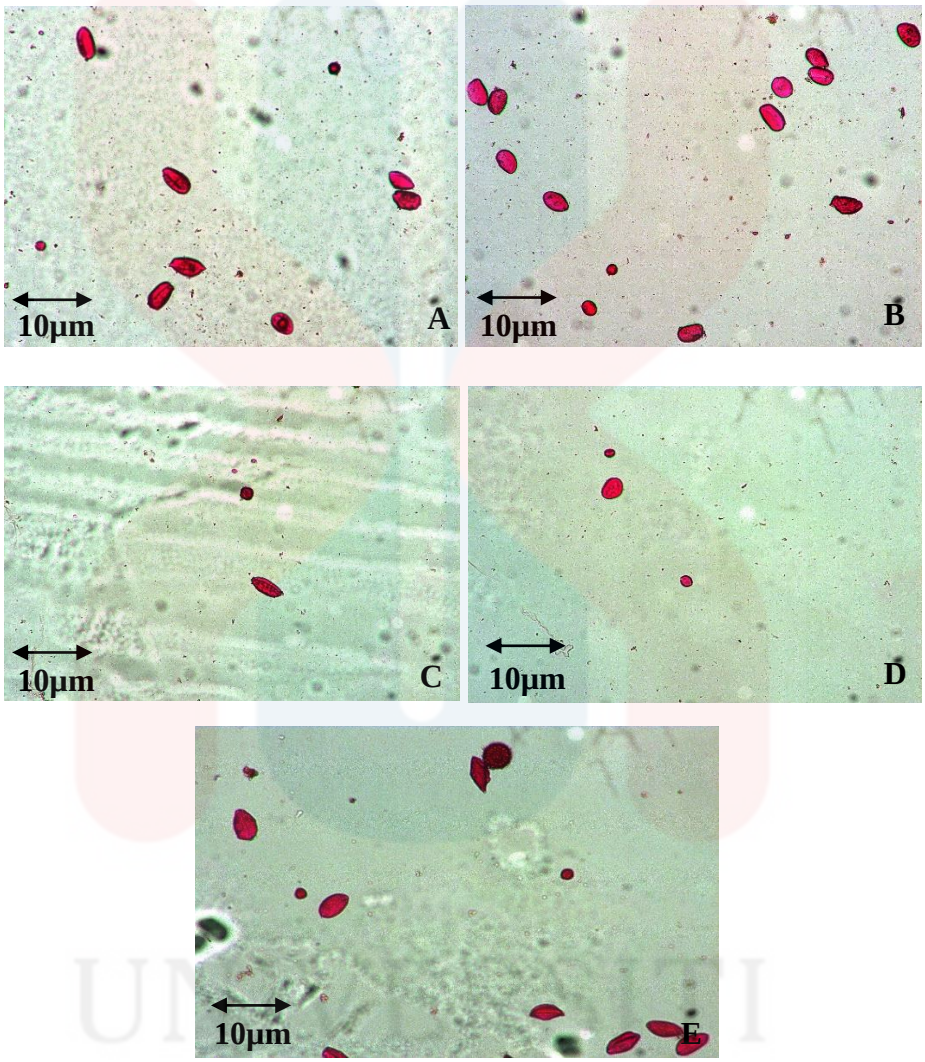

Figure 4.6 (a): Microscopic overviews of pollen density in honey sample from Hulu Langat 3 under 10x magnification. Overviews of A- Left edge of cover slip (top); B- Right edge of cover slip (top); C- Left edge of cover slip (bottom); D- Right edge of cover slip (bottom) and E- center of the cover slip (middle)

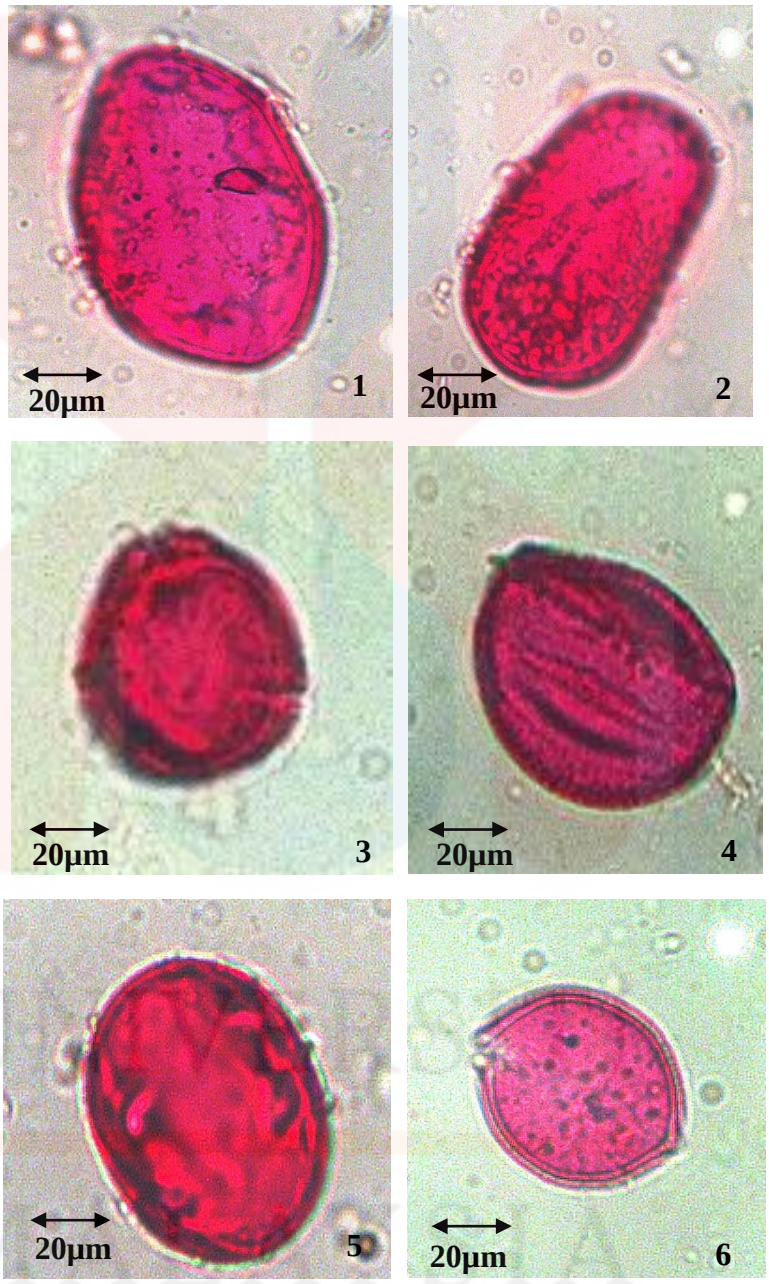

Figure 4.6 (b): Morphology of different pollen honey sample from Hulu Langat 3 under 40x magnification. 1- *Cocos nucifera* (Arecaceae); 2- *Commelina diffusa* (Commelinacaea);3- *Mangifera indica* (Anacardiaceae); 4- *Antigonon leptopus* (Polygonaceae) ;5- *Melastoma sp*. (Melastomataceae) and 6- *Clerodendrum inerme* (Lamiaceae)

Analysis on the honey samples collected from Hulu Langat 3 depicted the presence of 6 different types of pollen. Predominant taxon of pollen in this sample was *Melastoma sp* (Melastomataceae) followed by secondary pollen, *Cocos nucifera* (Arecaceae) up to 27.0% and *Mangifera indica* (Anacardiaceae), 16.6% pollen count and also other pollen occurred in this sample was important minor pollen, *Commelina diffusa* (Commelinaceae) and minor pollen, *Antigonon leptopus* (Polygonaceae) and *Clerodendrum inerme* (Lamiaceae) with 2.1% respectively.

Melastoma sp or in Malay term called 'Senduduk' had 3-colporate, prolate, Spheroid and radial symmetry in morphology. It is a native species found in secondary forest and forest fringes. The presence of the most abundant species pointed out that bees at Hulu Langat regions had more preference to forage flowers from such species as the Melastoma species in Peninsular Malayasia were very variable. Thus, *Cocos nucifera* and *Mangifera Indica* were identified as accompanying pollen where they were generally can be found around the beehive located. Stingless bees were known to be vital pollinator in tropical rainforest and also good candidates for supplying pollination amenities in agricultural ecosystem such as starfruits, mango, durian, watermelon, guava and coconut (Azmi et al., 2015).

This sample of honey was a unifloral honey. Besides, staining of the pollen had been successful using Glycerin jelly giving the pollen pinkish color under compound microscope with camera. Images of a few pollen did not appear so visible under the microscope observation due to the presence of debris and dirt on the microscopic slides.

4.7 *Honey sample Petaling Jaya, Selangor*

Table 4.7: The number of pollen and percentage of abundance of pollen types in the honey sample from Petaling Jaya (PJ2)

Plant species	**Family**	**No of pollen**	**Percentage of Abundance (%)**
Croton sp.	Euphorbiaceae	1	2.8%
Punica granatum	Punicaceae	1	2.8%
Ruta graveolens	Rutaceae	7	20.0%
Moringa oleifera	Moringaceae	2	5.7%
Cassia fistula	Myrtaceae	24	68.6%

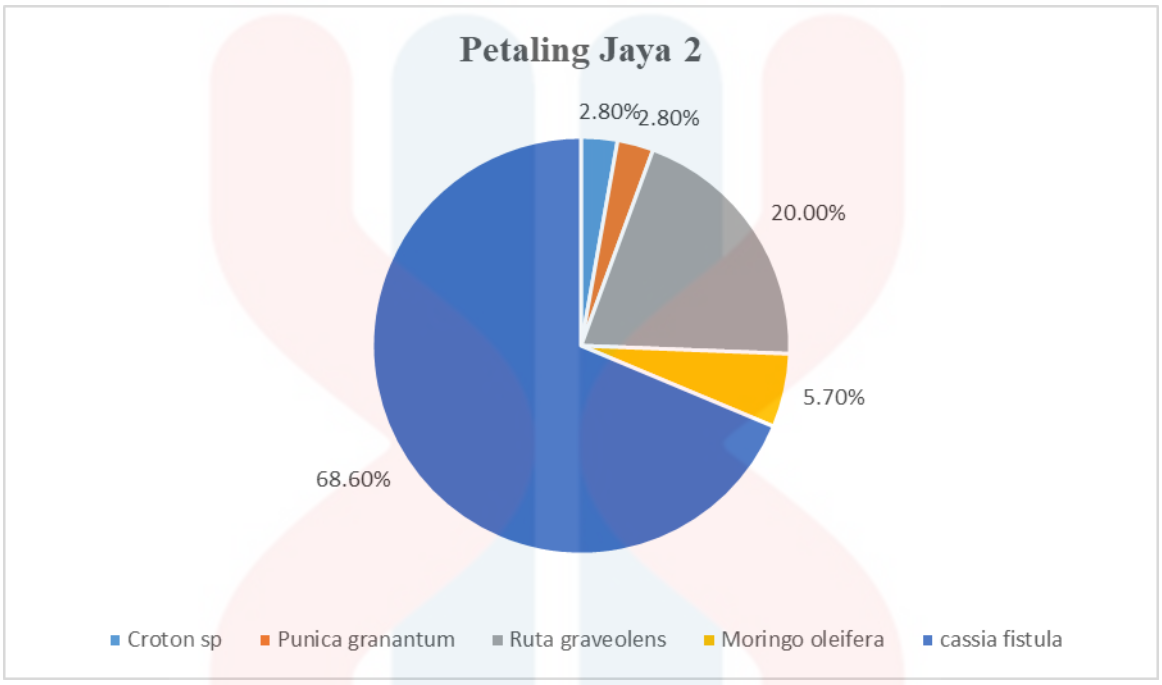

Figure 4.7: Pollen spectrum of honey sample from Petaling Jaya, Selangor

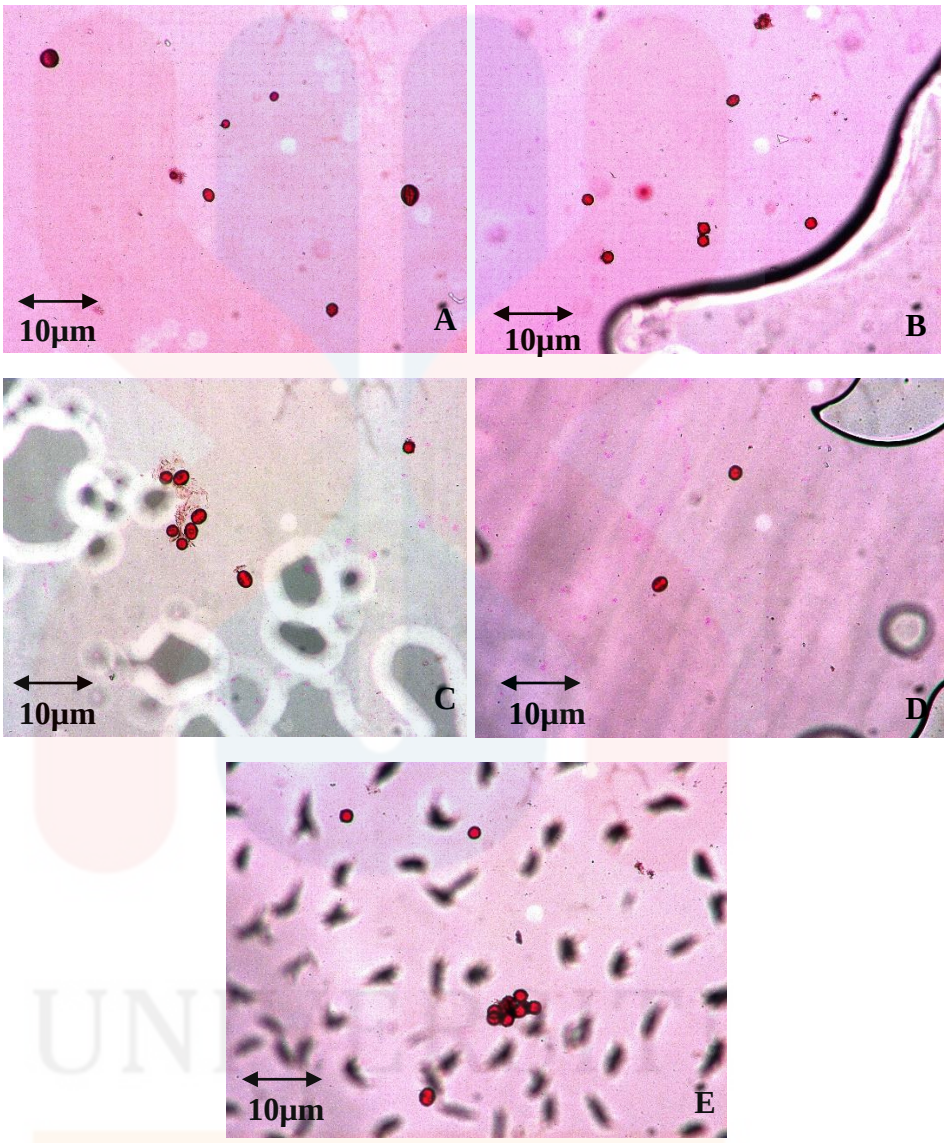

Figure 4.7 (a): Microscopic overviews of pollen density in honey sample from Petaling Jaya 2 under 10x magnification. Overviews of A- Left edge of cover slip (top); B- Right edge of cover slip (top); C- Left edge of cover slip (bottom); D- Right edge of cover slip (bottom) and E- center of the cover slip (middle)

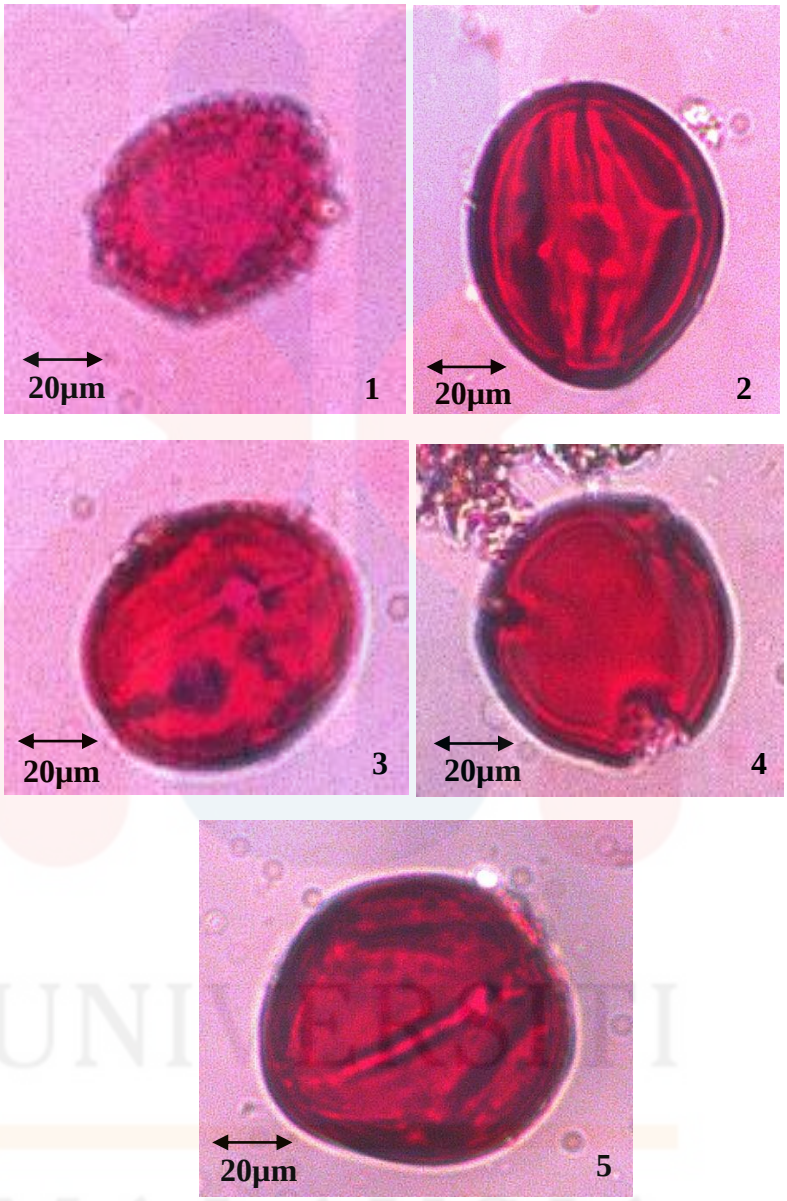

Figure 4.7 (b): Morphology of different pollen honey sample from Petaling Jaya 2 under 40x magnification. 1- *Croton sp.* (Euphorbiaceae); 2- *Punica granatum* (Punicaceae); 3- *Ruta graveolens* (Rutaceae); 4- *Moringa oleifera* (Moringaceae) and 5- *Cassia fistula* (Myrtacaeae)

Analysis on the honey samples from Petaling Jaya 2 based on the table 4.7 showed the occurrence of 5 different types of pollen, namely *Croton sp* (Euphorbiaceae), *Punica granatum* (Punicaceae), *Ruta graveolens* (Rutaceae), *Moringo oleifera* (Moringaceae) and *Cassia fistula* (Myrtaceae). The predominant pollen species collected in Petaling Jaya 2 was *Cassia fistula* with 68.6% of abundancy. In this sample only *Ruta graveolens* from Rutaceae family was categorised under the secondary pollen group due to its percentage abundance (16-45%) while, *Moringa oleifera* was categorized as important minor pollen pollen because of their pollen count were range from 3-16% and the rest of plant species the minor pollen which is < 3% were *Croton sp* and *Punica granatum.*

The honey sample was noticeable as unifloral honey as the most abundance pollen from plant species Cassia fistula or in Malay term called 'Dulang' from Fabaceae family. This plant was prevalently known as 'yellow shower tree' due to its exuberant racemose pending inflorescences (Saab et al., 2021). The presence of the most plentiful species proved that the plant species preferred by bees. Thus, plants with pollen flowers increased pollinator attraction generates a trade- off with the amount of pollen destined as food for. Pollen flowers was commonly pollinated by bees and propose a surplus of pollen as a special reward to pollinators instead of nectar and other floral resources bees (Saab et al., 2021). Moreover, pollen grains of *Croton sp* and *Punica granatum* were considered extremely poor in the honey sample collected in Petaling Jaya 2 based on the pollen grain frequency.

4.8 *Honey sample from Petaling Jaya, Selangor*

Table 4.8: The number of pollen and percentage of abundance of pollen types in the honey sample from Petaling Jaya (PJ3)

Plant species	**Family**	**No of pollen**	**Percentage of Abundance (%)**
Gliricida sepium	Fabaceae	8	17.8%
Ricinus communis	Euphorbiaaceae	14	31.1%
Coffea sp.	Rubiaceae	5	11.1%
Eucalyptus globus	Myrtaceae	2	4.4%
Solanum melongena	Solanaceae	8	17.8%
Taraxacum mongolicum	Asteraceae	1	2.2%
Sygyium jambos	Myrtaceae	1	2.2%
Acacia sp	Fabaceae	3	6.7%
Nelumbo nucifera	Nelumbonaceae	2	4.4%
Croton sp	Euphorbiaceae	1	2.2%

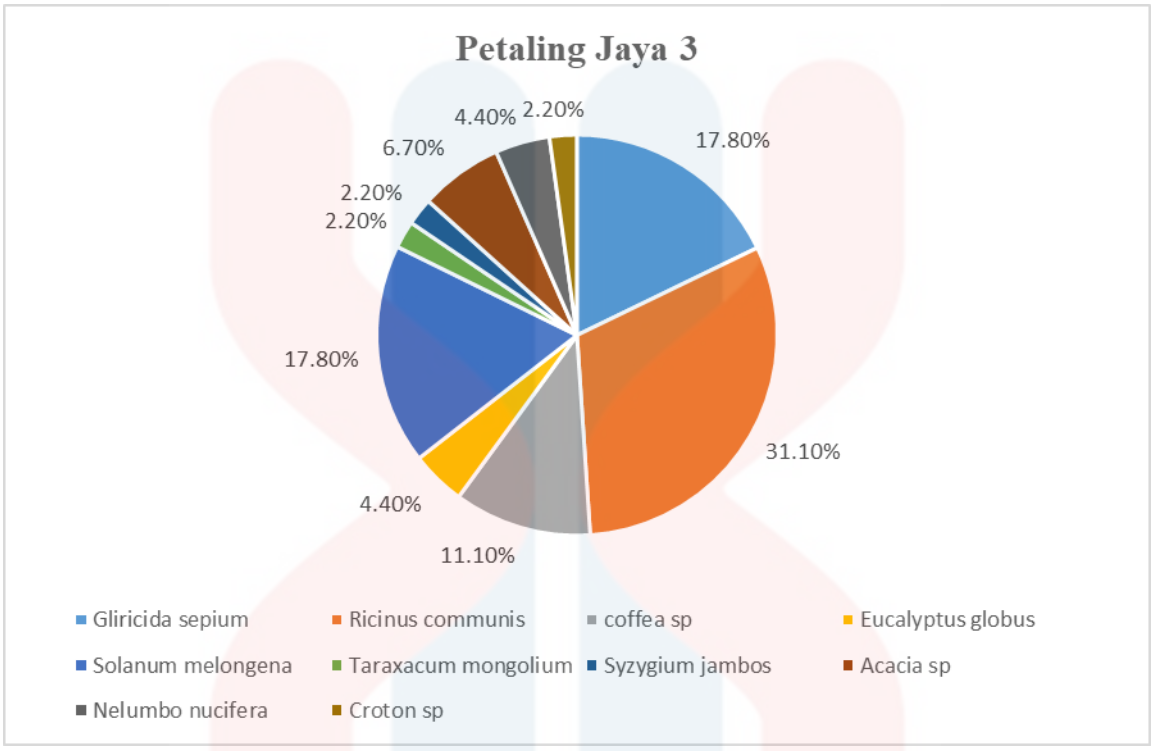

Figure 4.8: Pollen spectrum of honey sample from Petaling Jaya, Selangor

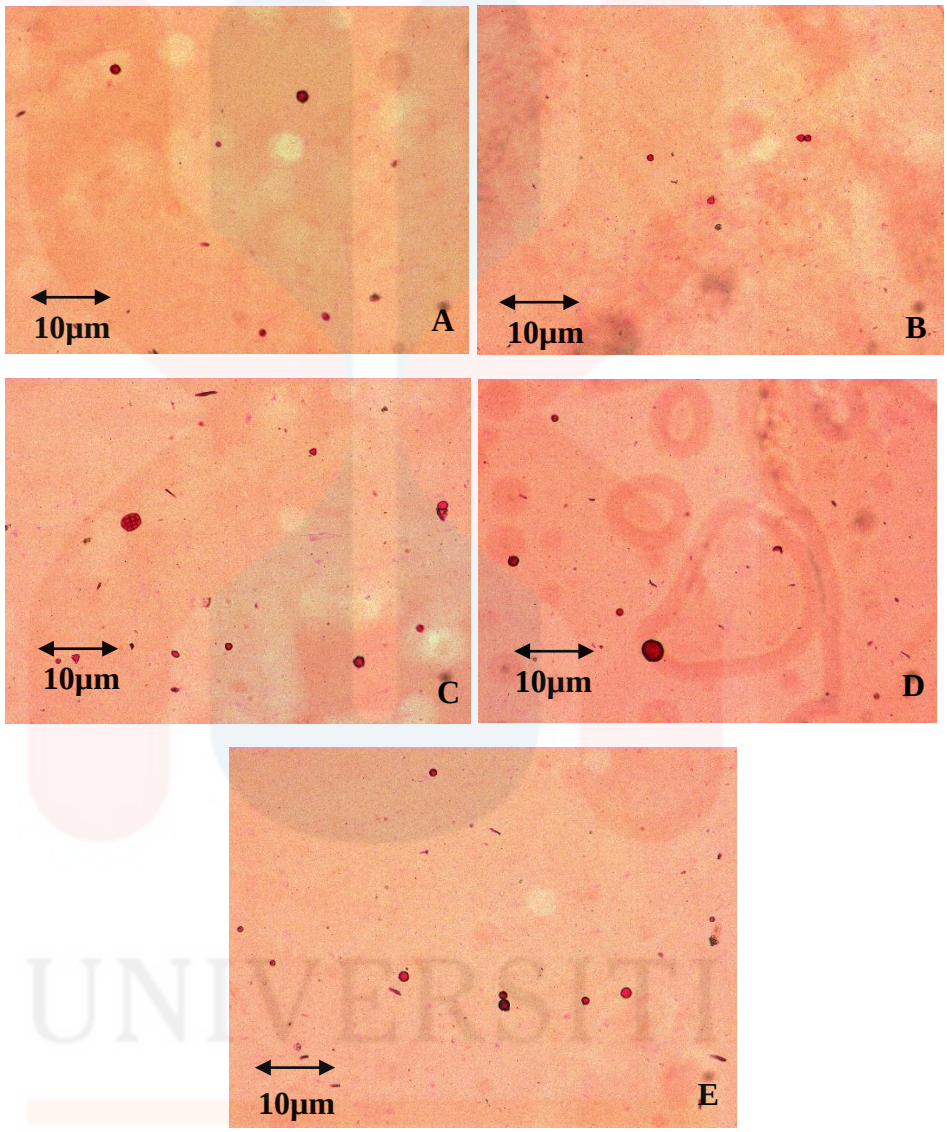

Figure 4.8 (a): Microscopic overviews of pollen density in honey sample from Petaling Jaya 3 under 10x magnification. Overviews of A- Left edge of cover slip (top); B- Right edgeof cover slip (top); C- Left edge of cover slip (bottom); D- Right edge of cover slip (bottom) and E- center of the cover slip (middle)

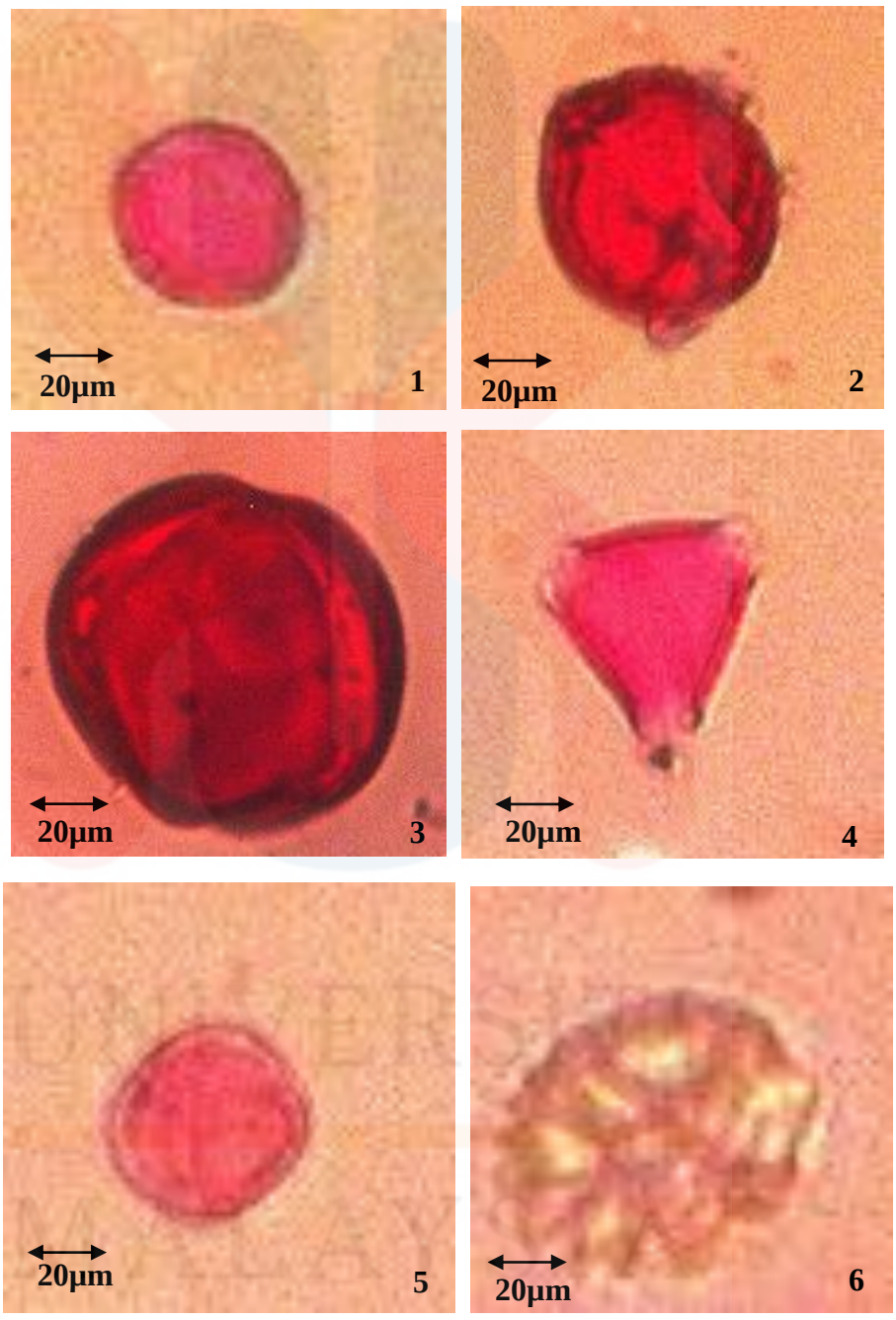

Figure 4.8 (b): Morphology of different pollen honey sample from Petaling Jaya 3 under 40x magnification. 1- *Gliricida sepium* (Fabaceae); 2- *Ricinus communis* (Euphorbiaceae); 3- *Coffee sp* (Rubiaceae); 4- *Eucalyptus globus* (Myrtaceae); 5- *Solanum melongena* (Solanaceae) and 6- *Taraxacum mongolicum* (Asteraceae)

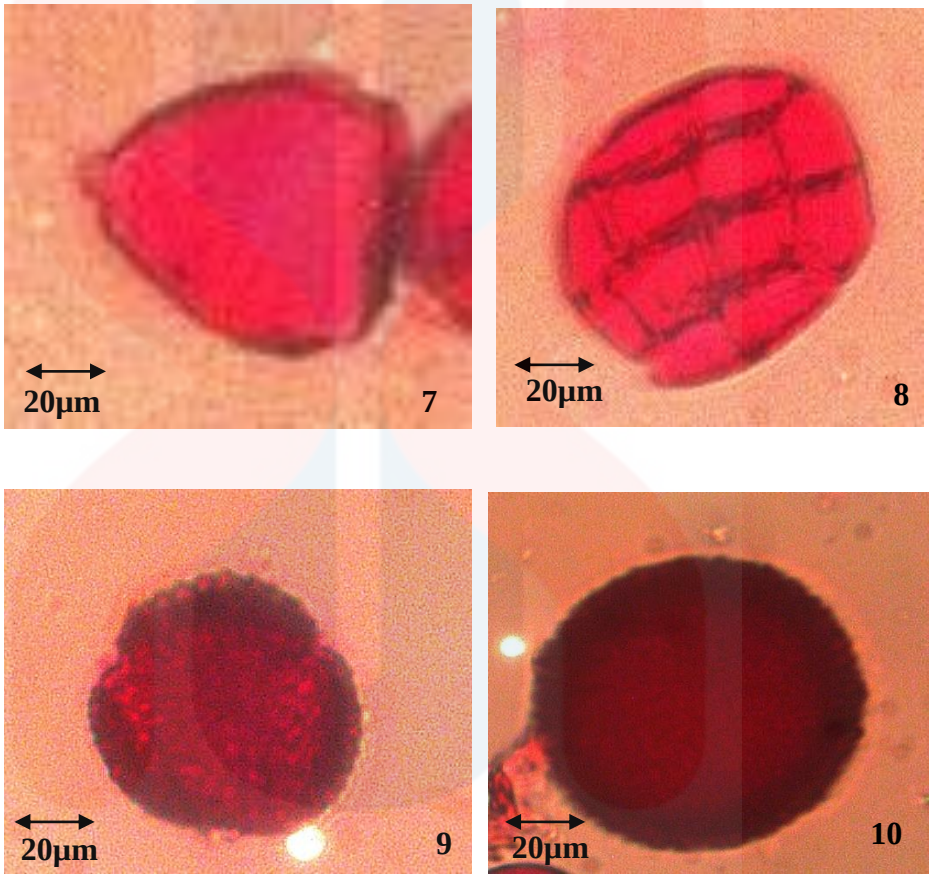

Figure 4.8 (c): Morphology of different pollen honey sample from Petaling Jaya 3 under 40x magnification. 7- *Sygyium jambos (*Myrtaceae); 8- *Acacia sp* (Fabaceae) 9- *Nelumbo nucifera* (Nelumbonaceae) and 10- *Croton sp* (Euphorbiaceae)

10 plant species belonging to 7 families were observed in the honey samples collected from Petaling Jaya 3 with 10 pollen types. In the honey samples from Petaling Jaya 3, pollen grains from *Ricinus communis* was more dominant (31.1%) followed by pollen grains from *Gliricida sepium* and *Solanum melongena* with 17.8% each pollen which is the dominant plant species in the area where beehives are located. Meanwhile, *Coffea sp.* (11.1%), *Acacia sp* (6.7%), *Eucalyptus globus* and *Nelumbo nucifera* (4.4%) were identified as important minor pollen and pollen grains from *Taraxacum mongolicum, Sygyium jambos* and *Croton sp* being the least with 2.2% frequency (Table 4.8).

Analysis had been done which showed that there was no predominant taxon of pollen found in Petaling Jaya 3. This samples contained a very little pollen than other sample collected from other location due their surrounding whereas it was located near to the industrial areas which limit the productivity of pollen. This sample had widened floral diversity in the region demonstrated abundant pollen composition of the honey in this area. The extra of bees foraging many plants were connected to the fact that pollen was the only proteic food within the beehive. In consequence, pollen plays an important role in feeding the colony (Abdulrahaman et al., 2013). Therefore, the amount of pollen found was also less.

4.9 *Honey sample Petaling Jaya, Selangor*

Table 4.9: The number of pollen and percentage of abundance of pollen types in the honey sample from Petaling Jaya (PJ4)

Plant species	**Family**	**No of pollen**	**Percentage of Abundance (%)**
Papaver somniferum	Papaveraceae	3	3.3%
Ricinus communis	Euphorbiaaceae	9	9.8%
Cocos nucifera	Arecaceae	3	3.3%
Rosa canina	Rosaceae	11	12.0%
Caesalpinia pulcherrima	Fabaceae	4	4.3%
Sygyium jambos	Myrtaceae	3	3.3%
Callistemon linearis	Myrtaceae	1	1.1%
Mimosa pundica	Moringaceae	5	5.4%
Ixora coccinea	Rubiaceaae	44	47.8%
Ipomea indica	Convolvulaceae	1	1.1%
Punica granatum	Punicaceae	2	2.4%

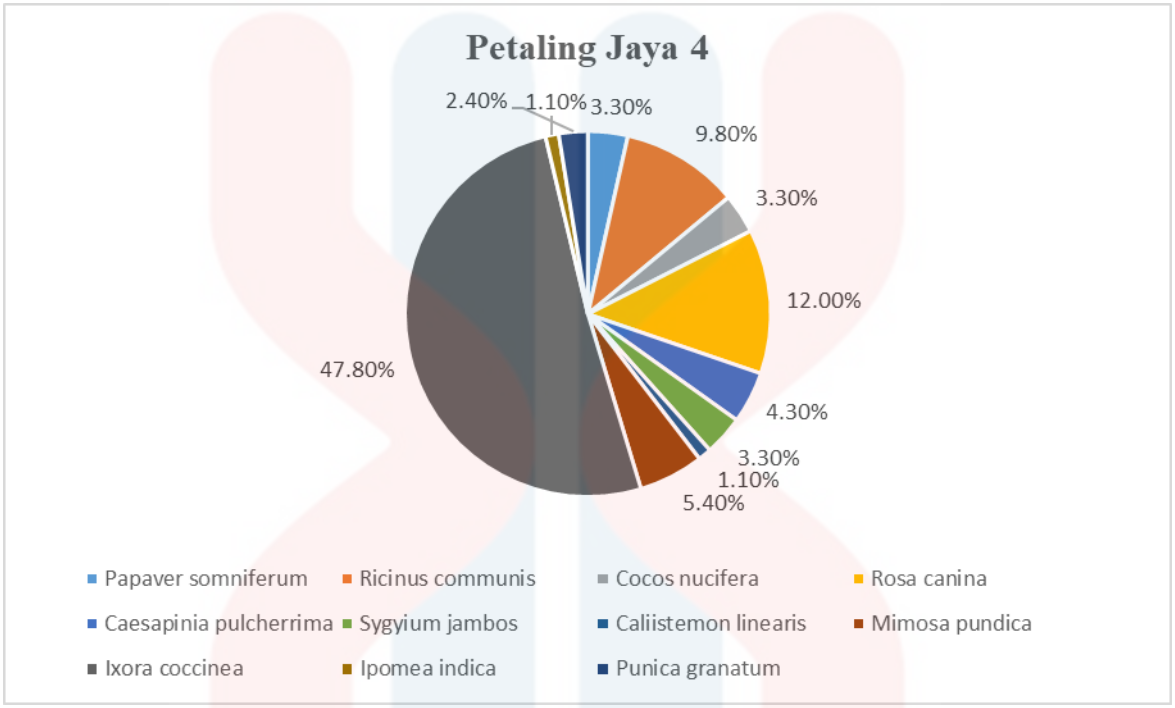

Figure 4.9: Pollen spectrum of honey sample from Petaling Jaya, Selangor

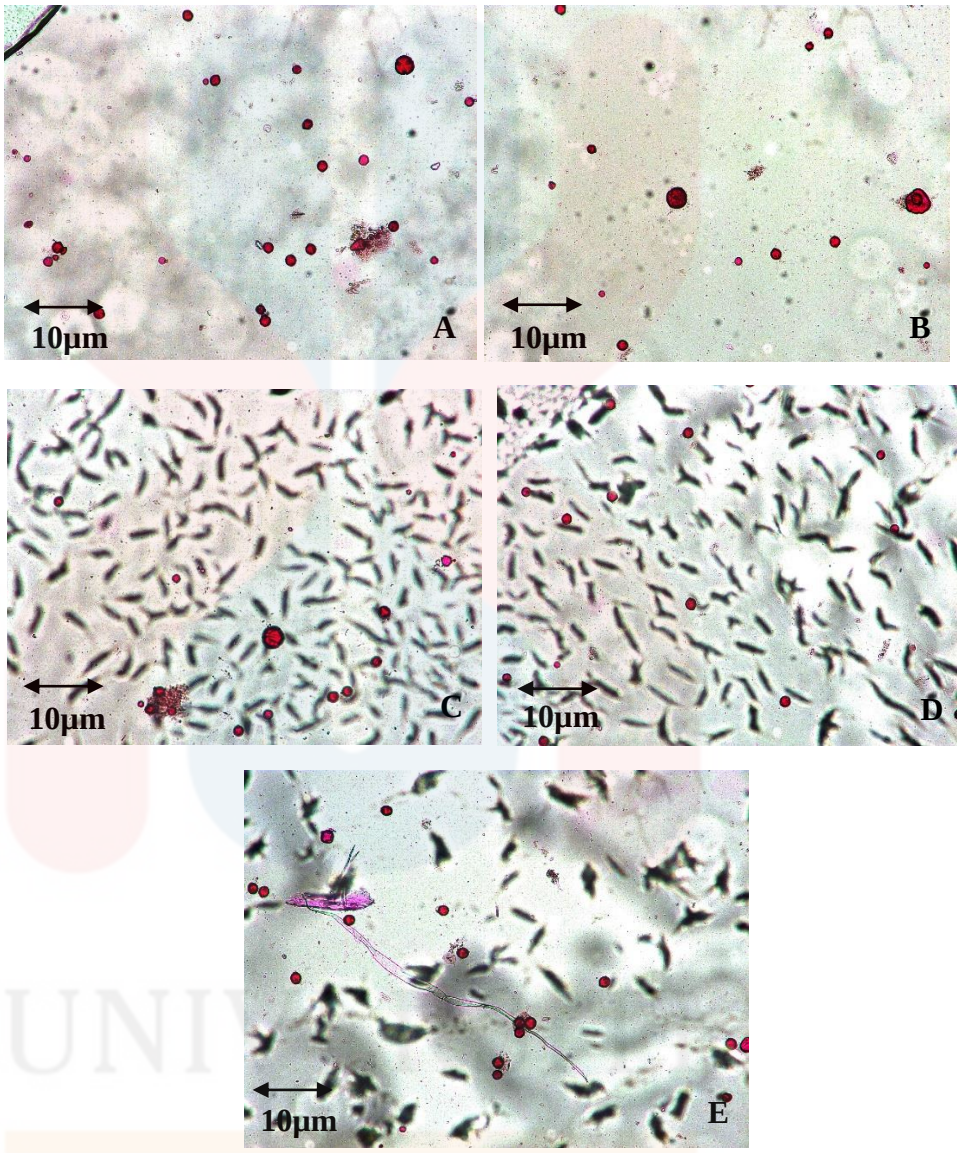

Figure 4.9 (a): Microscopic overviews of pollen density in honey sample from Petaling Jaya 4 under 10x magnification. Overviews of A- Left edge of cover slip (top); B- Right edge of cover slip (top); C- Left edge of cover slip (bottom); D- Right edge of cover slip (bottom) and E- centre of the cover slip (middle)

Figure 4.9 (b): Morphology of different pollen honey sample from Petaling Jaya 4 under 40x magnification. 1- *Papaver somniferum* (Papaveraceae); 2- *Ricinus communis* (Euphorbiaceae); 3- *Cocos nucifera* (Arecaceae); 4- *Rosa canina* (Rosaceae); 5- *Caesalpinia pulcherrima* (Fabaceae) and 6- *Syzygium jambos* (Myrtaceae)

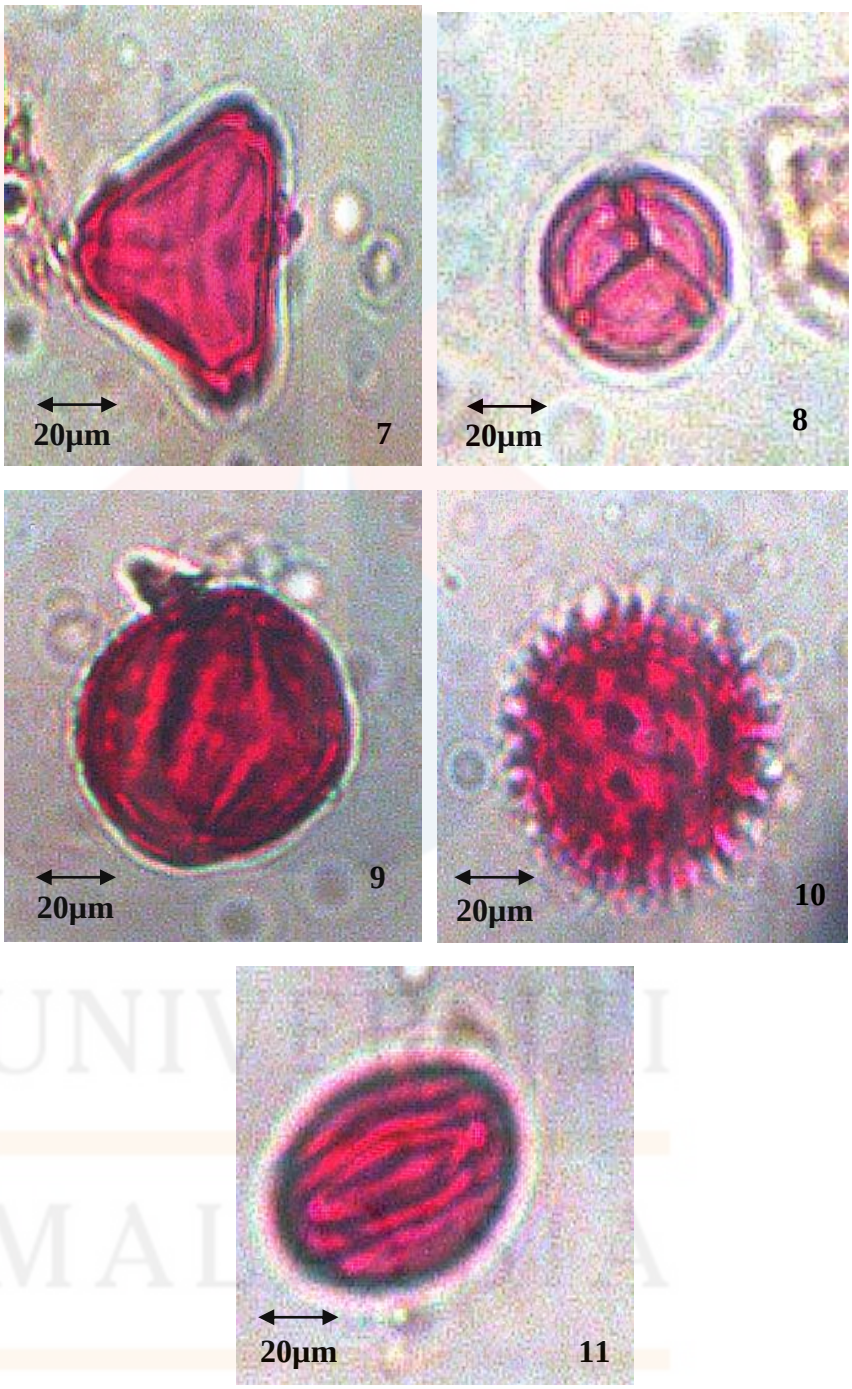

Figure 4.9 (c): Morphology of different pollen honey sample from Petaling Jaya 4 under 40x magnification. 7- *Callistemon linearis* (Myrtaceae) 8- *Mimosa pudica* (Moringaceae); 9- *Ixora coccinea* (Rubiaceae); 10- *Ipomea indica* (Convolvulaceae) and 11- *Punica granatum* (Punicaceae)

11 different types of pollen species were discovered from the sample in Petaling Jaya 4 from 10 families such as from *Papaver somniferum* from Papaveraceae family, *Ricinus communis* from Euphorbiaceae family, *Cocos nucifera* from Arecaceae family, *Rosa canina* from Rosaceae family, *Caesalpinia pulcherrima* from Fabaceae family, *Syzygium jambos* from Myrtaceae family, *Callistemon linearis* from Myrtaceae family, *Mimosa pudica* from Moringaceae family, *Ixora coccinea* from Rubiaceae family, *Ipomea indica* from Convolvulaceae family and *Punica granatum* from Punicaceae family. The predominant pollen at Petaling Jaya 4 was from the species of *Ixora coccinea* with percentage of 47.8%. The pollen structure was colpate-prolate, oralalongate and radial symmetry. Most of the samples contain *Ixora coccinea* plant species in their identification of samples. It is clearly shown this plant was contributed as an essential pollen source and funded a lots into honey bee secretion as well as serving them in improving the growth of apiculture business in Malaysia. In this sample, there are 7 types of important minor pollen had been observed which were *Rosa canina* (12.0%), *Ricinus communis* (9.8%), *Caesalpinia pulcherrima* (4.3%), *Mimosa pudica* (5.4%), *Papaver somniferum*, *Syzygium jambos* and *Cocos nucifera* with 3.3% each pollen. Meanwhile, *Punica granatum*, *Ipomea indica* and *callistemon linearis* were classified as minor pollen which pollen grain frequency less than 3%.

In this study, the most frequent pollen collected was *Ixora coccinea* which own several features that might influence the preference of pollen by the honeybees. *Ixora coccinea* have four to six-inch globular groups of bright red, orange, yellow, pink, or white tube-shaped flowers that bloom constantly under ideal conditions in full sunlight (Azmi et al., 2015). Thus, several studies found that flower attractiveness contribute positive impact on the abundance and regularity of the flower visitors. The honey samples from Petaling Jaya 4 was unifloral honey.as pollen grain frequenct more than 45%. Pollen analysis was one of the significant tool in defining the floral sources upon which the bees foraged to produce honey and it also embraces the study of pollen in the honey as well as the source of the pollen (Abdulrahaman et al., 2013).

4.10 *Honey sample from Bangi, Selangor*

Table 4.10: The number of pollen and percentage of abundance of pollen types in the honey sample from Bangi (BG2)

Plant species	**Family**	**No of pollen**	**Percentage of Abundance (%)**
Cassia fistula	Fabaceae	16	23.2%
Ruta graveolens	Rutaceae	48	69.6%
Syzygium jambos	Myrtaceae	2	2.9%
Mussaenda sp	Rubiaceae	1	1.4%
Eucalyptus globus	Myrtaceae	2	2.9%

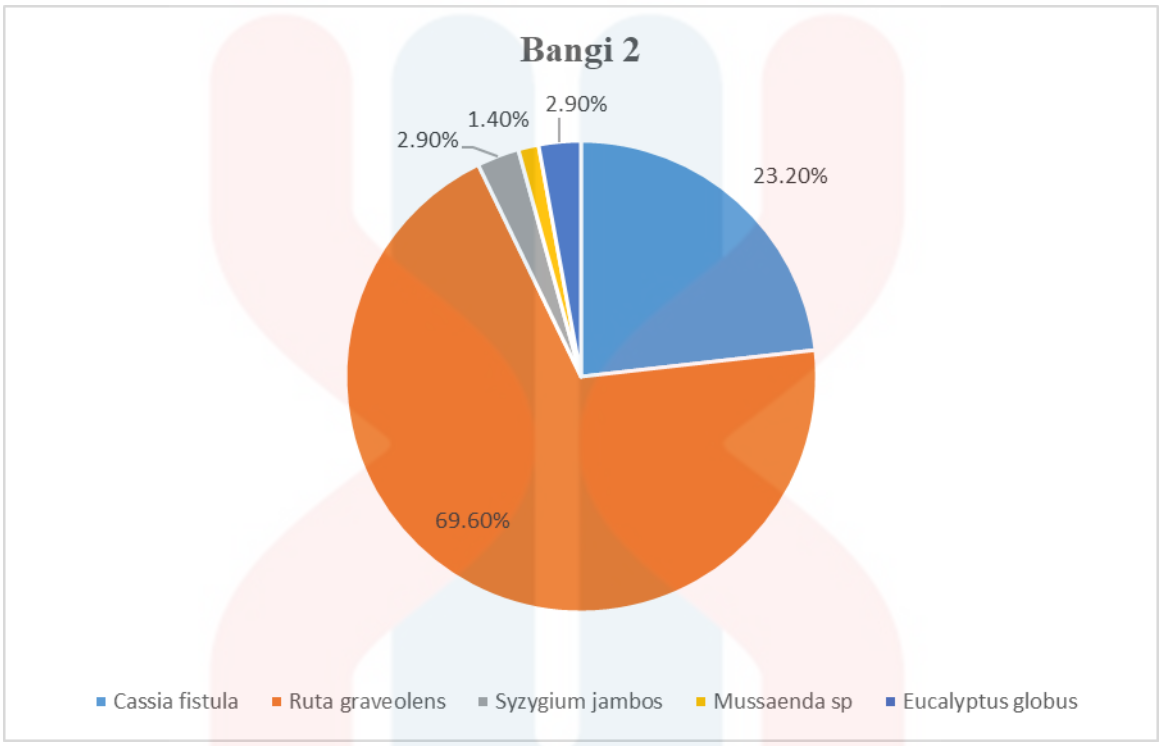

Figure 4.10: Pollen spectrum of honey sample from Bangi, Selangor

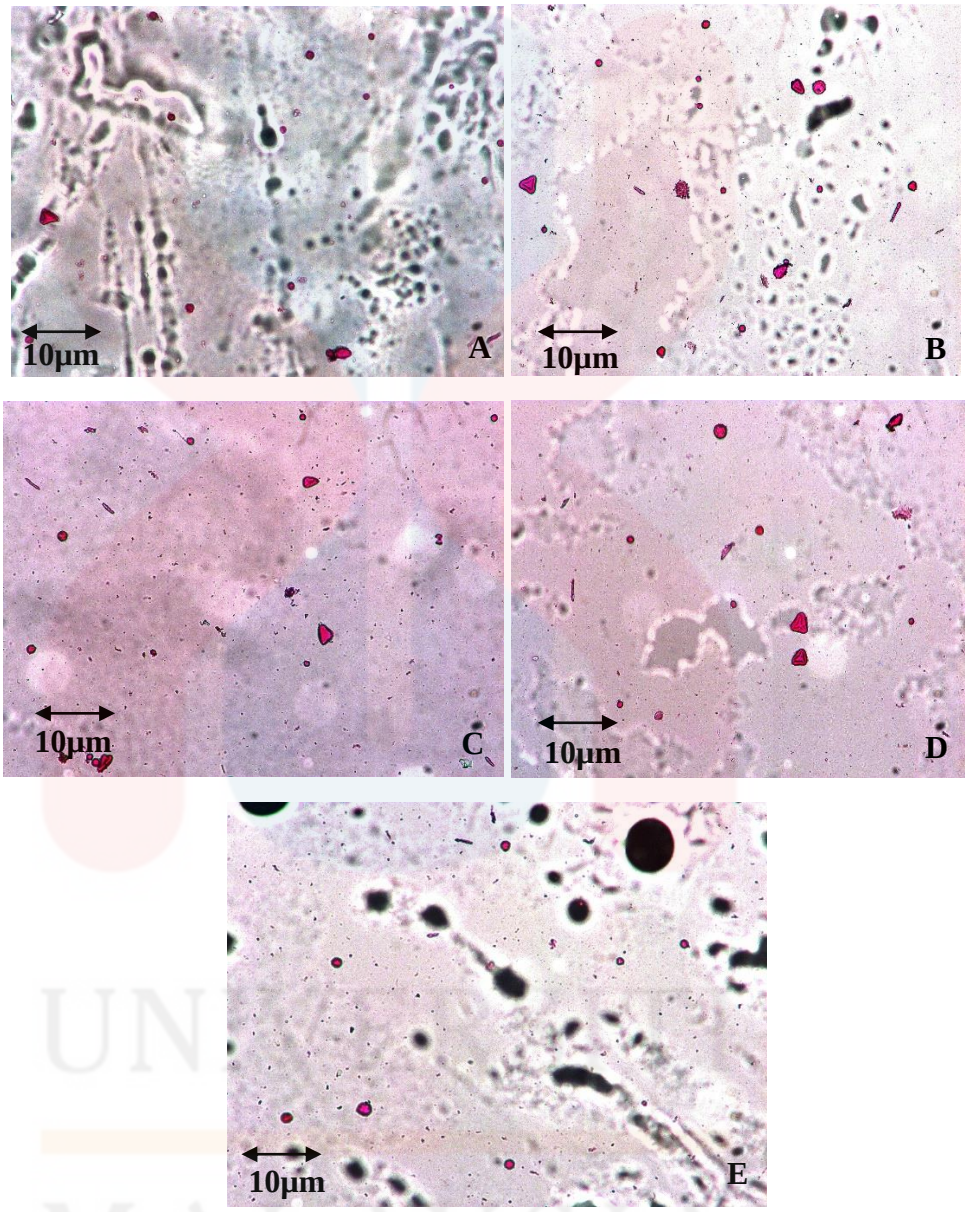

Figure 4.10 (a): Microscopic overviews of pollen density in honey sample from Bangi 2 under10x magnification. Overviews of A- Left edge of cover slip (top); B- Right edge of cover slip (top); C- Left edge of cover slip (bottom); D- Right edge of cover slip (bottom) and E-centre of the cover slip (middle)

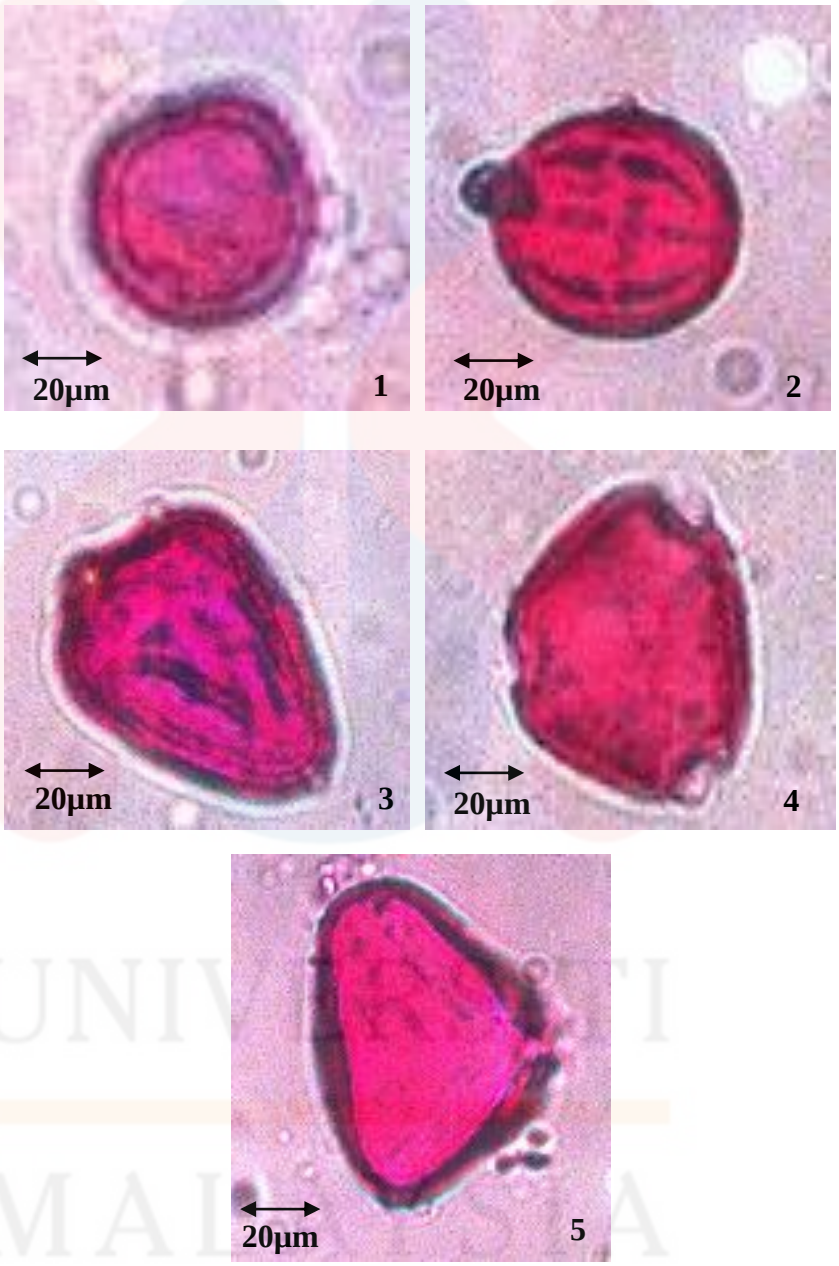

Figure 4.10 (b): Morphology of different pollen honey sample from Bangi 2 under 40x magnification. 1- *Cassia fistula* (Fabaceae); 2- *Ruta graveolens* (Rutaceae); 3- *Syzygium jambos* (Myrtaceae); 4- *Mussaenda sp* (Myrtaceae) and 5- *Eucalyptus globus* (Myrtaceae)

From the honey samples collected in Bangi 2, 5 different types of pollen species had been identified, namely *Cassia fistula* (Fabaceae), *Ruta graveolens* (Rutaceae), Syzygium jambos (Myrtaceae), *Mussaenda sp* (Myrtaceae) and *Eucalyptus globus* (Myrtaceae). The predominant pollen in this honey sample was *Ruta graveolens* which accounted up to 69.6% of the total pollen count. Therefore, this honey sample was unifloral honey. There was no secondary pollen has been observed but there was only 1 important minor pollen which was *Cassia fistula* while, there were 3 pollen species had been identified as minor pollen which were *Syzygium jambos*, *Eucalyptus globus* and *Mussaenda sp*.

Ruta graveolens, generally called rue was native to southern Europe. It was a glabrous, glaucous, woody-based, shrubby perennial with aromatic, fern-like, compound leaves. Rue was widely grown around urban in this region as an ornamental and it has been used as mosquito repellent. This plant species comprises of dull yellow floral. Honey bee was often found foraging the plant's yellow flowers for nectar and pollen. Based on the observation in Figure 4.10 (b) and Figure 4.10 (c), it was very apparent that honey bee in Bangi 2 region preferred to feed flowers from the species and families stated above as they play a vital role in bee nutrition. Pollen of numerous plants signifying potential source of nectar and pollen for the honeybees was an important obligation for the developing apiary (R et al., 2013).

4.11 *Honey sample from Hulu Langat, Selangor*

Table 4.11: The number of pollen and percentage of abundance of pollen types in the honey sample from Hulu Langat (HL4)

Plant species	**Family**	**No of pollen**	**Percentage of Abundance (%)**
Ixora coccinea	Rubiaceae	29	7.8%
Ricinus communis	Euphorbiaceae	311	83.4%
Cocos nucifera	Arecaceae	1	0.3%
Caesalpinia pulcherrima	Fabaceae	2	0.5%
Ipomoea indica	Convolvulaceae	1	0.3%
Mallotus apelta	Euphorbiaceae	29	7.8%

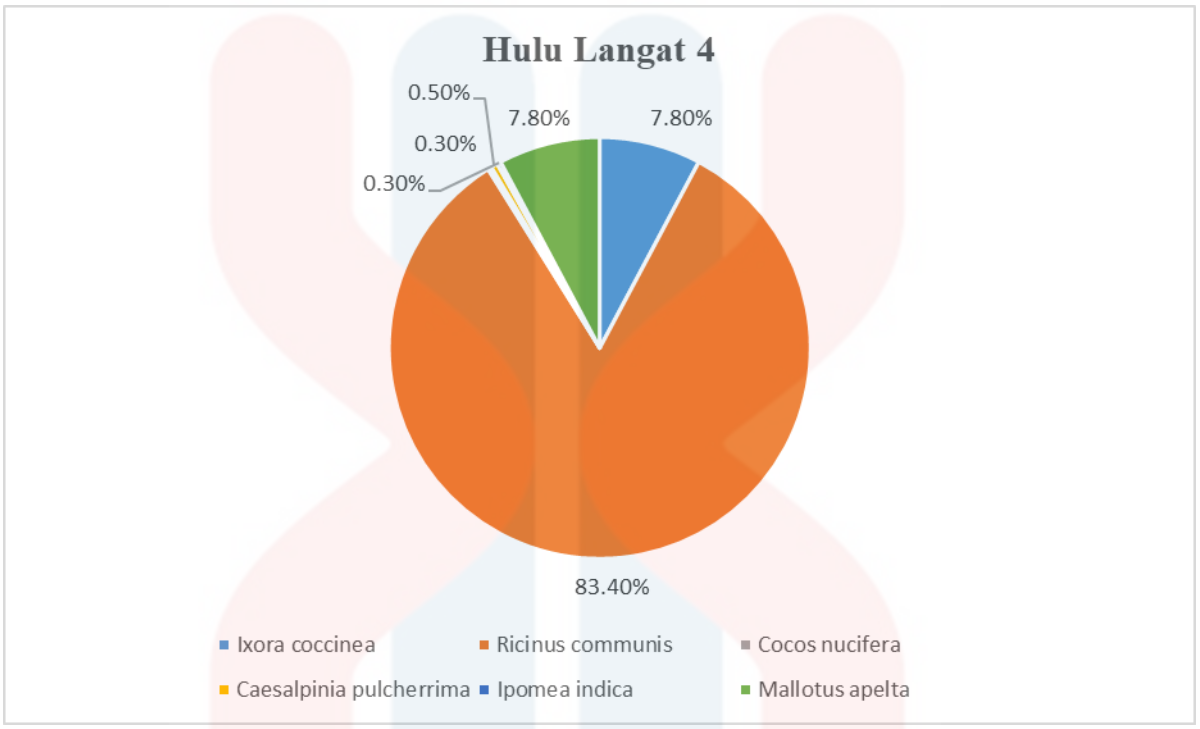

Figure 4.11: Pollen spectrum of honey sample from Hulu Langat, Selangor

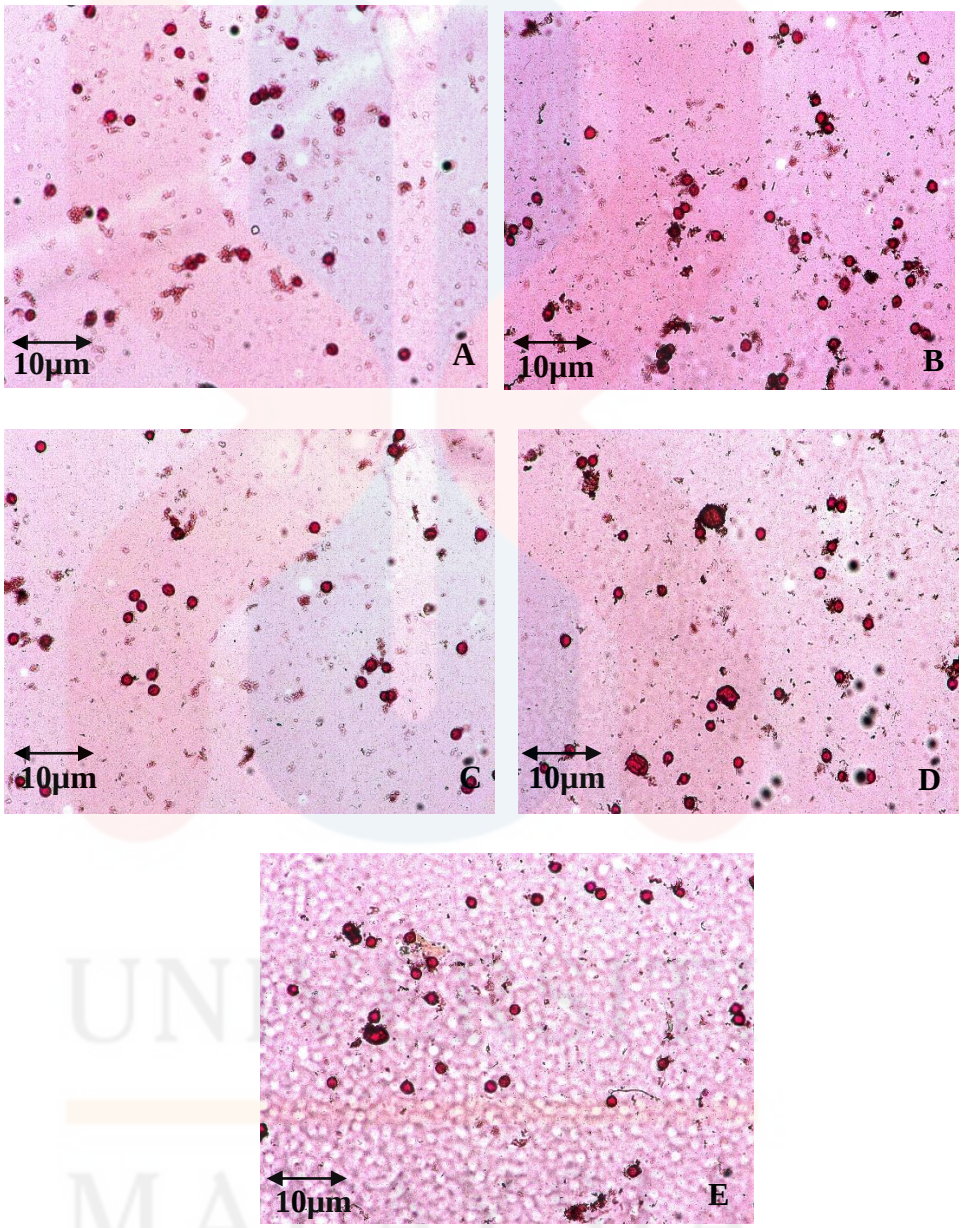

Figure 4.11 (a): Microscopic overviews of pollen density in honey sample from Hulu Langat 4 under 10x magnification. Overviews of A- Left edge of cover slip (top); B- Right edge of cover slip (top); C- Left edge of cover slip (bottom); D- Right edge of cover slip (bottom) and E- Centre of the cover slip (middle)

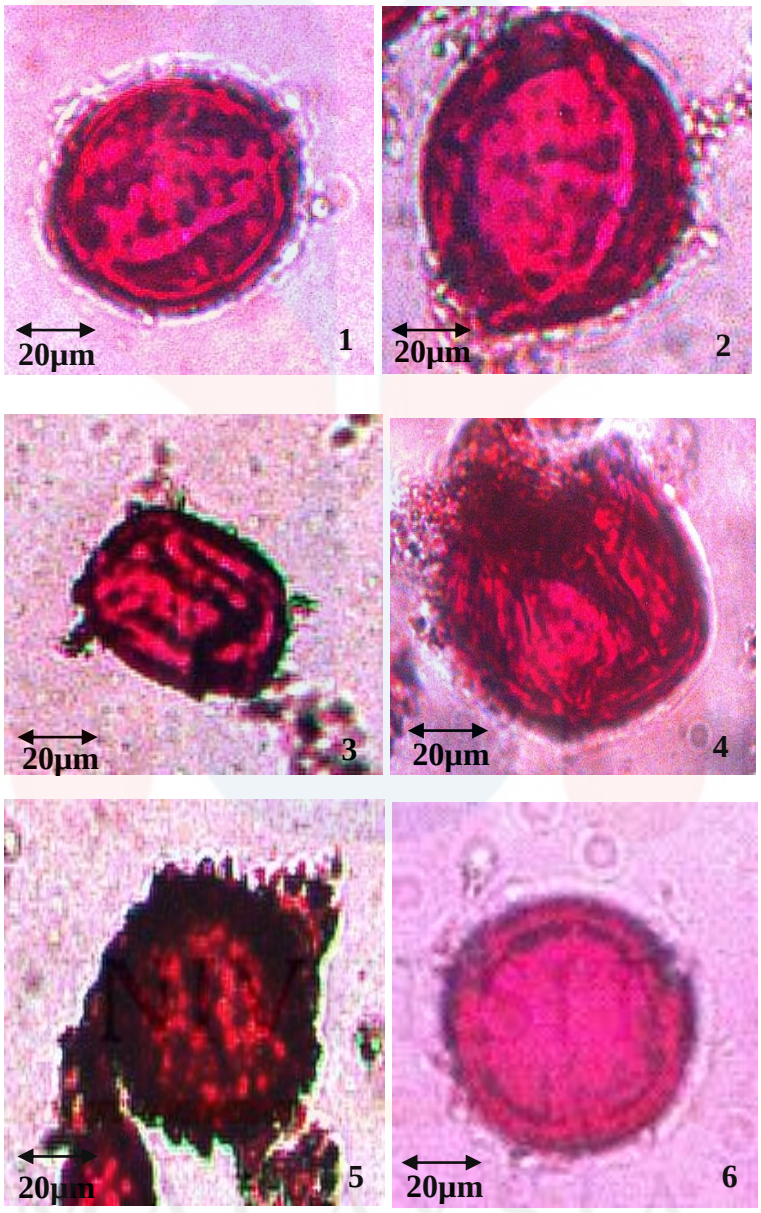

Figure 4.11 (b): Morphology of different pollen honey sample from Hulu Langat 4 under 40x magnification. 1- *Ixora coccinea* (Rubiaceae); 2- *Ricinus communis* (Euphorbiaceae); 3- *Cocos nucifera* (Arecaceae); 4- *Caesalpinia pulcherrima* (Fabaceae); 5- *Ipomoea indica* (Convolvulaceae) and 6- *Mallotus apelta* (Euphorbiaceae)

From the honey samples collected in Hulu Langat 4, there were 373 pollen grains found in a honey sample slide observed under the compound microscope with camera. 6 different types of pollen species were present in the sample, such as *Ixora coccinea* (Rubiaceae), *Ricinus communis* (Euphorbiaceae), *Cocos nucifera* (Arecaceae), *Caesalpinia pulcherrima* (Fabaceae), *Ipomoea indica* (Convolvulaceae) and *Mallotus apelta* (Euphorbiaceae). *Ricinus communis* got the highest percentage abundance of pollen in this sample which was 83.4%. The predominant pollen *Ricinus communis* or known as the castor bean was a species of Euphorbiaceae family. It has 3- Colporate, prolatespheroid, finely reticulate and bilateral symmetry of morphology (R et al., 2013). It adopts easily and cultivates in many regions as a mutual ruderal plant. Therefore, many of industrial were located at Hulu Langat. As a result, it grown commercially for the medicinal and industrial usage of its oil and for use in landscaping. *Cocos nucifera* and *Ipomea indica* plant species were the lowest percentage abundance which only got 0.3% of pollen spectrum. This pollens were categorized as minor pollen as the least frequency. Therefore, Hulu Langat 4 honey sample was considered as unifloral honey. Unifloral honey has a pollen spectrum where one species is major that takes accounts for more than 45% of the pollen grains and create in environments that encounter widespread flowering of a single melliferous plant species (Calaça et al., 2018).

4.12 *Honey sample from Shah Alam, Selangor*

Table 4.12: The number of pollen and percentage of abundance of pollen types in the honey sample from Shah Alam (SA1)

Plant species	**Family**	**No of pollen**	**Percentage of Abundance (%)**
Cocos nucifera	Arecaceae	14	19.7%
Spiraea miyabei	Rosaceae	8	11.3%
Heavea brasiliensis	Euphorbiaceae	3	4.2%
Eupatorium purpureum	Asteraceae	1	1.4%
Callistemon linearis	Myrtaceae	1	1.4%
Conium maculatum	Apiaceae	20	28.2%
Solanum Melongena	Solanaceae	6	8.5%
Punica granatum	Punicaceae	1	1.4%
Pongamia pinnata	Fabaceae	5	7.0%
Brassica campestris	Brassicaceae	19	26.8%
Tecoma stans	Bignoniaceae	1	1.4%

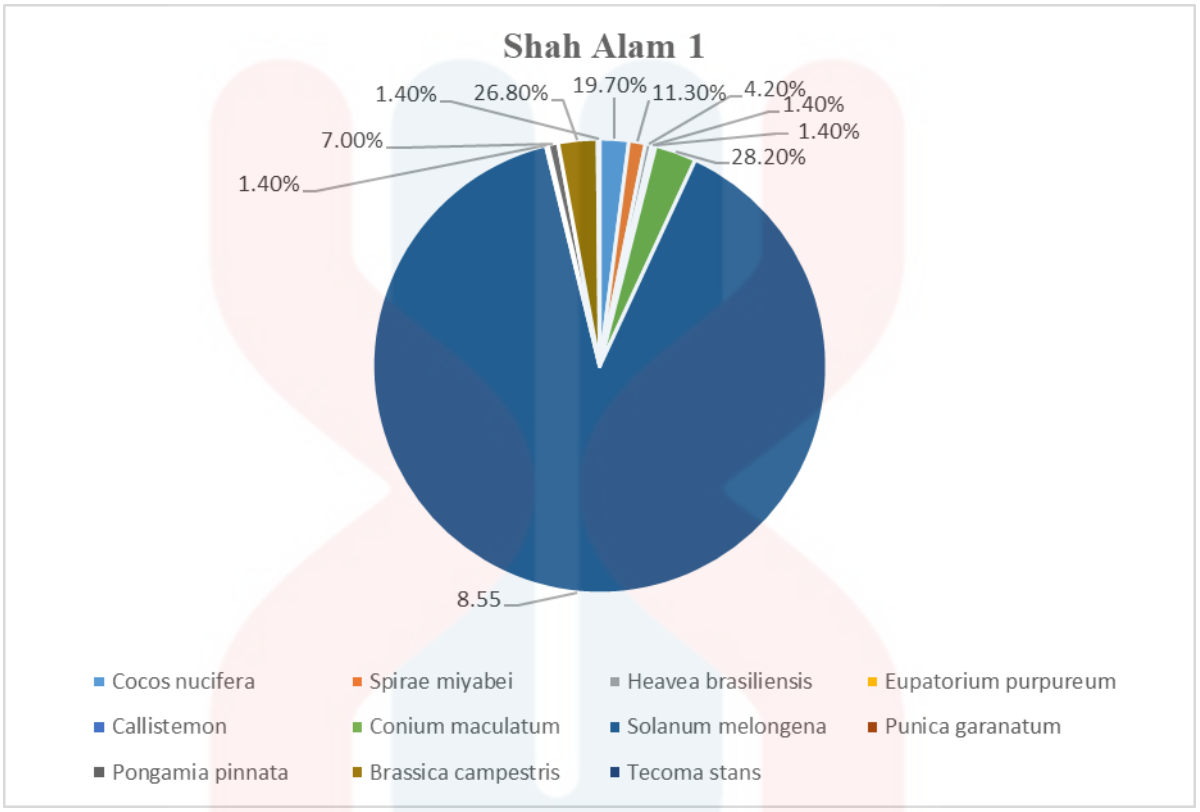

Figure 4.12: Pollen spectrum of honey sample from Shah Alam, Selangor

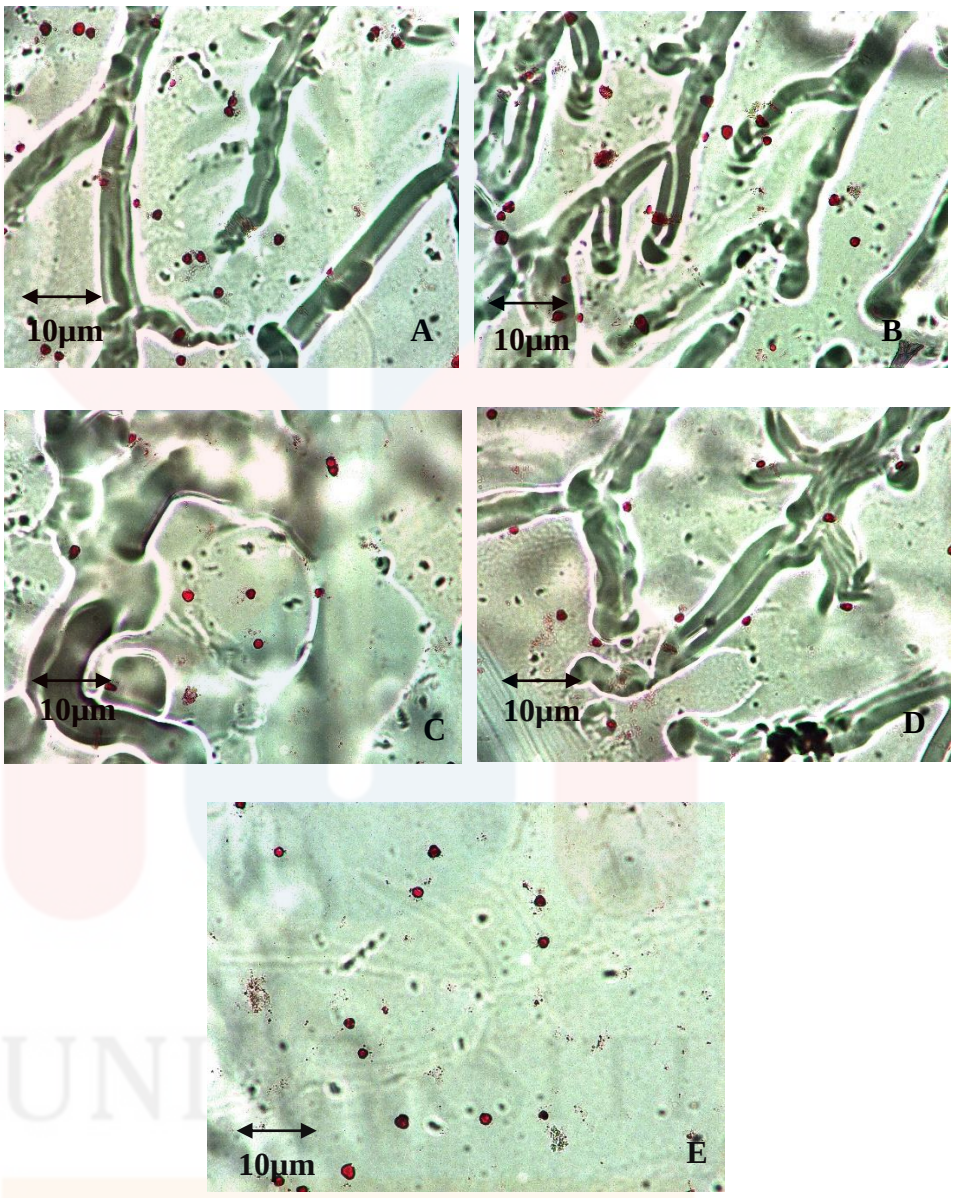

Figure 4.12 (a): Microscopic overviews of pollen density in honey sample from Shah Alam 1 under 10x magnification. Overviews of A- Left edge of cover slip (top); B- Right edge of cover slip (top); C- Left edge of cover slip (bottom); D- Right edge of cover slip (bottom) and E- centre of the cover slip (middle)

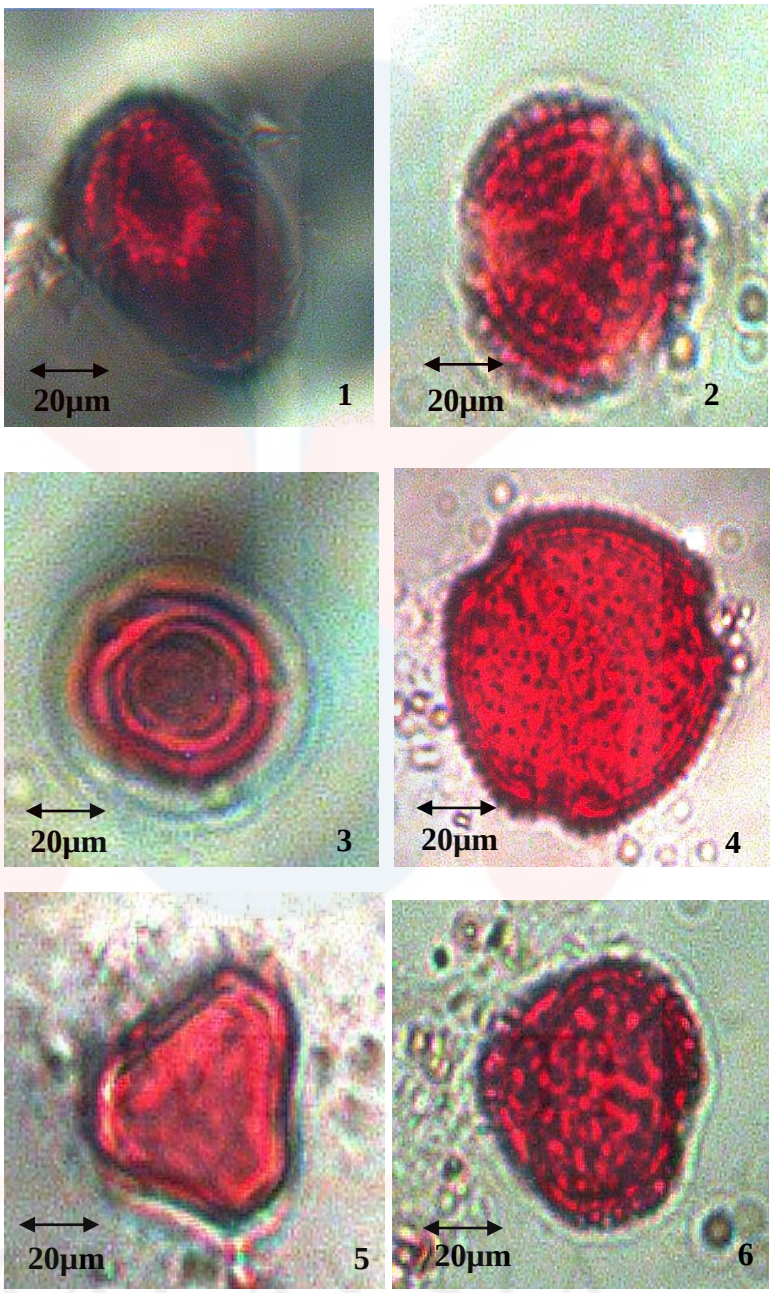

Figure 4.12 (b): Morphology of different pollen honey sample from Shah Alam 1 under 40x magnification. 1- *Cocos nucifera* (Arecaceae); 2- *Spiraea miyabei* (Rosaceae); 3- *Hevea brasiliensis* (Euphorbiaceae); 4- *Eupatorium purpureum* (Asteraceae); 5- *Callistemon linearis* (Myrtaceae) and 6- *Conium maculatum* (Apiaceae)

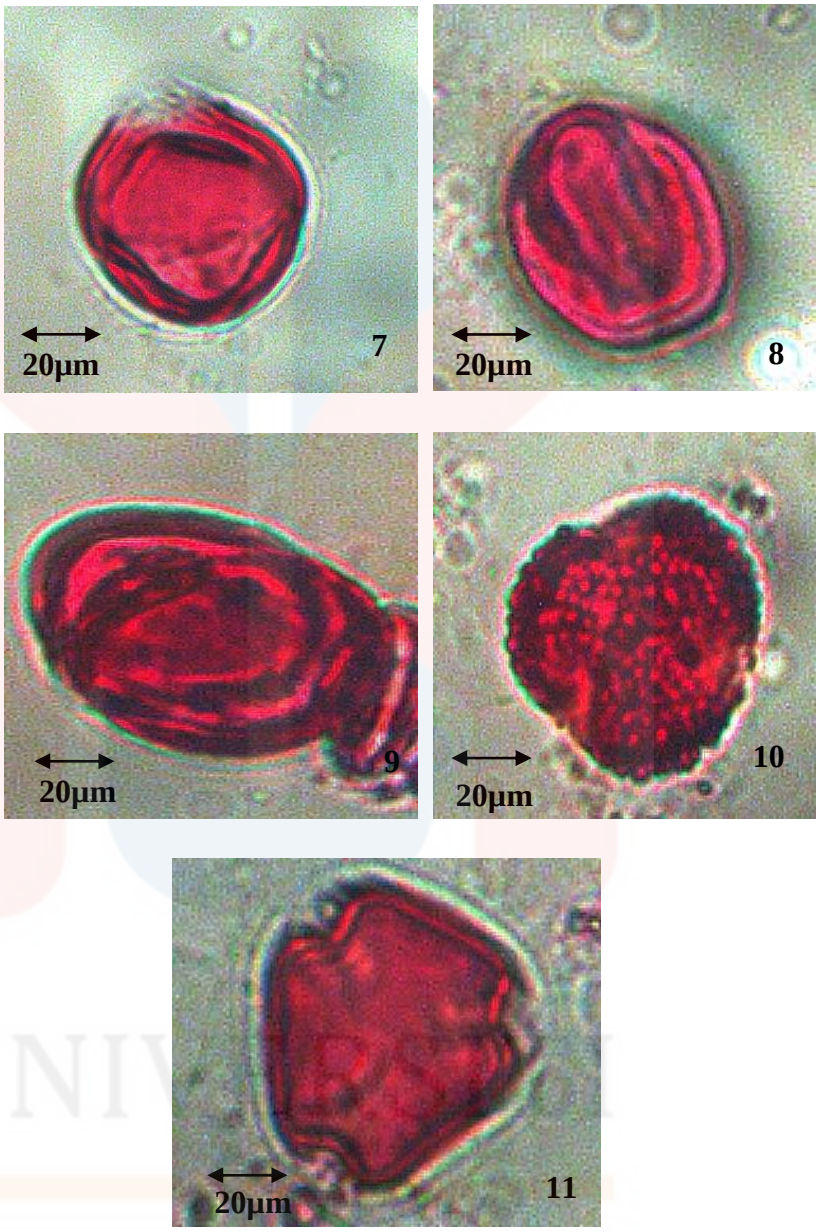

Figure 4.12 (c): Morphology of different pollen honey sample from Shah Alam 1 under 40x magnification. 7- *Solanum melongena* (Solanaceae); 8- *Punica granatum* (Punicaceae); 9- *Pongamia pinnata* (Fabaceae); 10- *Brassica compestris* (Brassicaceae) and 11- *Tecoma stans* (Bignoniaceae)

Table 4.12 showed the honey sample collected in Shah Alam 1 contain 11 different types of pollen species were discovered in it. Among the types of pollen species found *Cocos nucifera* (Arecaceae), *Spiraea miyabei* (Rosaceae), *Hevea brasiliensis* (Euphorbiaceae), *Eupatorium purpureum* (Asteraceae), *Callistemon linearis* (Myrtaceae), *Conium maculatum* (Apiaceae), *Solanum melongena* (Solanaceae), *Punica granatum* (Punicaceae), *Pongamia pinnata* (Fabaceae), *Brassica compestris* (Brassicaceae) and *Tecoma stans* (Bignoniaceae).

There was no predominant pollen species had been observed in this honey samples while, the most abundant pollen in the honey sample was *Conium maculatum* which accounted up to 28.2% of total pollen distribution. It was also known as Poison Hemlock. C. *maculatum* was an herbaceous biennial plant from Apiaceae family. It was a weed that grows along roadsides. It has large fern-like leaves, and resembles some wild edible plants. It was a good source of pollen as honey bee often visiting its flowers. Existence of a great variety of pollen in this honey sample showed the multiflorality of the honey from this area integrated with extensive of characterization of the various types of honey. *Brassica campestris and Cocos nucifera* (coconut) plant species were classified as the secondary pollen based on the preference of honeybees. This kind of plant species commonly found in Selangor's region. Thus, they were become favourable for foraging and wide range of insects' pollinators include honeybees always active on this plants.

Spiraea miyabei (Rosaceae), *Pongamia pinnata*, *Solanum Melongena* and *Heavea brasiliensis* (Euphorbiaceae) were categorized as minor pollen as they only got 11.3%, 7.0%, 8.5% and 4.2% from the overall pollen count. While, the rest of 4 different type of species from different family such as *Eupatorium purpureum* (Asteraceae), *Callistemon linearis* (Myrtaceae), *Punica granatum* (Punicaceae) and *Tecoma stans* (Bignoniaceae) with the least total percentage of abundance 1.4% were identified as important minor pollen in this Shah Alam 1's honey sample.

4.13 *Honey sample from Bangi, Selangor*

Table 4.13: The number of pollen and percentage of abundance of pollen types in the honey sample from Bangi (BG3)

Plant species	**Family**	**No of pollen**	**Percentage of Abundance (%)**
Melastoma sp	Melastomaceae	6	23.1%
Polianthes tuberosa	Asparagaceae	2	7.7%
Solanum melongena	Solanacaeae	9	34.6%
Psidium guajava	Myrtacaeae	1	3.9%
Bombax ceiba	Malvaceae	2	7.7%
Croton sp	Euphorbiaceae	3	11.5%
Punica granatum	Punicaceae	2	7.7%
Acacia sp	Fabaceae	1	3.9%

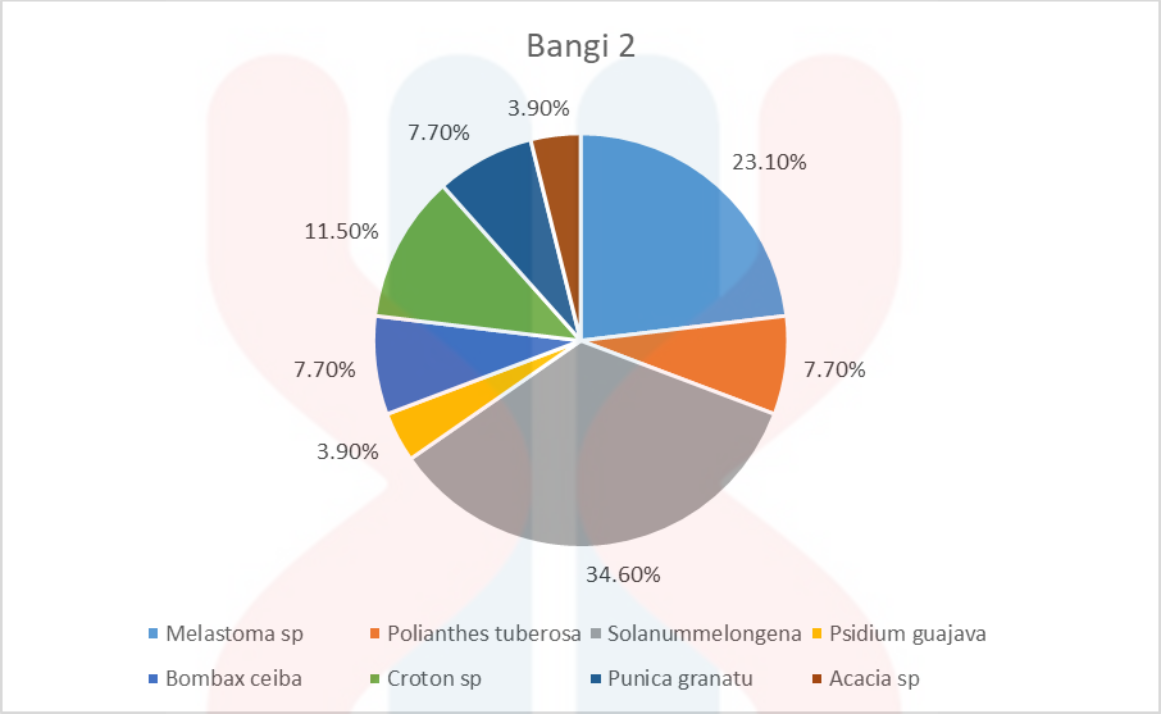

Figure 4.13: Pollen spectrum of honey sample from Bangi, Selangor

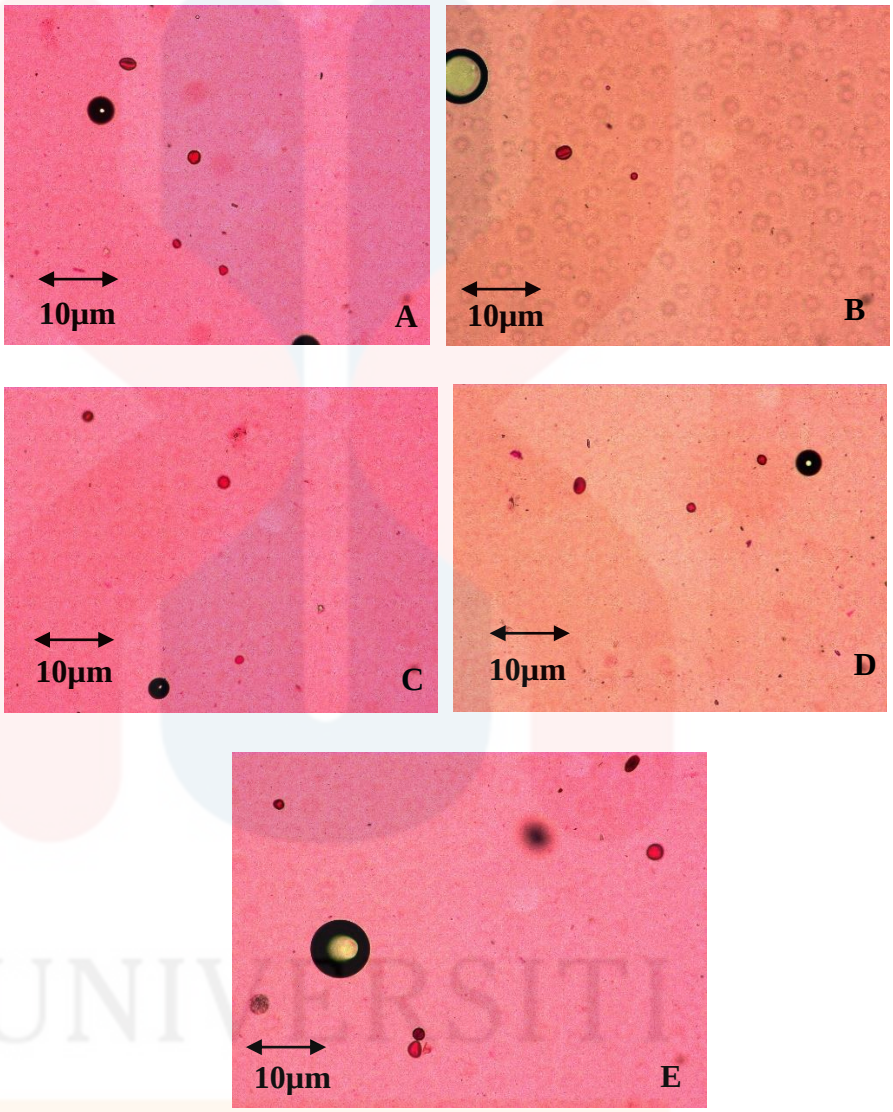

Figure 4.13 (a): Microscopic overviews of pollen density in honey sample from Bangi 3 under 10x magnification. Overviews of A- Left edge of cover slip (top); B- Right edge of cover slip (top); C- Left edge of cover slip (bottom); D- Right edge of cover slip (bottom) and E- centre of the cover slip (middle)

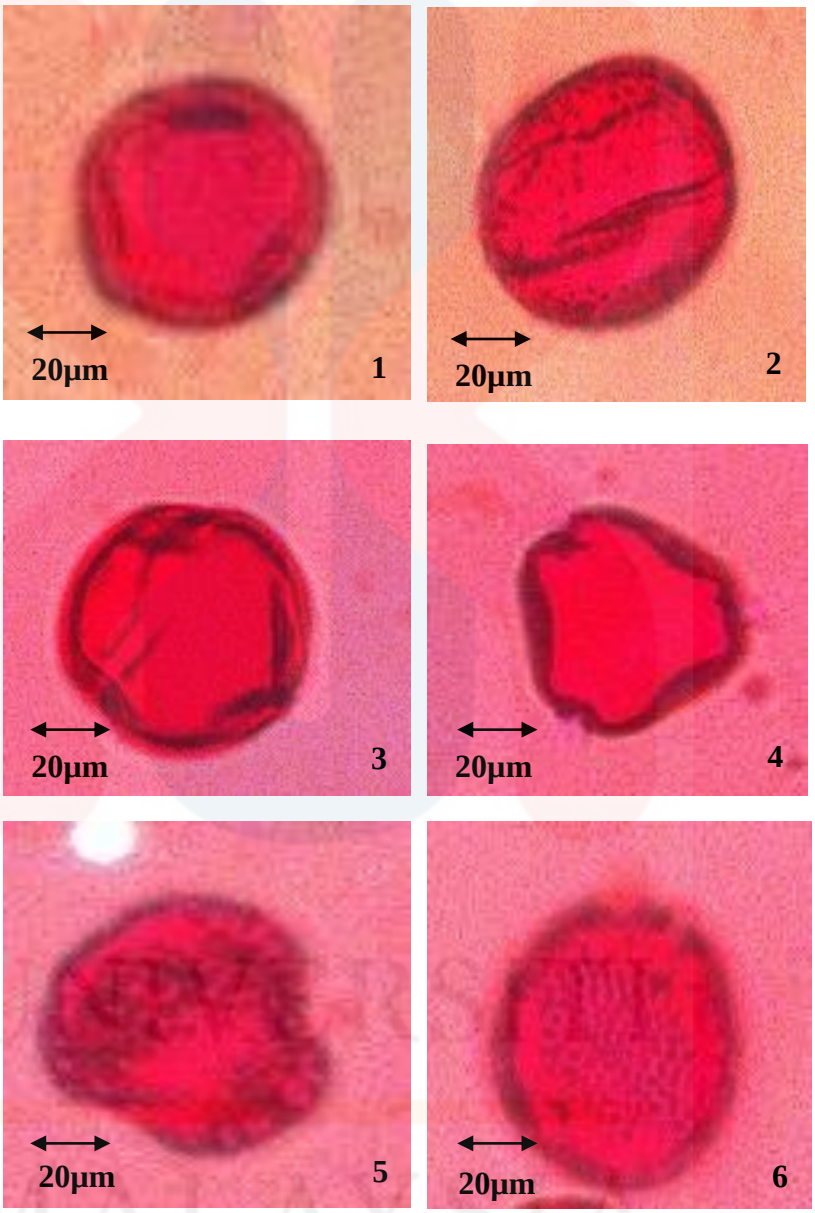

Figure 4.13 (b): Morphology of different pollen honey sample from Bangi 3 under 40x magnification. 1- *Melastoma sp* (Melastomaceae); 2- *Polianthes tuberosa* (Asparagaceae); 3- *Solanum melongena* (Solanaceae); 4- *Psidium guajava* (Myrtaceae); 5- *Bombax ceiba* (Malvaceae) and 6- *Croton sp* (Euphorbiaceae)

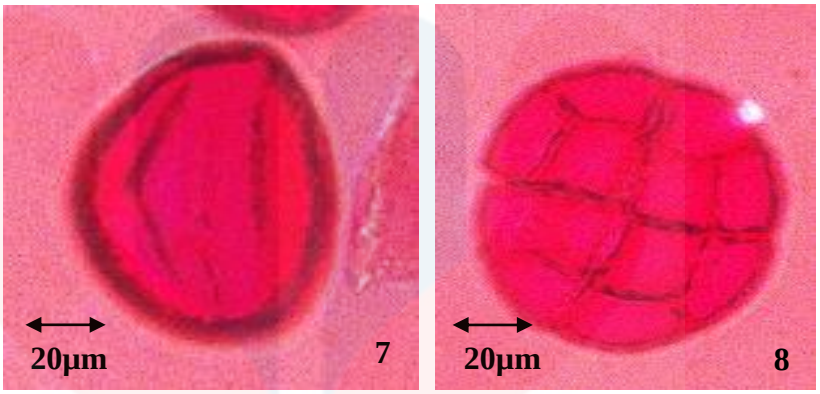

Figure 4.13 (c): Morphology of different pollen honey sample from Bangi 3 under 40x magnification. 7- *Punica granatum* (Punicaceae) and 8- *Acacia sp* (Fabaceae)

From the honey sample collected in Bangi 3 location, only 8 types of the pollen were successfully identified (Table 4.13). The most frequently collected pollen was from the following plant species: *Solanum melongena* (34.6%) and *Melastoma sp* (23.1%) (Figure 4.13 (a)). While the rest of pollen were considered as important minor pollens which consists of *Croton sp* (11.5%), *Polianthes tuberosa*, *Bombax ceiba*, and *Punica granatum* (7.7%) respectively. Also, *Acacia sp* and *Psidium guajava* contain same pollen grain frequency (3.9%).

There was no predominant pollen species had been identified in this honey sample. Thereby, *Solanum melongena* has been recognized as the most abundant pollen which accounted up to 34.6% of the pollen distribution. *Solanum melongena* or

commonly known as eggplant was a plant species in family solanaceae. It was widely grown in the region and crowned as King of vegetables and become an important ingredient in many dishes. Consequently, *Solanum melongena* possess several characteristics that influence the preference of pollen. It has Prolate, sub-spheroid, punctitegillate, furrows long, exinepsilate and thin and bilateral symmetry in morphology based on figure 4.13 (b). *Psidium guajava* and *Acacia sp* were the least with 3.9%. In Malay term called 'Jambu batu' and 'Pokok Akasia'. They were not often being seen in the Bangi area as the honey sample collected was an urban area. Eventually, the honeybees had their own preference for foraging activities. Besides, bees were more likely to visit plants with good nectar and appealing flora.

4.14 *Honey sample from Hulu Langat, Selangor*

Table 4.14: The number of pollen and percentage of abundance of pollen types in the honey sample from Hulu Langat (HL5)

Plant species	**Family**	**No of pollen**	**Percentage of Abundance (%)**
Cleidion brevipetiolatum	Euphorbiaceae	1	4.2%
Amaranthus lividus	Amaranthaceae	1	4.2%
Solanum melongena	Solanacaeae	5	20.8%
Ixora coccinea	Rubiaceae	17	70.8%

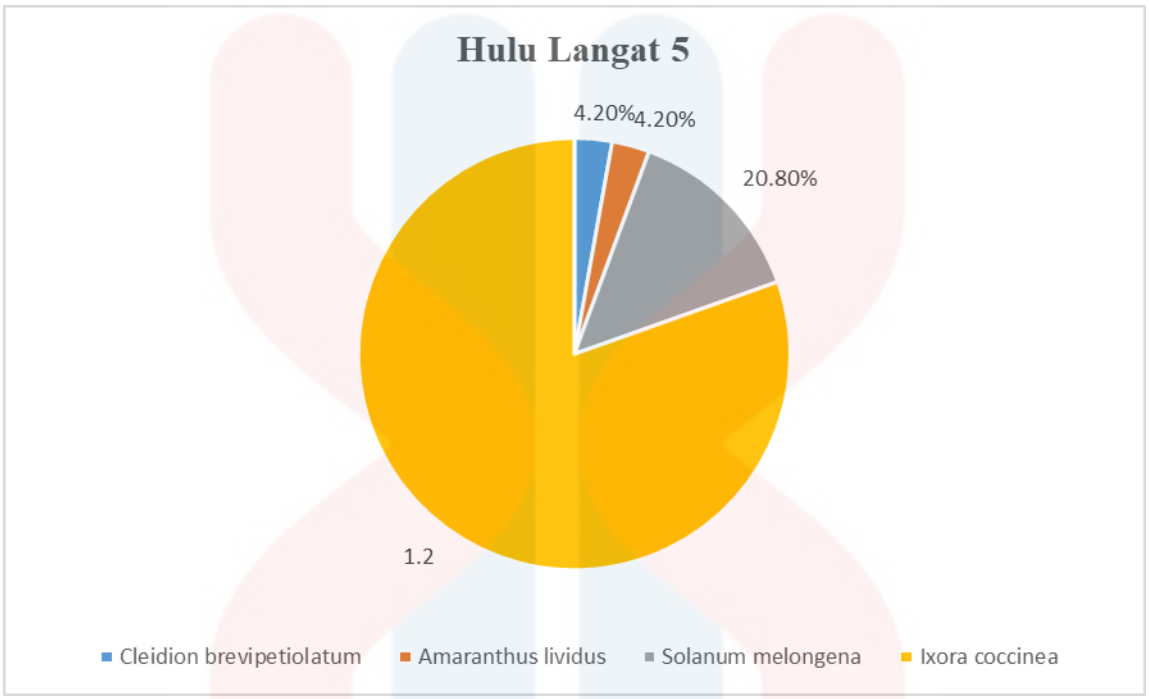

Figure 4.14: Pollen spectrum of honey sample from Hulu Langat, Selangor

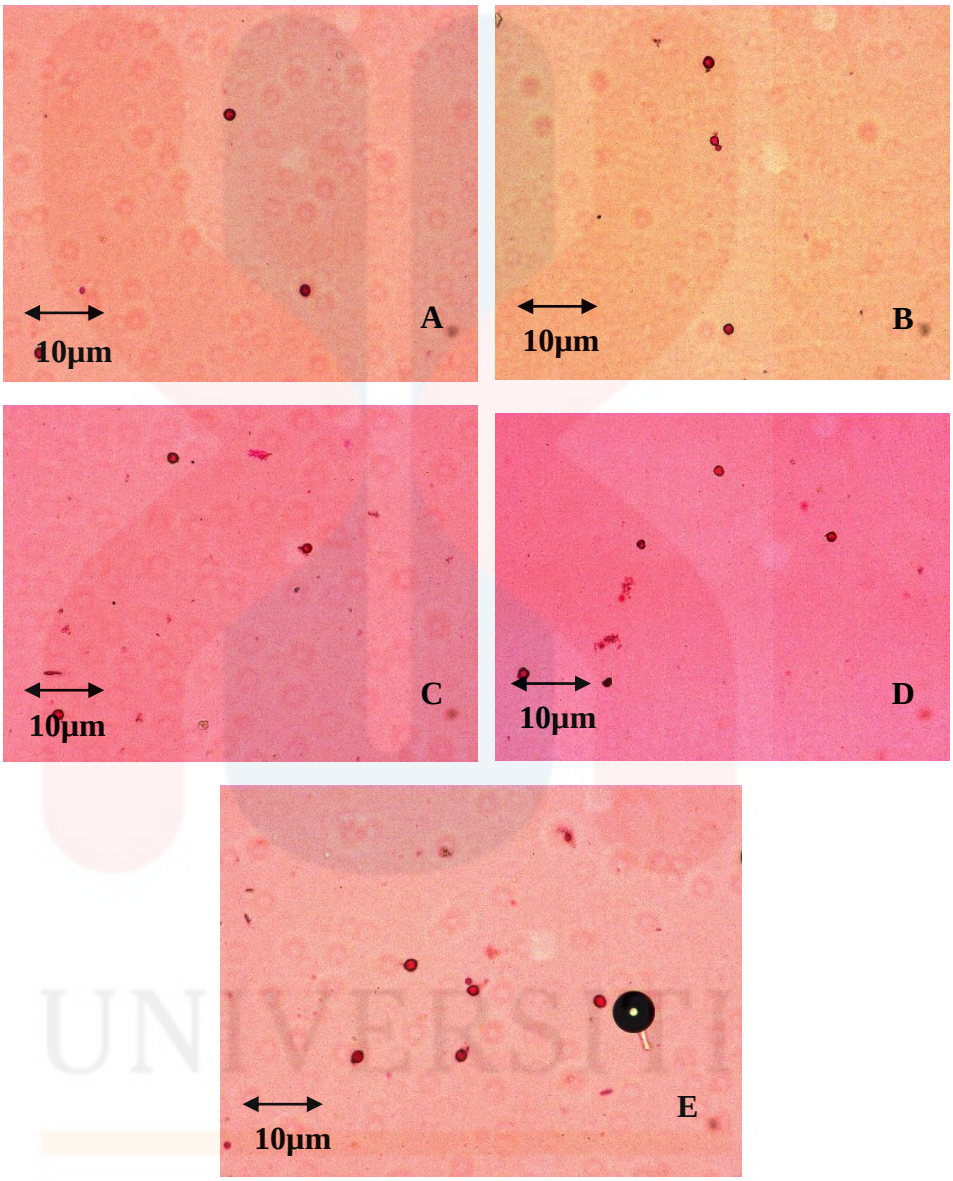

Figure 4.14 (a): Microscopic overviews of pollen density in honey sample from Hulu Langat 5 under 10x magnification. Overviews of A- Left edge of cover slip (top); B- Right edge of cover slip (top); C- Left edge of cover slip (bottom); D- Right edge of cover slip (bottom) and E- centre of the cover slip (middle)

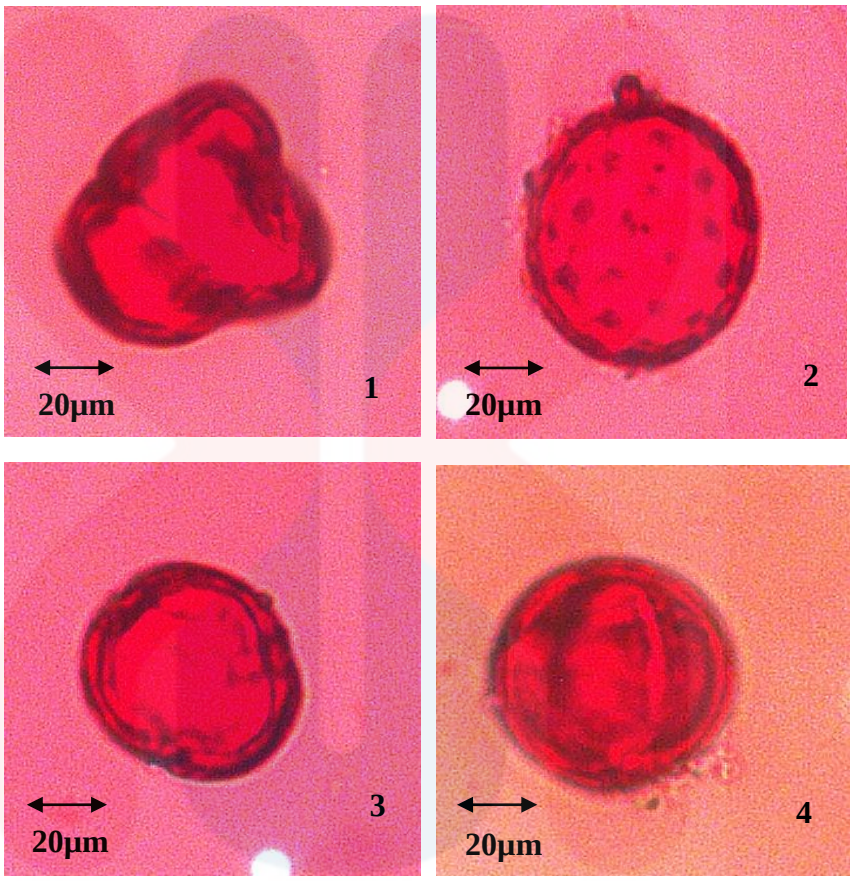

Figure 4.14 (b): Morphology of different pollen honey sample from Hulu Langat 5 under 40x magnification. 1- *Cleidion brevipetiolatum* (Euphorbiaceae); 2- *Amaranthus lividus* (Amaranthaceae); 3- *Solanum melongena* (Solanaceae) and 4- *Ixora coccinea* (Rubiaceae)

In the honey sample from Hulu Langat 5, only 4 different species of pollen were identified. There was a predominant in this honey sample was *Ixora coccinea* which had an abundancy 70.8%. *Ixora coccinea* or locally named as 'Jenjarum' from Rubiaceae family. It is a shrub that widely used as traditional medicine. It has four to six-inch globular clusters of bright red, orange, yellow, pink, or white tube-shaped flowers that bloom constantly under optimal conditions in full sunlight. Instead of color, recent studies also showed that shape and odor of flowers play important roles as the indications that enable the credit of rewarding resources. Azmi et al., (2015) also stated that flower attractiveness a positive effect on the number of pollinators. The pollen of *Ixora coccinea* was 3-colporate, prolate, spheroid shape, and radial symmetry. Next, *Solanum melongena* was the secondary pollen with 20.8% pollen count. Crops from Solanaceae family were common examples of bee-pollinated crops. Even though Solanum flowers lack of nectar, they manage a comparatively wealthy pollen load for bees that visit them (Jayasinghe et al., 2017).

There was also other important minor pollen source in this honey such as *Cleidion brevipetiolatum* (Euphorbiaceae) and *Amaranthus lividus* (Amaranthaceae) were the least frequency with 4.2% (Table 4.14). *Cleidon brevipetiolatum* was a species of tree in the Euphorbiaceae. Euphorbiaceae has approximately 8000 species with over 300 types. It was classified in the subfamily Acalyphoideae where pollen grain was tricolporate while pollen of *Amaranthus lividus* was poplyporate in its morphology whereas many pores were

dispersed throughout the pollen wall. Indian Amaranth was a perennial herb with yellow flowers which can be found near roadsides and open waste grounds. Therefore, it was critical to examine a large number of pollen grains from one family in order to gain a whole knowledge of different types within that family (Yang et al., 2020). It was clearly proved that the honey sample was grouped as multifloral honey due to the presence of different variety of pollen of this area.

4.15 *Honey sample from Batang Berjuntai, Selangor*

Table 4.15: The number of pollen and percentage of abundance of pollen types in the honey sample from Batang berjuntai (BB)

Plant species	**Family**	**No of pollen**	**Percentage of Abundance (%)**
Ruta graveolens	Rutaceae	2	1.9%
Solanum melongena	Solanaceae	68	63.0%
Cocos nucifera	Arecaceae	7	6.5%
Durio zibenthinus	Malvaceae	31	28.7%

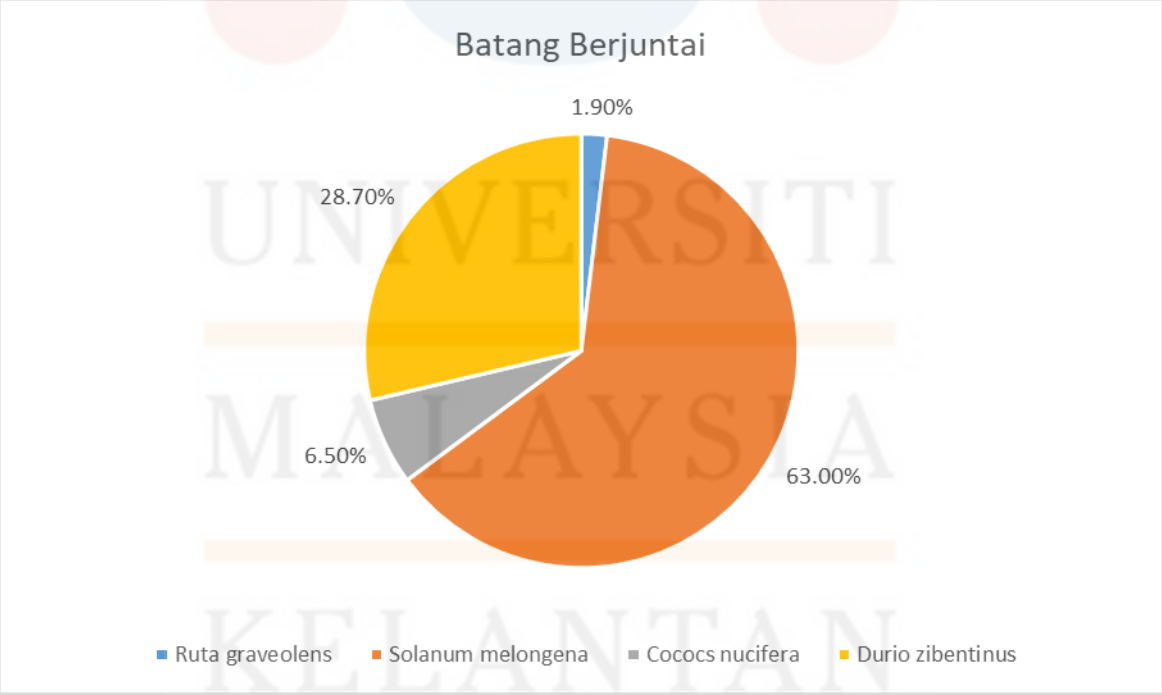

Figure 4.15: Pollen spectrum of honey sample from Batang Berjuntai, Selangor

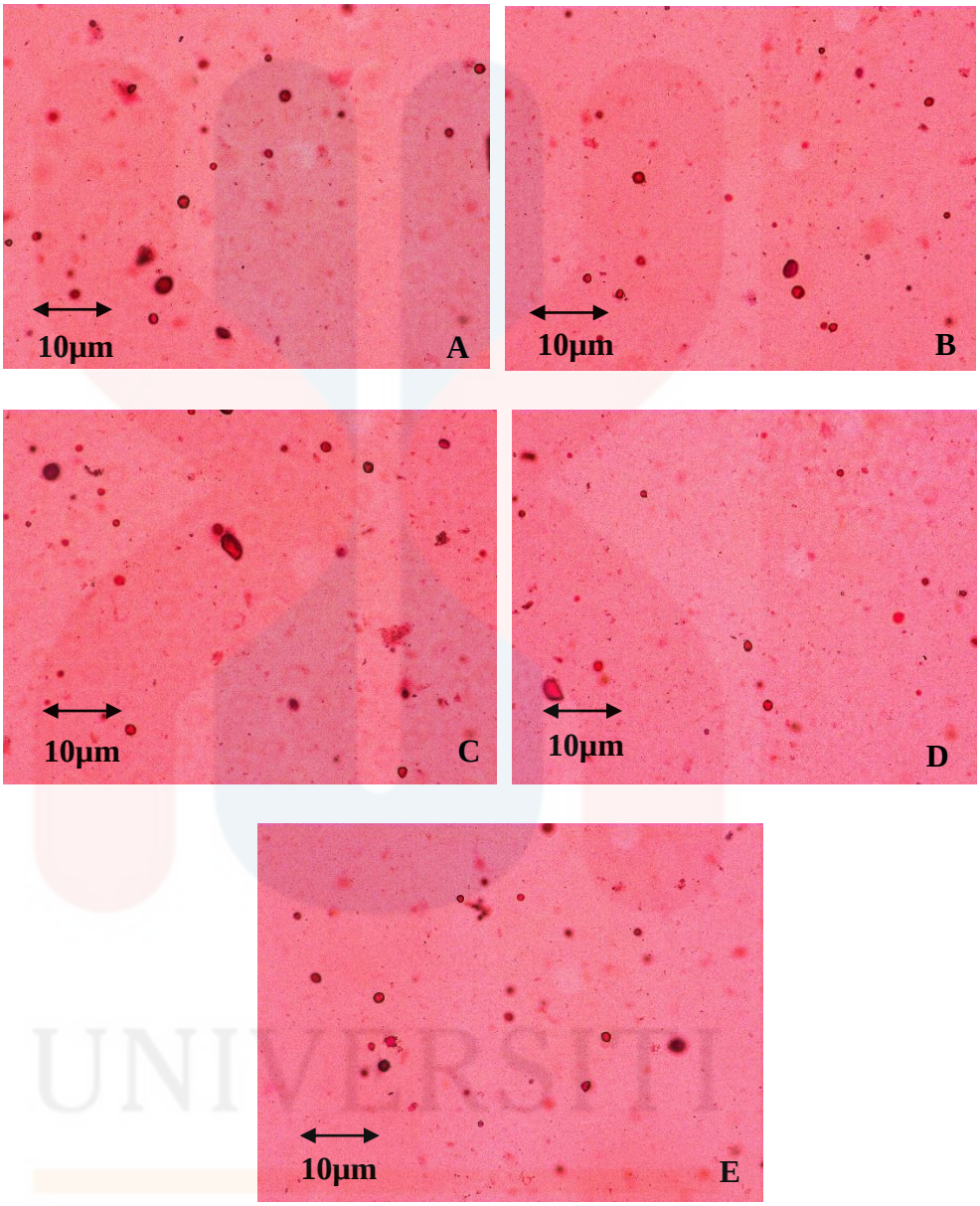

Figure 4.15 (a): Microscopic overviews of pollen density in honey sample from Batang Berjuntai under 10x magnification. Overviews of A- Left edge of cover slip (top); B- Right edge of cover slip (top); C- Left edge of cover slip (bottom); D- Right edge of cover slip (bottom) and E- centre of the cover slip (middle)

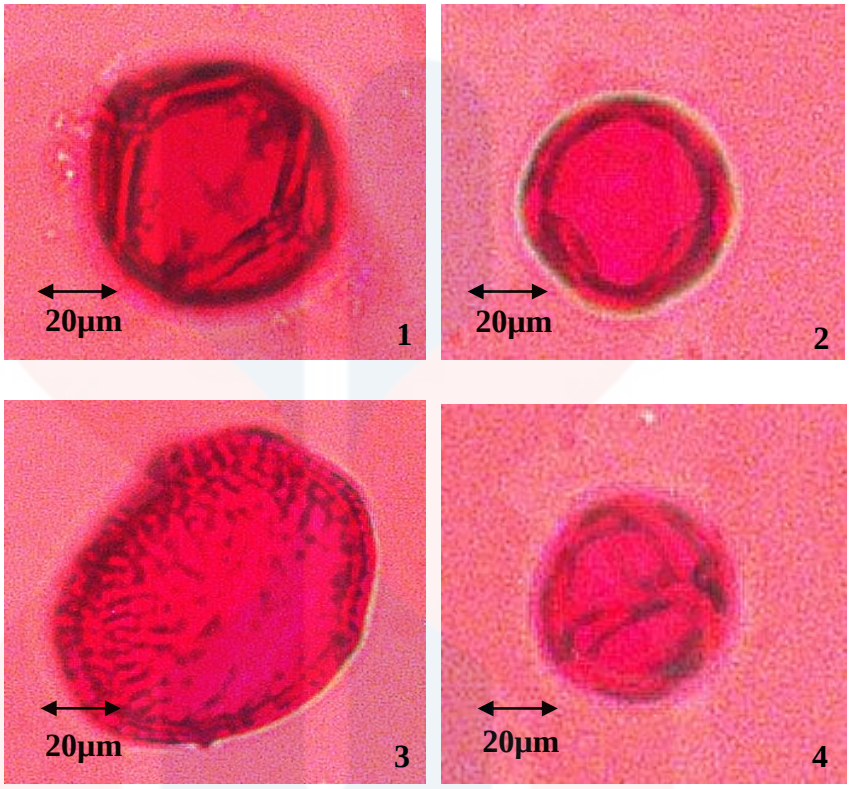

Figure 4.15 (b): Morphology of different pollen honey sample from Batang Berjuntai under 40x magnification. 1- *Ruta graveolens* (Rutaceae); 2- *Solanum melongena* (Solanaceae); 3- *Cocos nucifera* (Arecaceae) and 4- *Durio zibenthinus* (Malvaceae)

4 different types of pollen species were recognized from the sample in Batang Berjuntai such as *Ruta graveolens* (Rutaceae), *Solanum melongena* (Solanaceae), *Cocos nucifera* (Arecaceae) and *Durio zibenthinus* (Malvaceae). The highest percentage of pollen at Batang Berjuntai was from the species of *Solanum melongena* with 63.0%. The pollen grain was Prolate, sub-spheroid, punctitegillate, furrows long, exinepsilate and thin and bilateral symmetry (Figure 4.15 (b)). Most of the location in Selangor of honey sample

collected had this kind of plant species. It was clearly displayed that this plant is one of the essential pollen source and had donated to a great extent into honey bee secretion plus helping them in improving the growth of apiculture business in Malaysia. In this sample, there was only 1 type of secondary pollen had been observed which Durio zibenthinus with abundancy percentage 28.7%. The genus Durio is the Malayan name for the durian fruit. It was a native plant in Malaysian tropical rainforests. It was vital fruit tree grown in villages and agricultural plantations. It has bright yellow flowers that blooms at night and releasing strong odor to attract pollinators. *Durio zibenthinus* has strong and nice smell. Thus, this would indicate why these flower selected by honeybees in this current study. Flower scent and scent chemical profiles were identified as crucial attributes in captivating the pollinators (Azmi et al., 2015).

Besides, *Cocos nucifera* was classified as important minor pollen with 6.5% pollen distribution. The total pollen found in this sample a bit less compared to the amount of pollen found in other samples as the area was prominent for industrial activities. Hence, this location has less floral distinction than other area. Furthermore, *Ruta graveolens* has the least of percentage of abundance with 1.9%. Therefore, bees at Batang berjuntai region have less plants to forage in search of nectar and pollen.

The honey samples collected from Batang Berjuntai was Unifloral honey as it consists a pollen spectrum where one species was predominant. This microscopic analysis pollen of plants forged by bees allow to determine the source of honey in the area (Roopa et al., 2013).

4.16 *Honey sample from Petaling Jaya, Selangor*

Table 4.16: The number of pollen and percentage of abundance of pollen types in the honey sample from Petaling Jaya (PJ5)

Plant species	**Family**	**No of pollen**	**Percentage of Abundance (%)**
Acacia sp	Fabaceae	3	1.9%
Syzygium jambos	Myrtaceae	17	10.8%
Ricinus communis	Euphorbiaceae	4	2.5%
Moringo oleifera	Moringaceae	121	77.1%
Cocos nucifera	Arecaceae	4	2.5%
Cyperus laevigatus	Cyperaceae	6	3.8%
Croton sp	Euphorbiaceae	1	0.6%
Tecoma stans	Bignoniaceae	1	0.6%

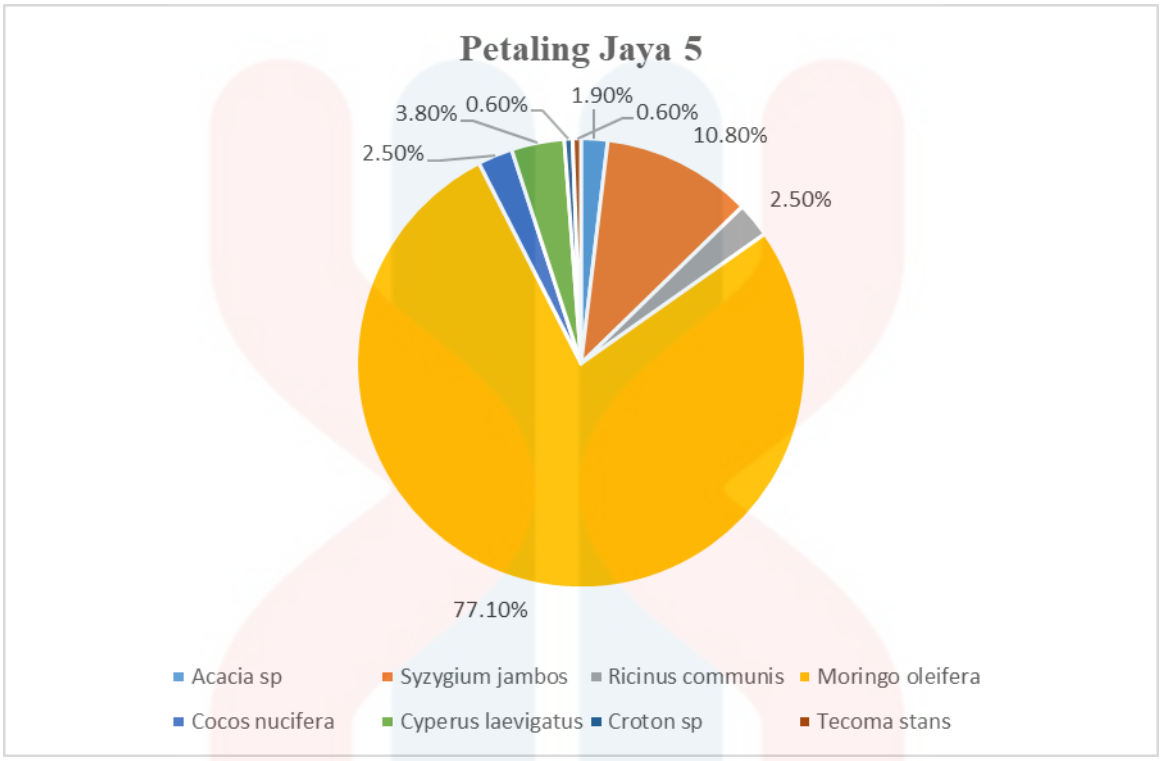

Figure 4.16: Pollen spectrum of honey sample from Petaling Jaya, Selangor

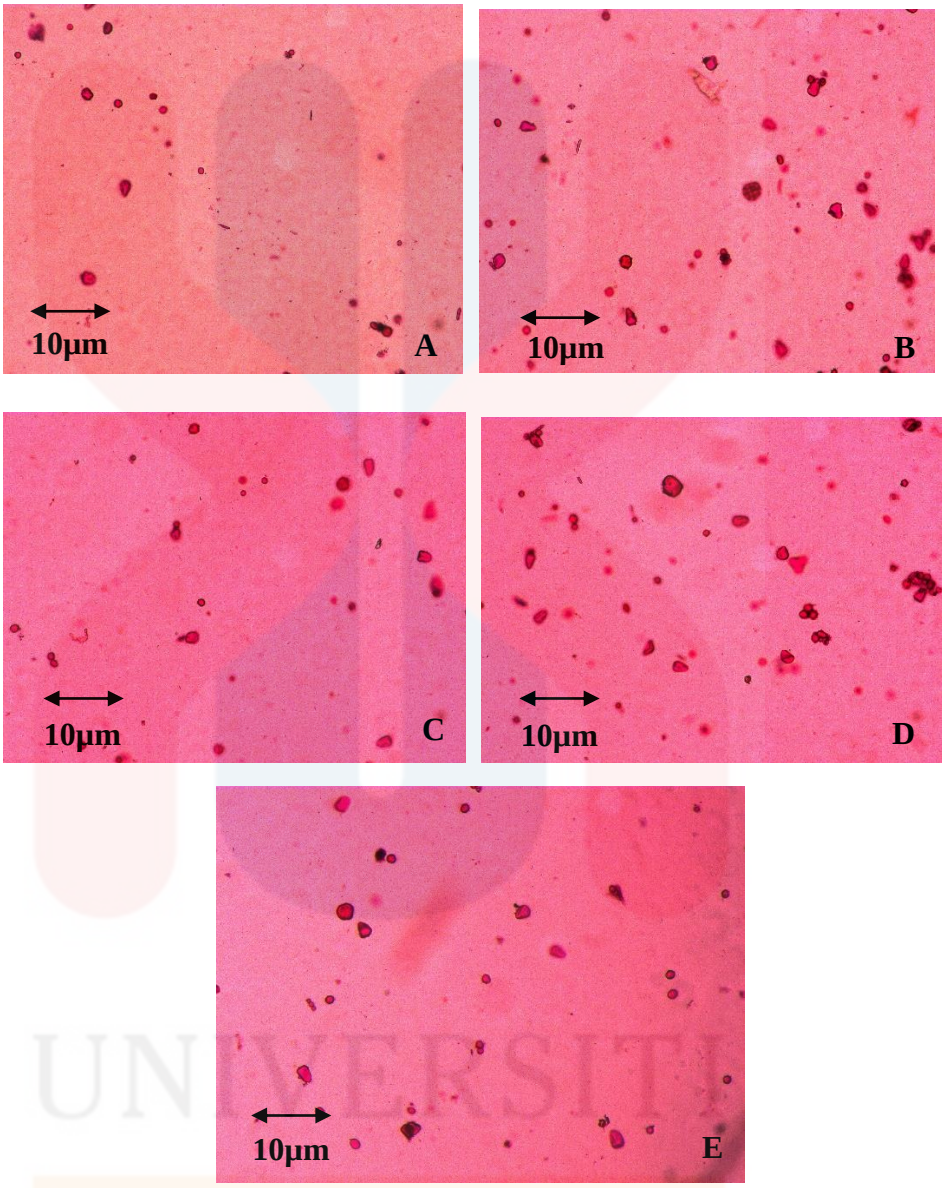

Figure 4.16 (a): Microscopic overviews of pollen density in honey sample from Petaling Jaya 5 10x magnification. Overviews of A- Left edge of cover slip (top); B- Right edge of cover slip (top); C- Left edge of cover slip (bottom); D- Right edge of cover slip (bottom) and E- centre of the cover slip (middle)

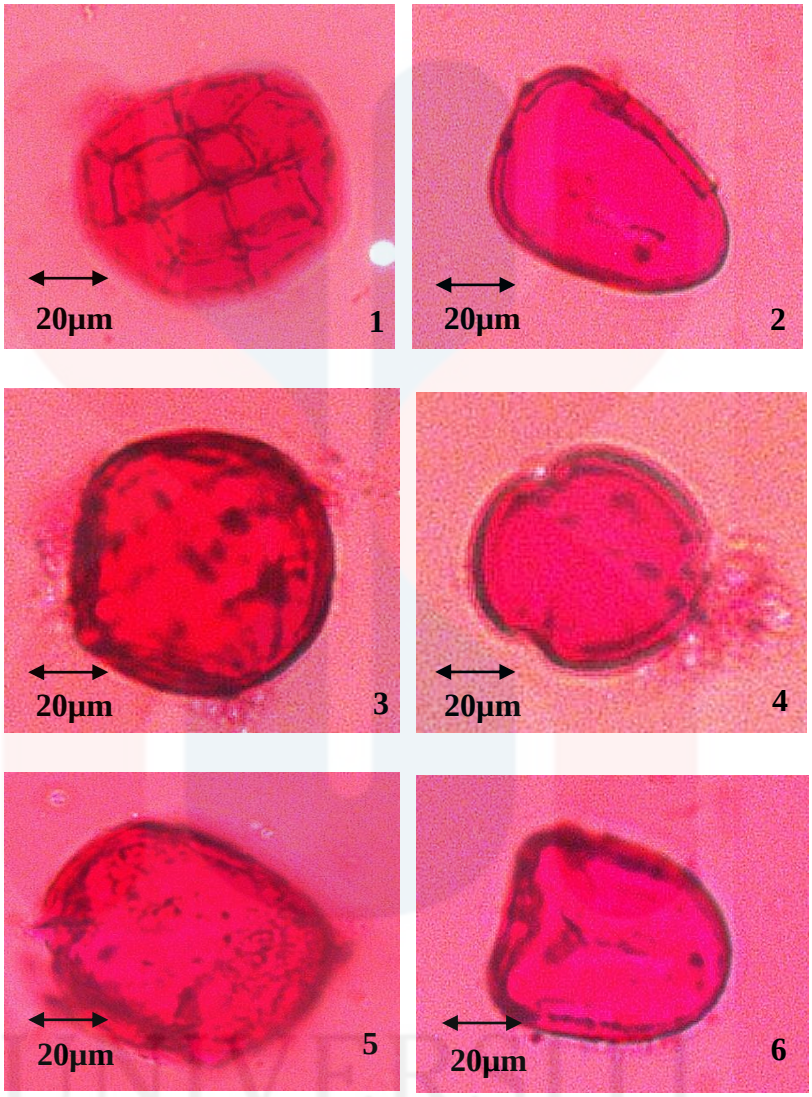

Figure 4.16 (b): Morphology of different pollen honey sample from Petaling Jaya 5 under 40x magnification. 1- *Acacia sp* (Fabaceae); 2- *Syzygium jambos* (Myrtaceae); 3- *Ricinus communis* (Euphorbiaceae); 4- *Moringo oleifera* (Moringaceae); 5- *Cocos nucifera* (Arecaceae) and 6- *Cyperus laevigatus* (Cyperaceae)

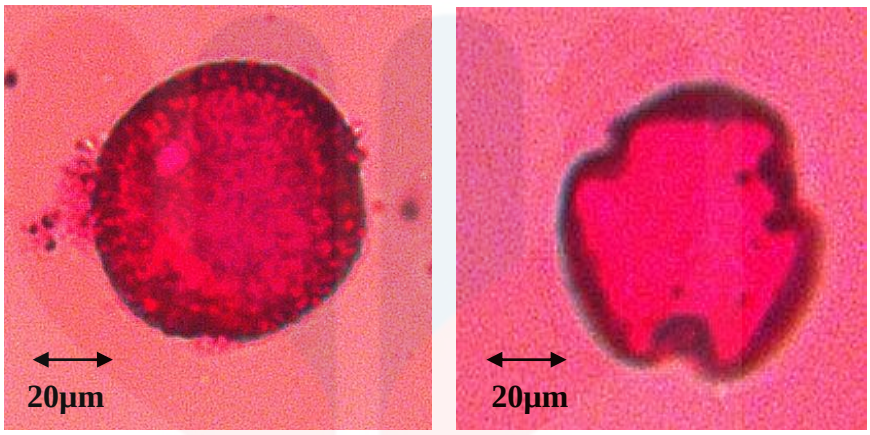

Figure 4.16 (c): Morphology of different pollen honey sample from Petaling Jaya 5 under 40x magnification. 7- *Croton sp* (Euphorbiaceae) and 8- *Tecoma stans* (Bignoniaceae)

From the honey samples collected in Petaling Jaya 5, there were 157 pollen grains found in slide under the compound microscope with camera. 8 different types of pollen species were present in the sample, such as *Acacia sp* (Fabaceae), *Syzygium jambos* (Myrtaceae), *Ricinus communis* (Euphorbiaceae), *Moringo oleifera* (Moringaceae), *Cocos nucifera* (Arecaceae), *Cyperus laevigatus* (Cyperaceae), *Croton sp* (Euphorbiaceae) and *Tecoma stans* (Bignoniaceae) based on table 4.16. *Moringo oleifera* got the highest percentage abundance of pollen in this sample which was 77.1%. *Moringo oleifera* is a scientific name of the family Moringaceae. It was originated from Indian and Malaysian people commonly called 'Pokok Kelar' as this plant species widely grown around the area. It has periporate shape and psilate exine surface. Moringa contain high nutritional value due

to the occurrence of a variation of important phytochemicals present in every part of the tree to meet demand for either dietary and commercialization (Gopalakrishnan et al., 2016). *Croton sp* and *Tecoma stans* were plant species that have the lowest percentage abundance which only got 0.6% of pollen spectrum. This two pollens had a retipilate and Tri-zonocolpate, morphological structure respectively.

The honey sample from Petaling Jaya 5 was unifloral honey as the total frequency of pollen grain more than 45% and they possess substantial commercial value since them owning exact physicochemical and organoleptic attributes, and specific tastes and flavors. Eventually, Petaling Jaya 5 has the most total pollen count compared with other location in Petaling Jaya. Pollen analysis of honey assist in generating a microscopic fingerprint of the environment from which the honey came and can serve as criteria for quality and origin control (Calaça et al., 2018).

4.17 *Honey sample from Bangi, Selangor*

Table 4.17: The number of pollen and percentage of abundance of pollen types in the honey sample from Bangi (BG4)

Plant species	**Family**	**No of pollen**	**Percentage of Abundance (%)**
Ricinus communis	Euphorbiaceae	560	31.8%
Amaranthus lividus	Amaranthaceae	6	0.3%
Ruta graveolens	Rutaceae	629	35.7%
Elaeis guinensis	Arecaceae	4	0.2%
Moringa pterygosperma	Moringaceae	126	7.2%
Ixora congesta	Rubiaceae	150	8.5%
Ipomoea indica	Convolvulaceae	6	0.3%
Gliricida sepium	Fabaceae	5	0.3%
Syzygium jambos	Myrtaceae	3	0.2%
Cocos nucifera	Arecaceae	6	0.3%
Tridax procumbens	Asteraceae	8	0.5%
Agava sp	Asparagaceae	6	0.3%
Acacia sp	Fabaceae	1	0.1%

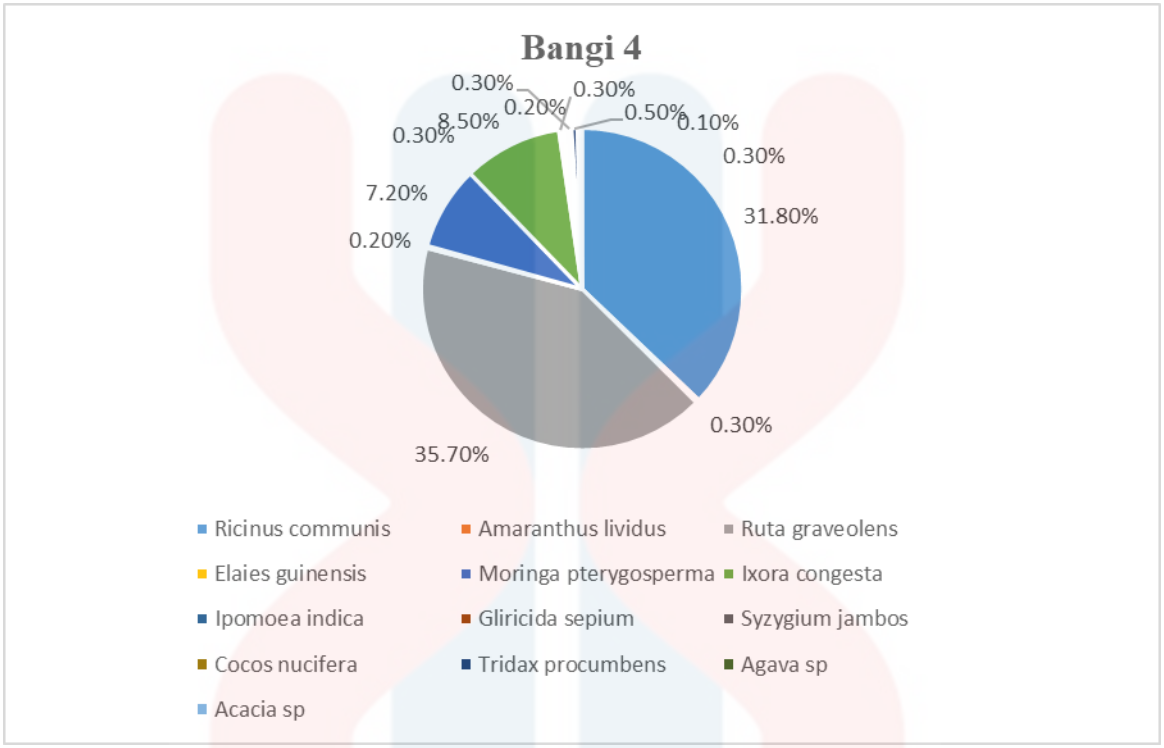

Figure 4.17: Pollen spectrum of honey sample from Bangi, Selangor

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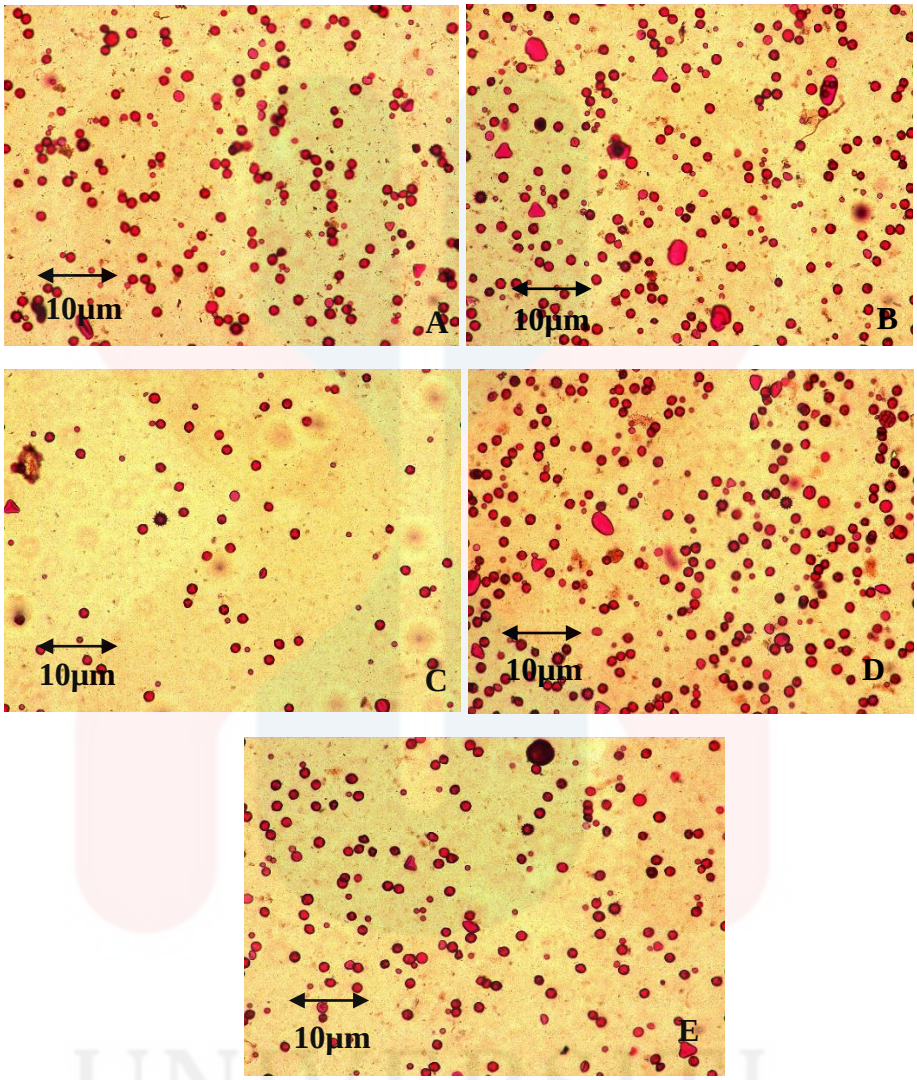

Figure 4.17 (a): Microscopic overviews of pollen density in honey sample from Bangi 4 10x magnification. Overviews of A- Left edge of cover slip (top); B- Right edge of cover slip (top); C- Left edge of cover slip (bottom); D- Right edge of cover slip (bottom) and E- centre of the cover slip (middle)

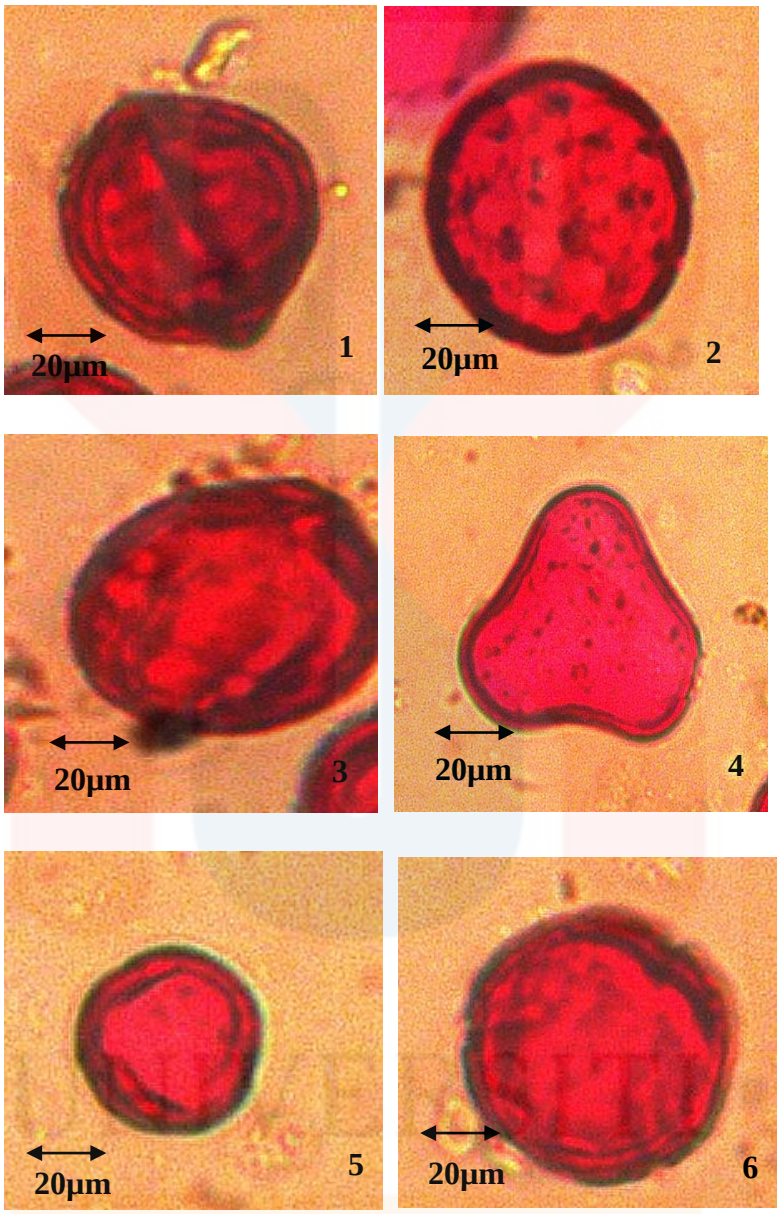

Figure 4.17 (b): Morphology of different pollen honey sample from Bangi 4 under 40x magnification. 1- *Ricinus communis* (Euphorbiacea); 2- *Amaranthus lividus* (Amaranthaceae); 3- *Ruta graveolens* (Rutaceae); 4- *Elaeis guinensis* (Arecaceae); 5- *Moringa pterygosperma* (Moringaceae) and 6- *Ixora congesta* (Rubiaceae)

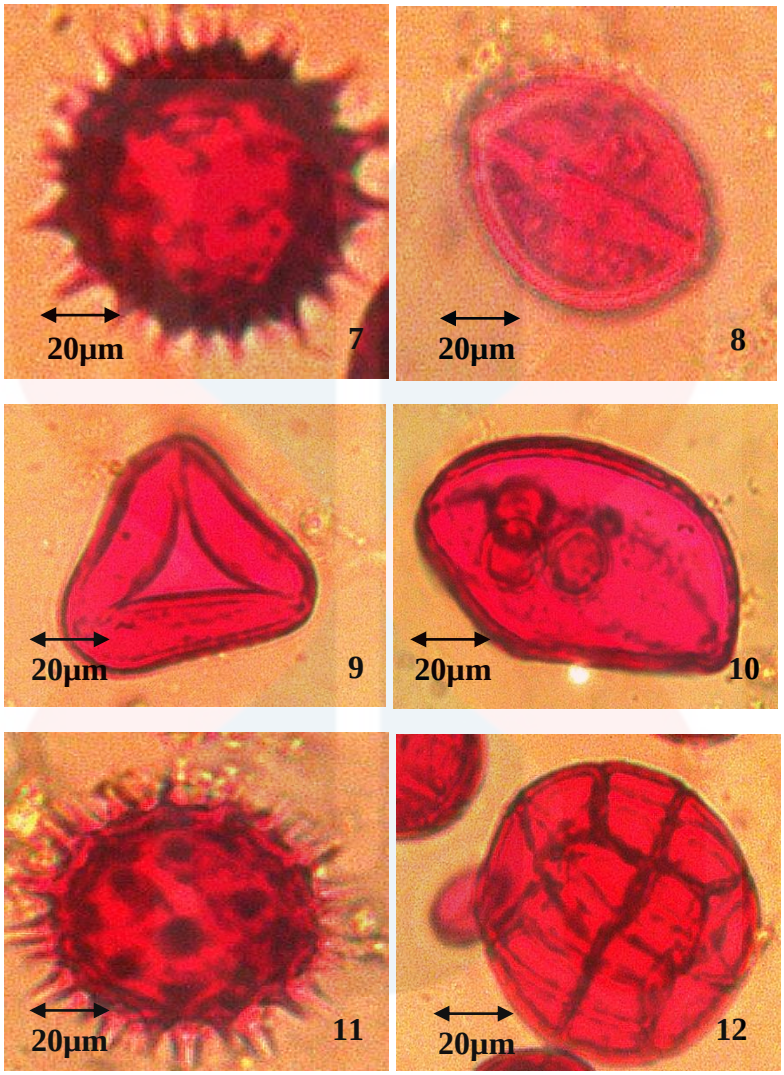

Figure 4.17 (c): Morphology of different pollen honey sample from Bangi 4 under 40x magnification. 7- *Ipomoea indica* (Convolvulaceae); 8- *Gliricida sepium* (Fabaceae); 9- Syzygium jambos (Myrtaceae); 10- *Cocos nucifera* (Arecaceae); 11- *Tridax procumbens* (Asteraceae) and 12- *Acacia sp* (Fabaceae)

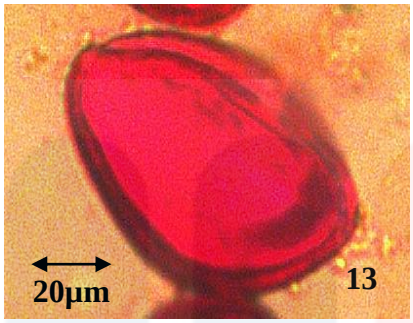

Figure 4.17 (d): Morphology of different pollen honey sample from Bangi 4 under 40x magnification. 13- *Agava sp* (Asparagaceae)

Analysis on the honey sample from Bangi 4 area revealed the presence of 13 different types of pollen species. Among the types of pollen species found in this sample were *Ricinus communis* (Euphorbiacea), *Amaranthus lividus* (Amaranthaceae), *Ruta graveolens* (Rutaceae), *Elaeis guinensis* (Arecaceae), *Moringa pterygosperma* (Moringaceae), *Ixora congesta* (Rubiaceae), *Ipomoea indica* (Convolvulaceae), *Gliricida sepium* (Fabaceae), *Syzygium jambos* (Myrtaceae), *Cocos nucifera* (Arecaceae), *Tridax procumbens* (Asteraceae), *Acacia sp* (Fabaceae) and *Agava sp* (Asparagaceae). *Ruta graveolens* was more dominant pollen in this honey sample with an abundancy of 35.7% followed closely by pollen grains from *Ricinus communis* 31.8% which is the dominant plant species in the area where beehives are placed, and pollen grains from *Acacia sp* being the least with 0.1% frequency (Table 4.17). *Ruta graveolens* has a colporoidate shape and exinepsilate surface.

Ixora congesta and *Moringa pterygosperma* were an example of important minor pollen present in Bangi 4. The pollen grain is pericolpate and subolate in morphology. *Ixora congesta* was a scientific name in Rubiaceae family. People locally called it 'Jarum-jarum'. This plant was widely distributed in Peninsular Malaysia. It has bright yellow and turning reddish-orange shape of flower that seasonally produced. Yet, it was scentless as a result honey bees were not preferring for foraging. Therefore, Flower scent is important factor to attract the pollinators. While, *Moringa pterygosperma* has tricolporate and spheroidal in shape.

The rest 9 species were categorized as minor pollen due to their pollen count which were less than 3% such as *Amaranthus lividus* (Amaranthaceae), *Elaeis guinensis* (Arecaceae), *Ipomoea indica* (Convolvulaceae), *Gliricida sepium* (Fabaceae), *Syzygium jambos* (Myrtaceae), *Cocos nucifera* (Arecaceae), *Tridax procumbens* (Asteraceae), *Acacia sp* (Fabaceae) and *Agava sp* (Asparagaceae). Also, the honey samples collected from Bangi 4 of different location of Selangor was multifloral as did not have any predominant taxa and as it has different proportions of pollen taxon distribution. Thus, Bangi 4 contain highest total pollen count compared to other honey sample that had been observed.

4.18 *Honey sample from Hulu Langat, Selangor*

Table 4.18: The number of pollen and percentage of abundance of pollen types in the honey sample from Hulu Langat (HL6)

Plant species	**Family**	**No of pollen**	**Percentage of Abundance (%)**
Cassia fistula	Fabaceae	11	6.4%
Ixora congesta	Rubiaceae	2	1.2%
Tridax procumbens	Asteraceae	10	5.8%
Ruta graveolens	Rutaceae	7	4.0%
Ipomoea indica	Convolvulaceae	1	0.6%
Rosa canina	Rosaceae	4	2.3%
Mimosa pudica	Fabaceae	74	65.5%
Acacia sp	Fabaceae	4	2.3%

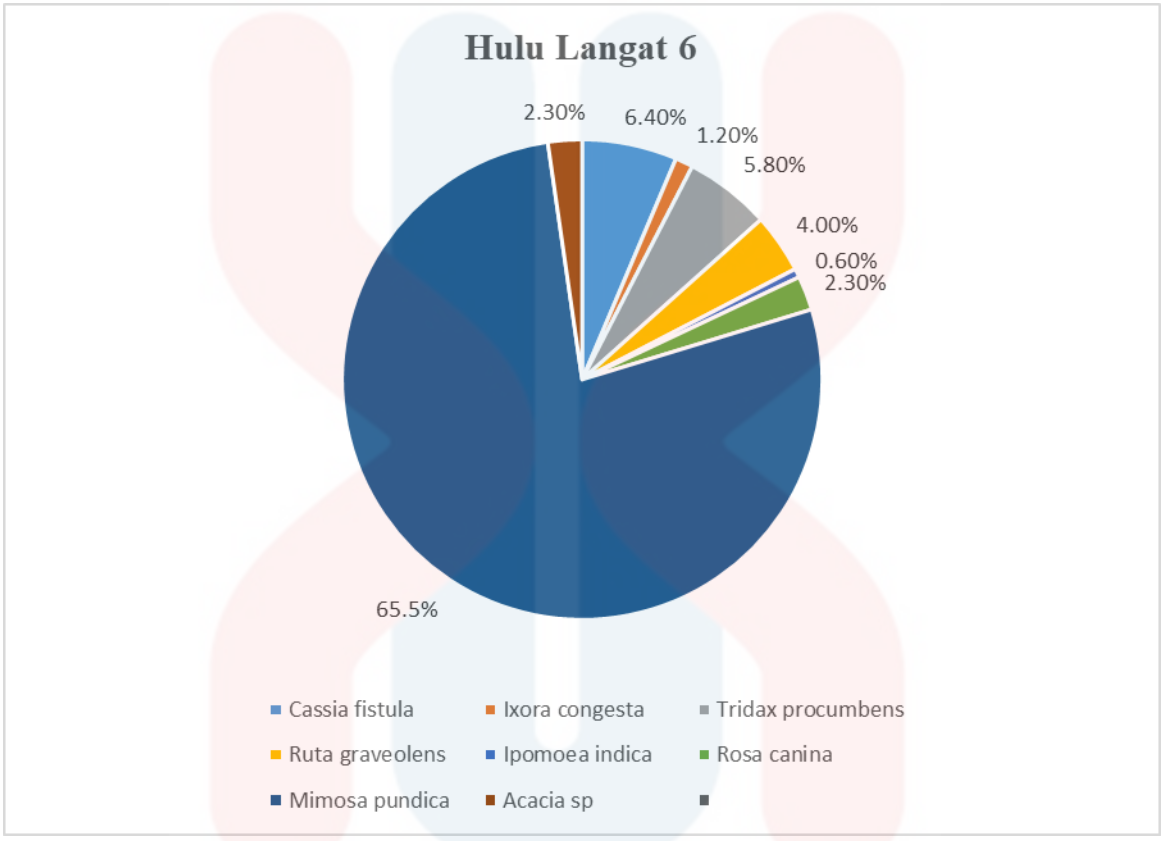

Figure 4.18: Pollen spectrum of honey sample from Hulu Langat, Selangor

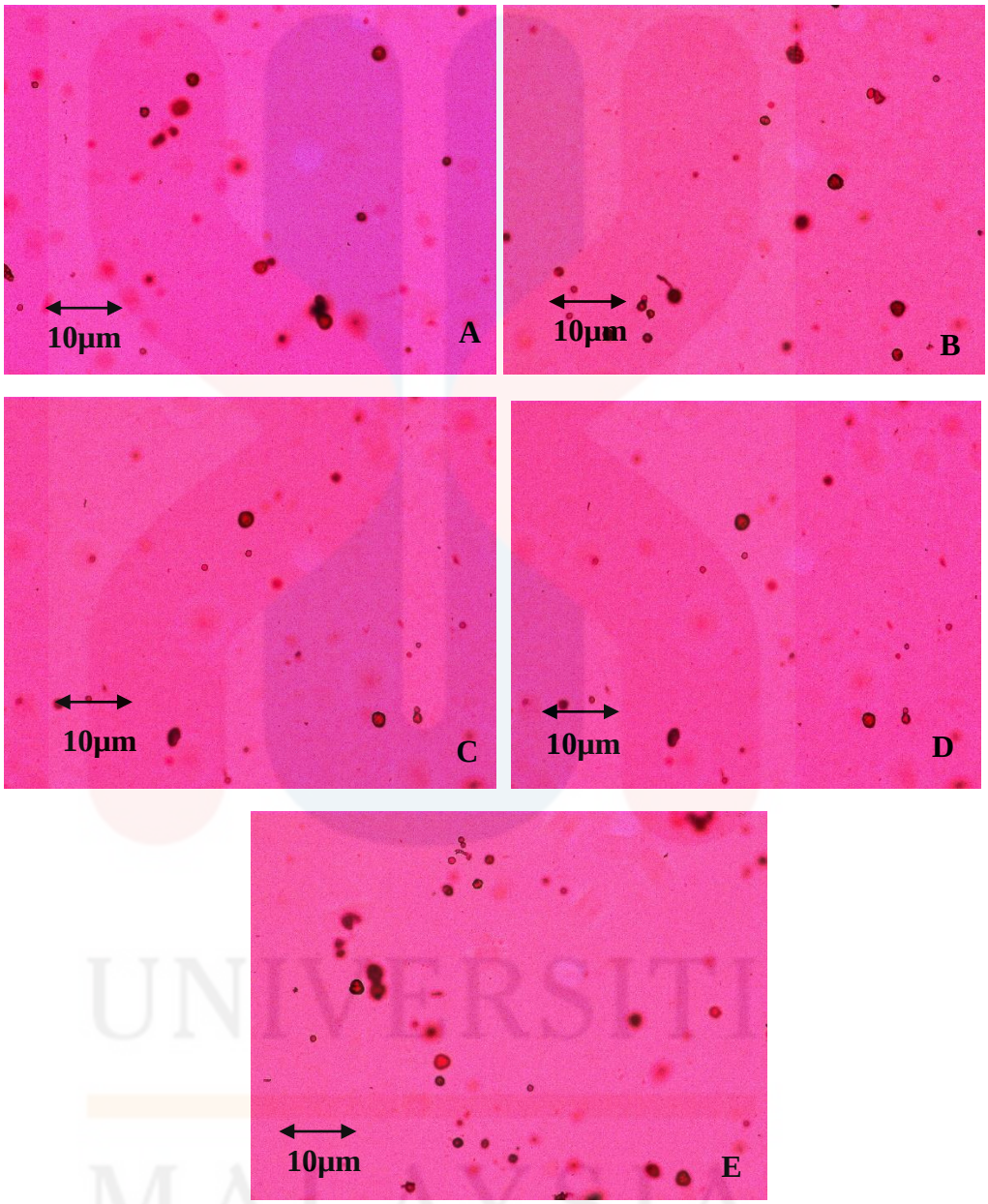

Figure 4.18 (a): Microscopic overviews of pollen density in honey sample from Hulu Langat 6 10x magnification. Overviews of A- Left edge of cover slip (top); B- Right edge of cover slip (top); C- Left edge of cover slip (bottom); D- Right edge of cover slip (bottom) and E- centre of the cover slip (middle)

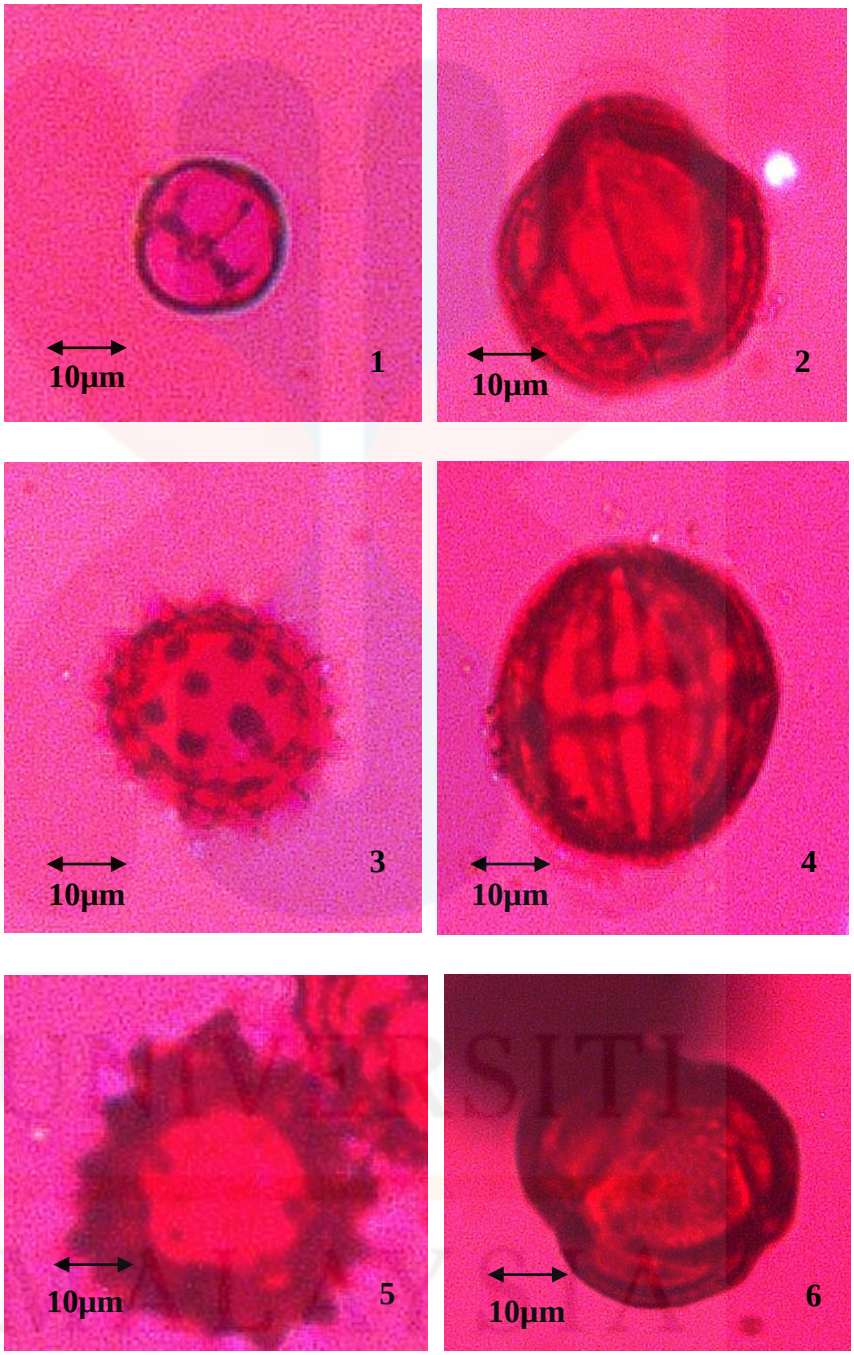

Figure 4.18 (b): Morphology of different pollen honey sample from Hulu Langat 6 under 40x magnification. 1- *Cassia fistula* (Fabaceae); 2- *Ixora congesta* (Rubiaceae); 3- *Tridax procumbens* (Asteraceae); 4- *Ruta graveolens* (Rutaceae); 5- *Ipomoea indica* (Convolvulaceae) and 6- *Rosa canina* (Rosaceae)

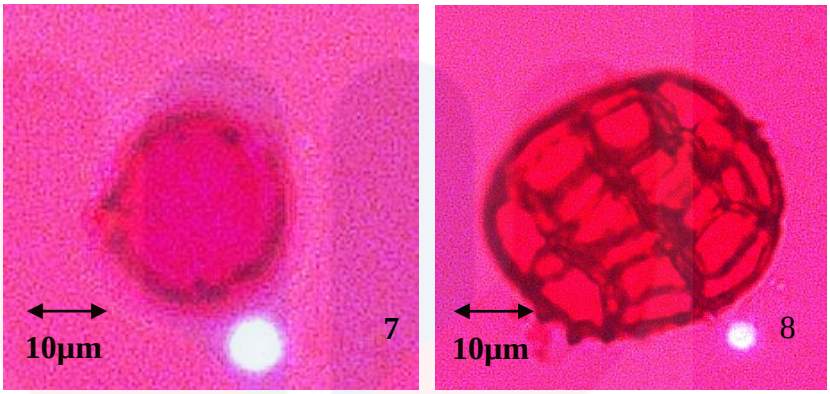

Figure 4.18 (c): Morphology of different pollen honey sample from Hulu Langat 6 under 40x magnification. 7- *Mimosa pudica* (Fabaceae) and 8- *Acacia sp* (Fabaceae)

There were 8 different types of pollen identified from the honey collected in Hulu Langat 6. The most abundance plant species was Mimosa pudica from Fabaceae with percentage abundance, 65.5%. In Malay term, it's called as 'Pokok Semalu'. This result clearly proved that honeybees preferred to forage this plant which flowers intermittently throughout the year at Hulu Langat district. *Mimosa pudica* was a native of tropical America and naturalized nearly all through the Malaysia include Selangor state. This plant has the smallest pollen among other species. The pollen grain exists in tetrads with spheroidal shape. Also, it was pantocolpate pollen grain. Obviously, size of flower was the other factor of pollen preferences. Other samples that contributed as important minor pollen such as *Cassia fistula*, *Tridax procumbens*, and *Ruta graveolens*. The least amount of pollen has been found in this sample was came from Convolvulaceae family with plant species of *Ipomea indica*

pollen. It had Pantoporate morphological structure with echinate surface. Based on the result shown, eventually the honey bees in Hulu Langat region more preferred to forage flowers from the species and families mentioned above compared to others. Honey bee forage for pollen and nectar to support their colonies as well as producing honey (Ara Begum et al., 2021). This honey sample collected area was unifloral as these samples had achieved predominant proportion of pollen taxon distribution where the percentage of abundance reached more than 45%. Presence of variation of pollen spectrum makes it possible to gain clear evidence of determining the particular geographical location.

4.19 *Honey sample from Shah Alam, Selangor*

Table 4.19: The number of pollen and percentage of abundance of pollen types in the honey sample from Shah Alam (SA2)

Plant species	**Family**	**No of pollen**	**Percentage of Abundance (%)**
Cocos nucifera	Arecaceae	1	0.5%
Mimosa pundica	Fabaceae	7	3.2%
Commelina diffusa	Commelinaceae	205	92.3%
Solanum melongena	Solanaceae	2	0.9%
Gliricida sepium	Fabaceae	2	0.9%
Mimusops elengi	Sapotaceae	4	1.8%
Ruta graveolens	Rutaceae	1	0.5%

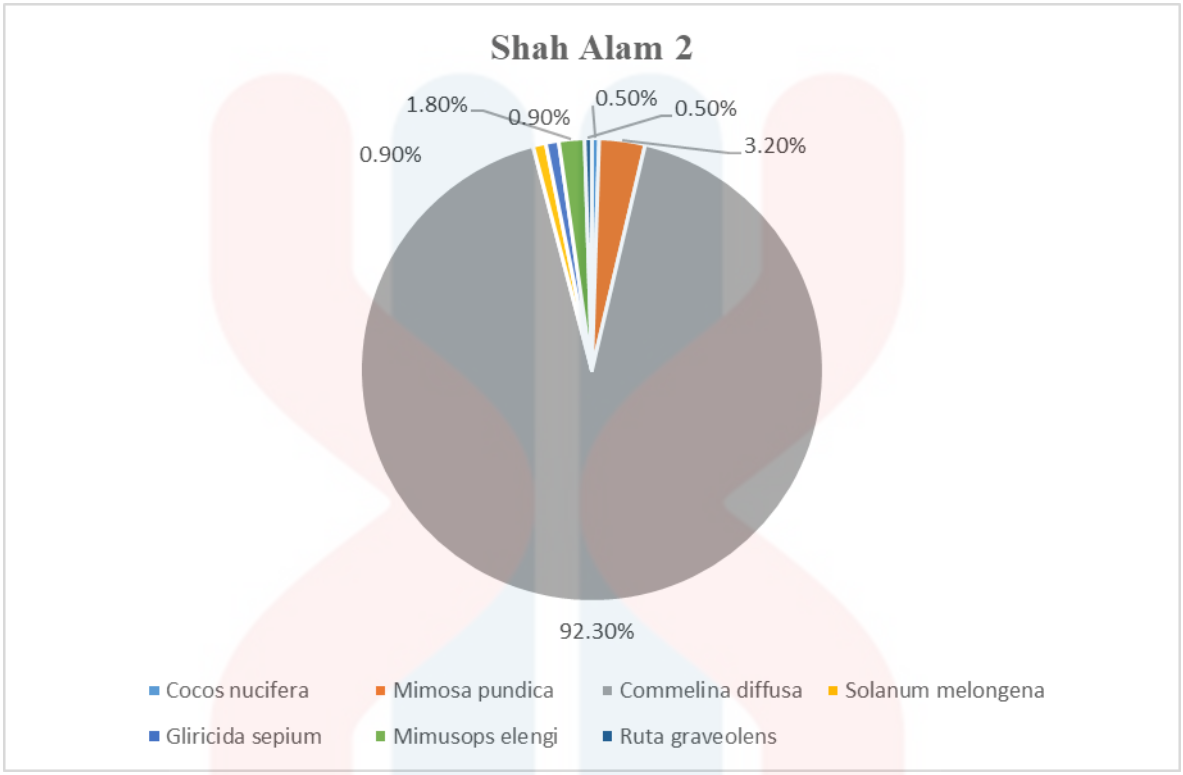

Figure 4.19: Pollen spectrum of honey sample from Shah Alam, Selangor

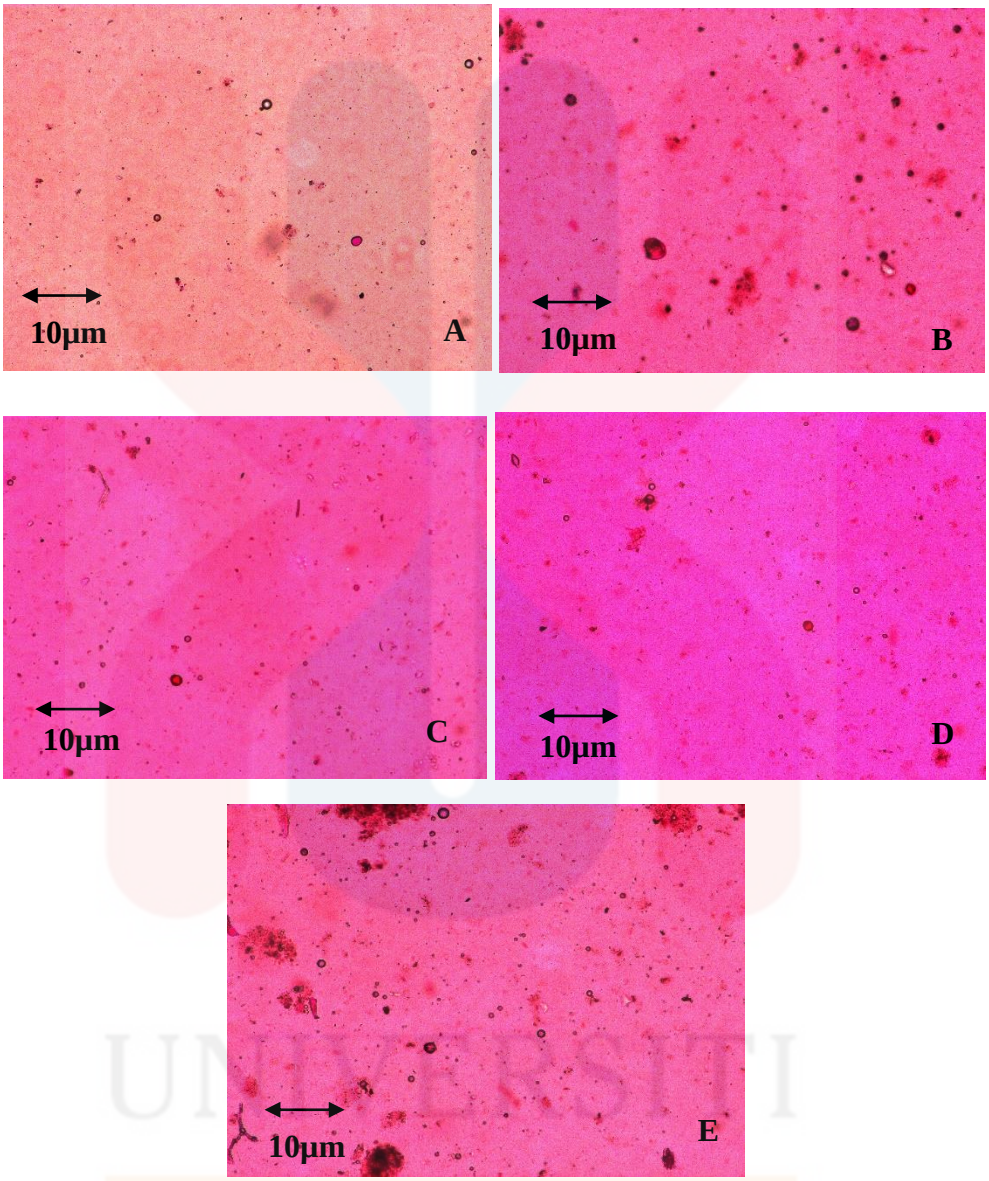

Figure 4.19 (a): Microscopic overviews of pollen density in honey sample from Shah Alam 2 under 10x magnification. Overviews of A- Left edge of cover slip (top); B- Right edge of cover slip (top); C- Left edge of cover slip (bottom); D- Right edge of cover slip (bottom) and E- centre of the cover slip (middle)

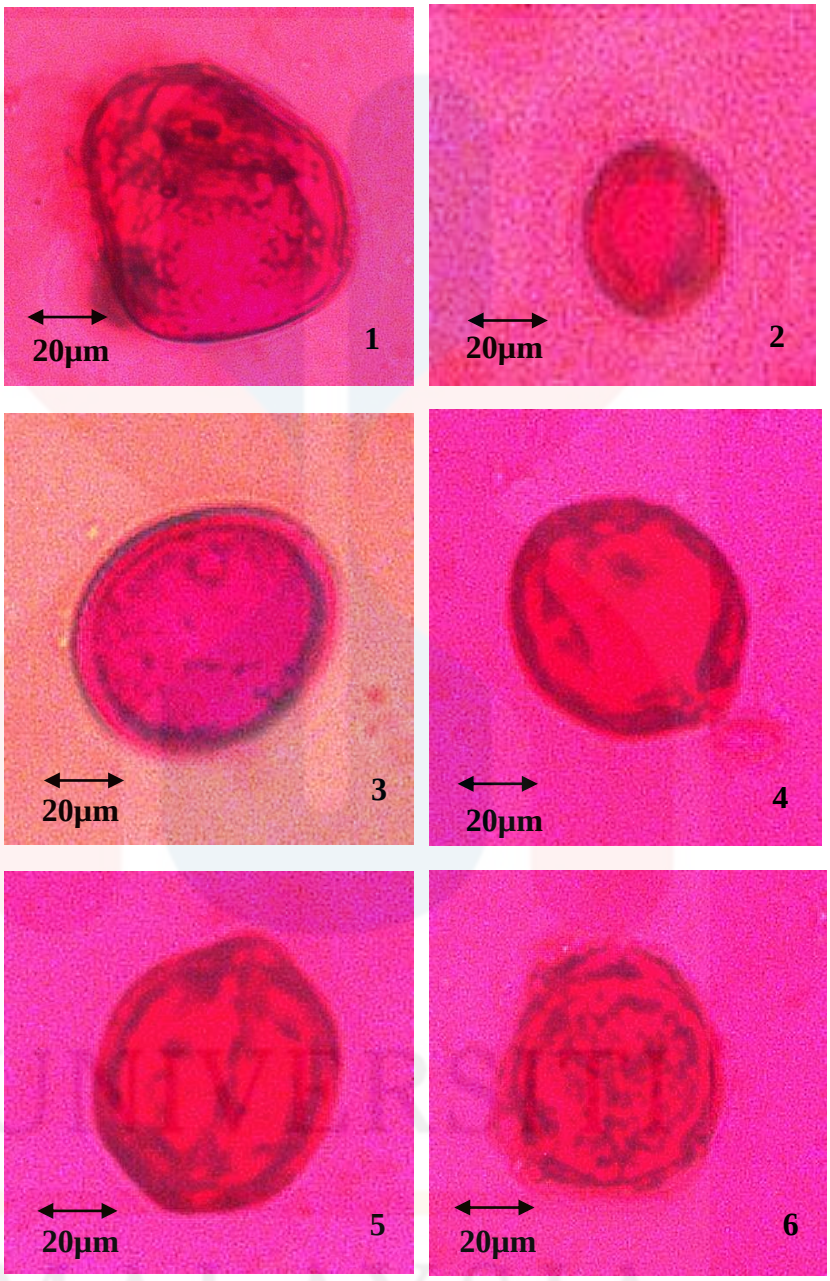

Figure 4.19 (b): Morphology of different pollen honey sample from Shah Alam 2 under 40x magnification. 1- *Cocos nucifera* (Arecaceae); 2- *Mimosa pundica* (Fabaceae); 3- *Commelina diffusa* (Commelinaceae); 4- *Solanum melongena* (Solanaceae); 5- *Gliricida sepium* (Fabaceae) and 6- *Mimusops elengi* (Sapotaceae)

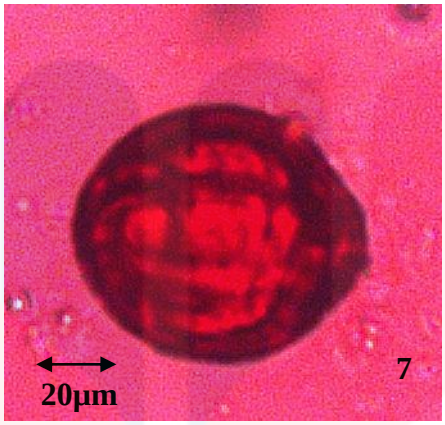

Figure 4.19 (c): Morphology of different pollen honey sample from Shah Alam 2 under 40x magnification. 7- *Ruta graveolens* (Rutaceae)

From the honey samples collected in Shah Alam 2, there were 222 pollen grains found in a slide under the compound microscope with camera. 7 different types of pollen species were present in the sample, such as *Cocos nucifera* (Arecaceae), Mimosa pundica (Fabaceae), *Commelina diffusa* (Commelinaceae), *Solanum melongena* (Solanaceae), *Gliricida sepium* (Fabaceae), *Mimusops elengi* (Sapotaceae) and *Ruta graveolens (*Rutaceae). *Commelina diffusa* got the highest percentage abundance of pollen in this sample which was 92.3%. It has elliptical shape with narrow furrow on one side. The flowers were usually brightly colored comprises of blue color of the petals, as well as the bright yellow color of the lateral and central anthers stipulates their ability to attract honeybees. Moreover, flower enchantment can influence on the abundancy and frequency of the flower pollinators.

Cocos nucifera and *Ruta graveolens* plant species were identified as the lowest percentage abundance which only got 0.5% of pollen spectrum. Pollen grain of *Cocos nucifera* had was a pantocolpate with spheroidal in shape. Meanwhile, *Ruta Graveolens* was colporoidate with exinepsilate in shape. Therefore, honey sample from Shah Alam 2 was unifloral honey since, there was a predominant pollen identified and *Commelina diffusa* was considered rich in the honey sample of this region. Melissopalynology was useful for recognizing the flowers used by honeybees and develop the preservation status of economically plants (Roopa et al., 2013).

4.20 *Honey sample from Rawang, Selangor*

Table 4.20: The number of pollen and percentage of abundance of pollen types in the honey sample from Rawang (RG)

Plant species	**Family**	**No of pollen**	**Percentage of Abundance (%)**
Garnicia hombroniana	Clusiaceae	2	1.7%
Punica granatum	Punicaceae	14	11.8%
Solanum melongena	Solanaceae	26	21.8%
Pongamia pinnata	Fabaceae	16	13.4%
Bauhinia purpurea	Fabaceae	34	28.6%
Cocos nucifera	Arecaceae	12	10.1%
Ixora congesta	Rubiaceae	11	9.2%
Lolium multiflorum	Poaceae	2	1.7%
Moringo oleifera	Moringaceae	1	0.8%
Tecoma stans	Bignoniaceae	1	0.8%

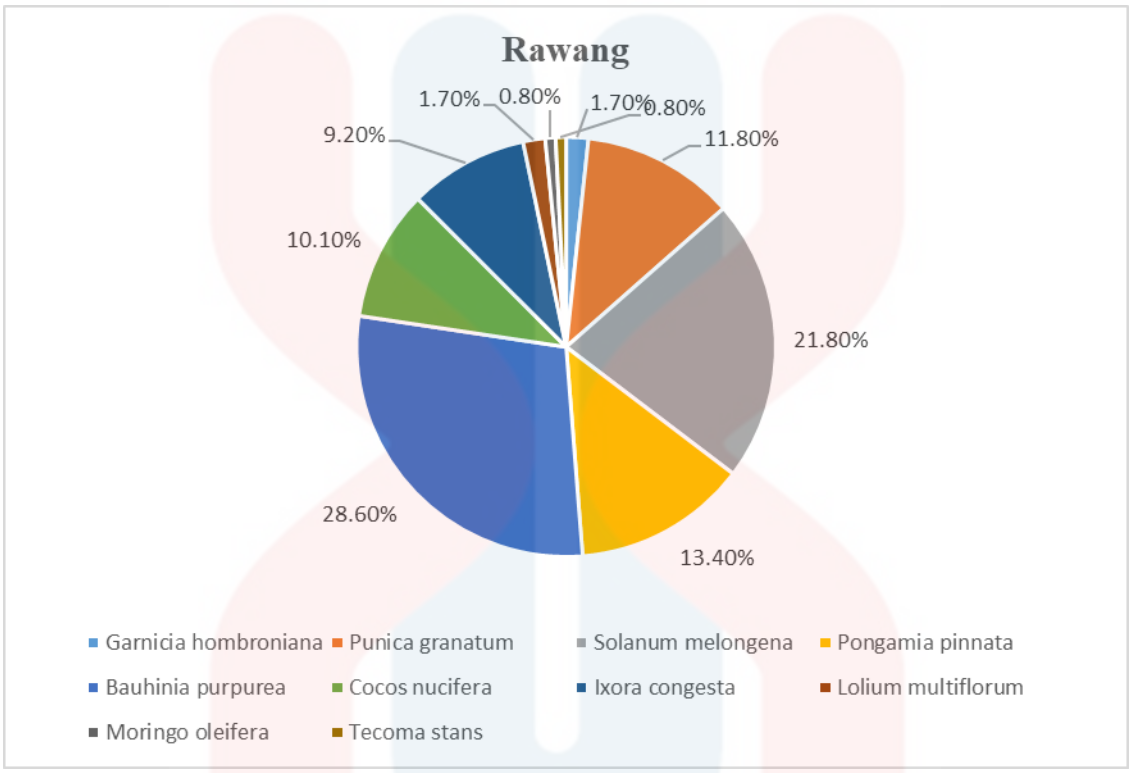

Figure 4.20: Pollen spectrum of honey sample from Rawang, Selangor

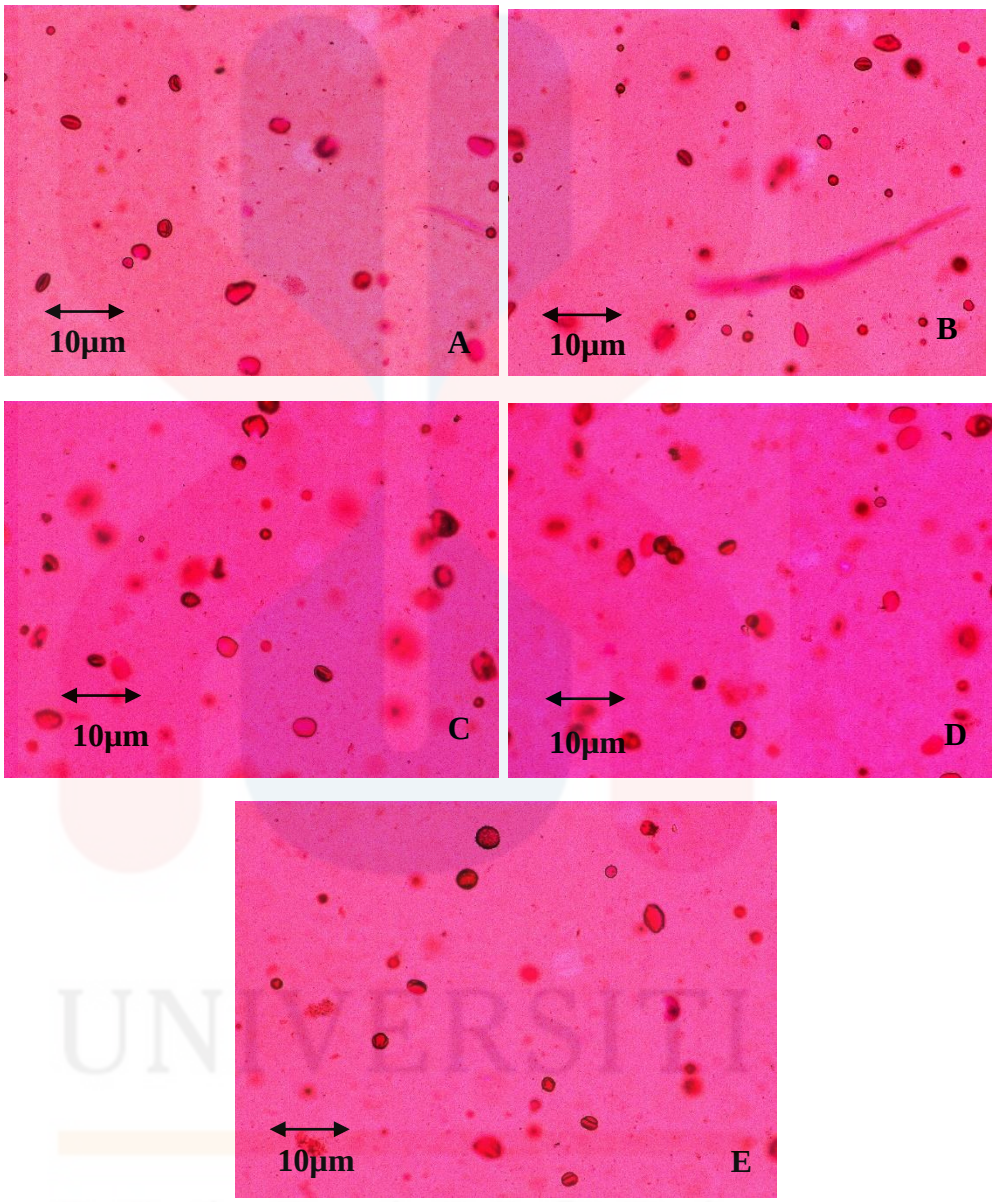

Figure 4.20 (a): Microscopic overviews of pollen density in honey sample from Rawang under 10x magnification. Overviews of A- Left edge of cover slip (top); B- Right edge of cover slip (top); C- Left edge of cover slip (bottom); D- Right edge of cover slip (bottom) and E- centre of the cover slip (middle)

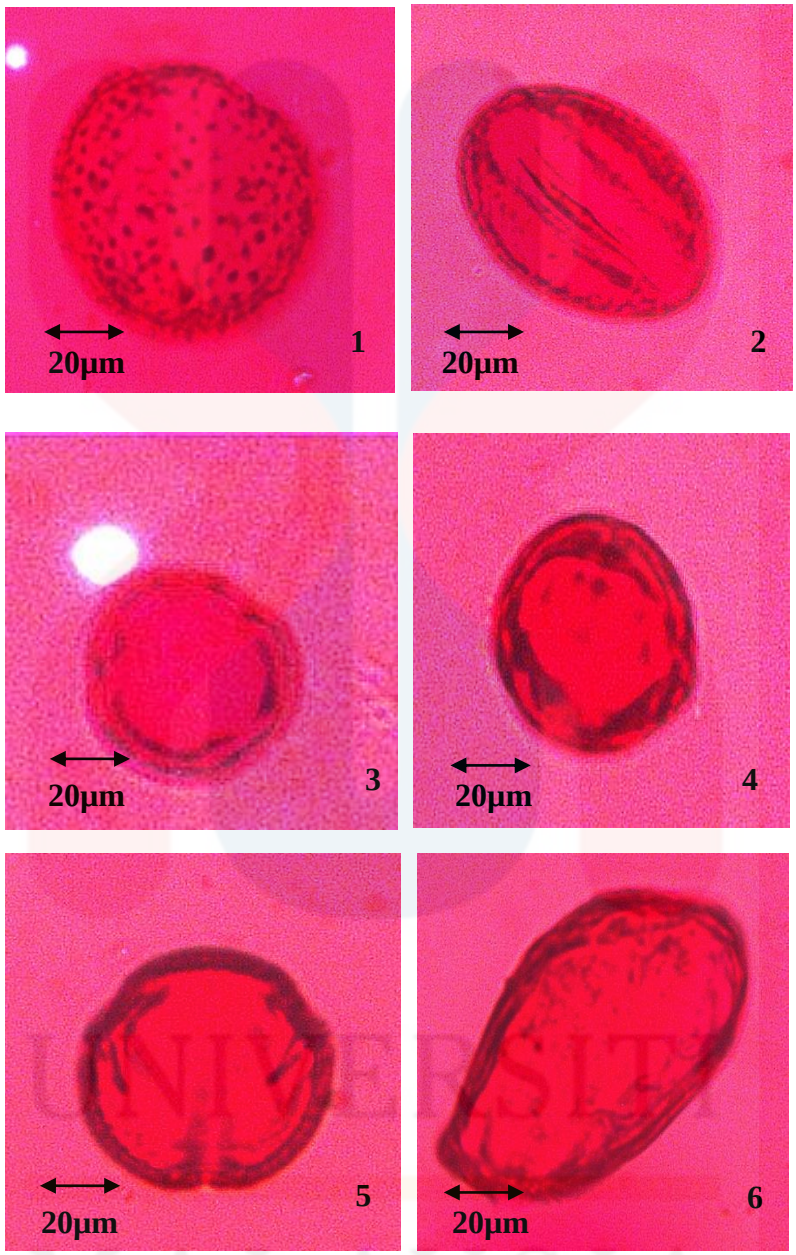

Figure 4.20 (b): Morphology of different pollen honey sample from Rawang under 40x magnification. 1- *Garnicia hombroniana* (Clusiaceae); 2- *Punica granatum* (Punicaceae); 3- *Solanum melongena* (Solanaceae); 4- *Pongamia pinnata* (Fabaceae); 5- *Bauhinia purpurea* (Fabaceae) and 6- *Cocos nucifera* (Arecaceae)

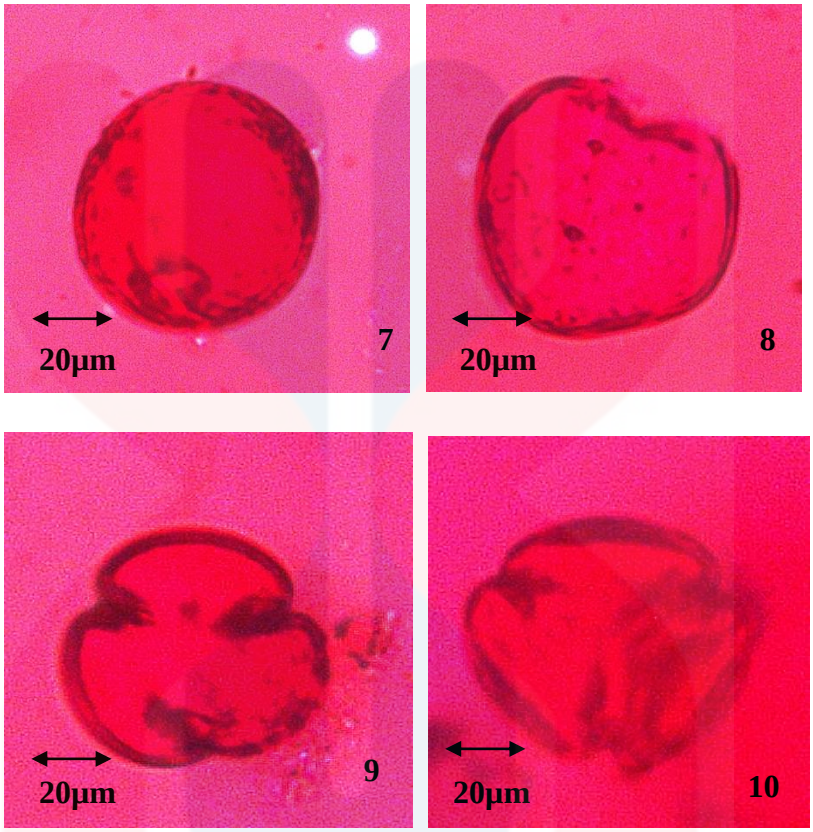

Figure 4.20 (c): Morphology of different pollen honey sample from Rawang under 40x magnification. 7- *Ixora congesta* (Rubiaceae); 8 - *Lolium multiflorum* (Poaceae) ;9- *Moringa oleifera* and 10- *Tecoma stans* (Bignoniaceae)

Honey sample from Rawang, Selangor consists of 10 different types of pollen (table 4.20) which the highest percentage of pollen was from *Bauhinia purpurea* flowers. The pollen from this plant species accounted up to 28.6% followed closely by *Solanum melongena* with 21.8% of the total pollen abundance overall. This two plant species classified as secondary pollen grain with pollen grain frequency range 16-45%. Bauhinia or 'Mountain Ebony' has been widely planted as a garden, park and roadside ornamental tree in many warm temperate and Sub-tropical country include in Malaysia. The pollen grain was prolate-spheroidal shape with striato-retibulate ornamention. Besides, *Pongamia pinnata*, *Punica granatum*, *Cocos nucifera* and *Ixora congesta* were known as important minor pollen (3-16%) while *Garnicia hombroniana* (1.7%), *Lolium multiflorum* (1.7%), Moringo oleifera (0.8%) and *Tecoma stans* (0.8%) were present as minor pollen (less than 3%). It can be deducted that honey sample from Rawang was multifloral due to the absence of predominant taxon of pollen. Each of the local flora has their own trait that represented honey in form of pollen spectrum from different types of honey samples collected. Hence, the excessive honey bees foraging many plants were associated to the fact that pollen was the only proteic food as a result the pollen plays an essential role in nurturing the colony (Abdulrahaman et al., 2013).

4.21 *Honey sample from Hulu Langat, Selangor*

Table 4.21: The number of pollen and percentage of abundance of pollen types in the honey sample from Hulu Langat (HL7)

Plant species	**Family**	**No of pollen**	**Percentage of Abundance (%)**
Solanum melongena	Solanaceae	242	92.0%
Callistemon linearis	Myrtaceae	2	0.8%
Psidium guajava	Myrtaceae	1	0.4%
Acacia sp	Fabaceae	14	5.3%
Garcinia hombroniana	Clusiaceae	2	0.8%
Rosa canina	Rosaceae	1	0.4%
Terminalia arjuna	Combretaceae	1	0.4%

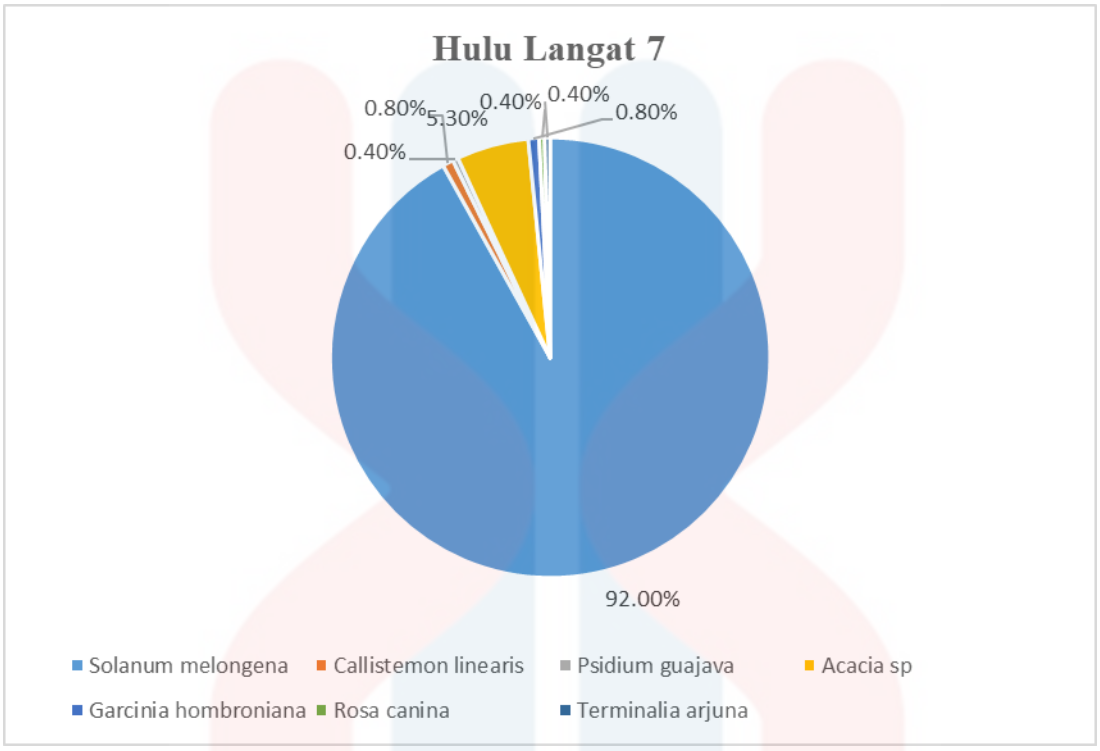

Figure 4.21: Pollen spectrum of honey sample from Hulu Langat, Selangor

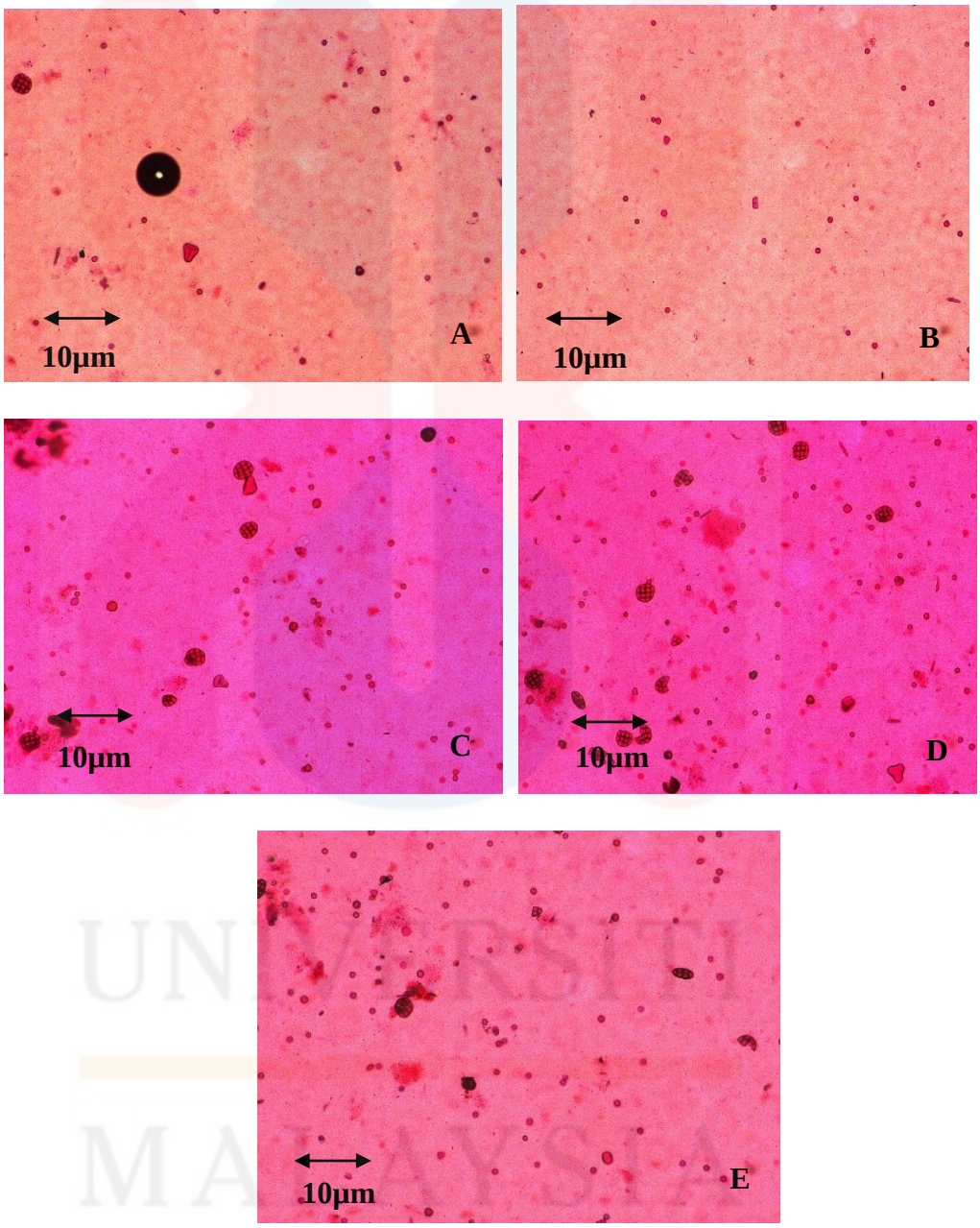

Figure 4.21 (a): Microscopic overviews of pollen density in honey sample from Hulu Langat 7 under 10x magnification. Overviews of A- Left edge of cover slip (top); B- Right edge of cover slip (top); C- Left edge of cover slip (bottom); D- Right edge of cover slip (bottom) and E- centre of the cover slip (middle)

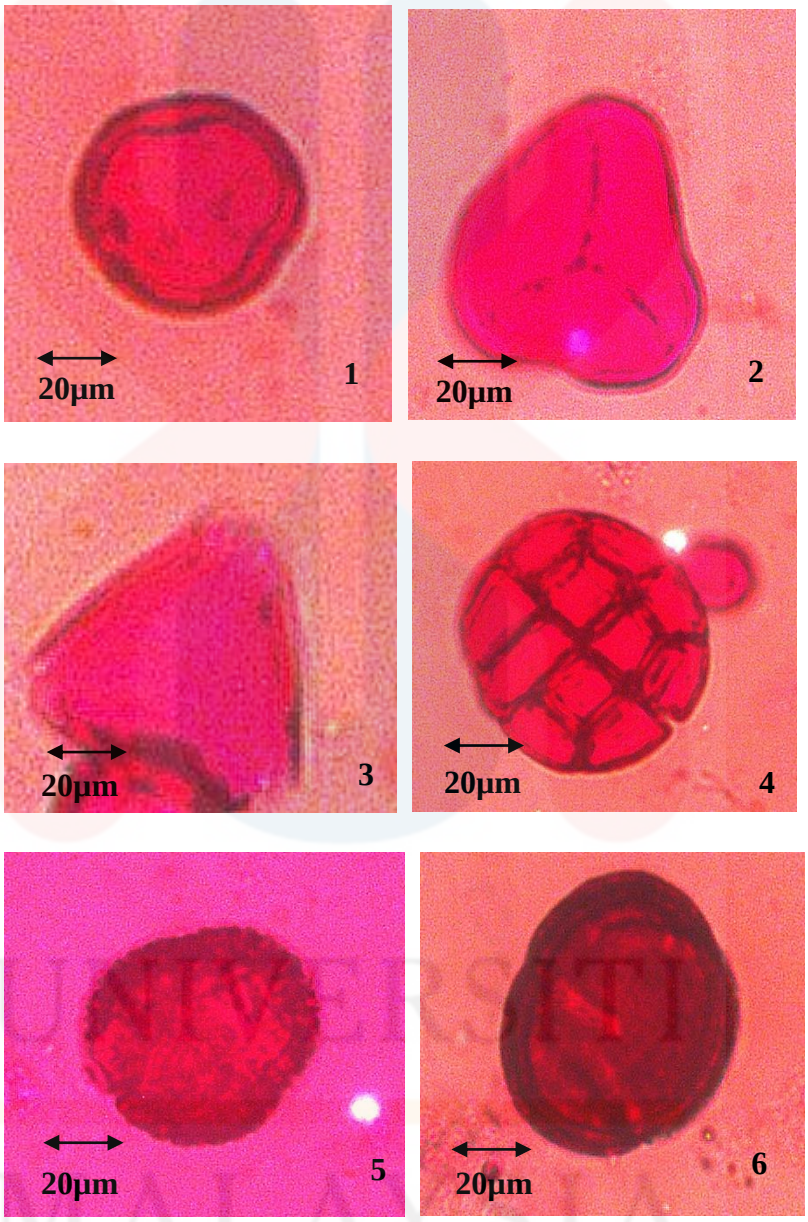

Figure 4.21 (b): Morphology of different pollen honey sample from Hulu Langat 7 under 40x magnification. 1- *Solanum melongena* (Solanaceae); 2- *Callistemon linearis* (Myrtaceae); 3- *Psidium guajava* (Myrtaceae); 4- *Acacia sp* (Fabaceae); 5- *Garcinia hombroniana* (Clusiaceae) and 6- *Rosa canina* (Rosaceae)

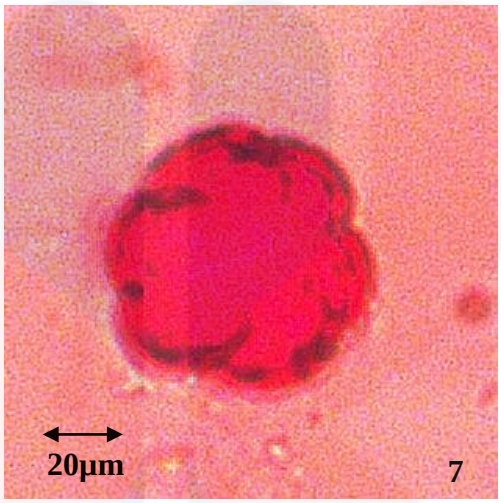

Figure 4.21 (c): Morphology of different pollen honey sample from Hulu Langat 7 under 40x magnification. 7- *Terminalia arjuna* (Combretaceae)

Analysis on the honey samples collected from Hulu Langat represented the presence of 7 different types of pollen (table 4.21). Predominant taxon of pollen in this sample was *Solanum melongena* (Solanaceae) with 92.0% of the overall total pollen count. *Acacia sp* was identified as the only important minor pollen with 5.8% and the rest of 5 pollen were classified as minor pollen with less than 3% percentage abundance of pollen grain. *Solanum melongena* has a prolate, sub-spheroid shape with a punctitegillate exinepsilate surface. The presence of the greater species revealed that bees at Hulu Langat 7 regions had more preference to forage flowers from such species. This sample of honey was a unifloral honey. Furthermore, identification of pollen types was done by mounting using glycerin jelly giving the pollen pinkish color under compound microscope with camera. However, images

of a few pollen did not appear so visible due to the presence of debris and dirt on the microscopic slides. Therefore, it can be concluded that pollen analysis plays an essential part in understanding variety of crops that were foraged by bees also crucial in sustainable development of apiculture industry in Malaysia. Presence of other pollen indicated the diversification of floral at Hulu Langat region of Selangor.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

Analysis on the 20 honey samples from 6 different locations of Selangor state in Malaysia revealed that occurrence of 55 types of pollen from 30 different plant families. 10 of honey samples were unifloral and 10 honey samples were multifloral. Based on the result, each of the location of the samples have their variety of pollen honey. The predominant pollen will be categorized as unifloral according to the percentage of pollen abundance is > 45%. Most of the samples had at least one predominant pollen especially in each of the unifloral samples while, there is absence of predominant pollen in multifloral honey. There are 2 types of pollen that are very famous which continuously or repeated be found on this pollen analysis which are *Solanum melongena* and *Cocos nucifera*. Through this information, it would be easier to distinguish the presence of imported honeys. Pollen acts as a blueprint in honey revealing its geographical and botanical origin which provides essential information for future beekeeping and the development of apiculture in Malaysia in order to improve the

value and quality of honey. Therefore, this pollen analysis study important to understand which plants are used as a food source for bees. Yet, it provides the necessary information as well as estimate the potential for beekeeping in an area. Therefore, it promotes the sustainability of the beekeeping industry. Therefore, more often melissopalynological studies should be carried out to gain more information and became more understand about the floral density of Malaysia that contribute towards the production of high quality of local honeys. As the recommendation for future research, the cover slip was placed on top of it and the already warmed liquid glycerin holding the sample was sealed with nail polish as sealants to keep the sample from drying out. Futhermore, the application of scanning electron microscopy (SEM) is another microscopic method that allows for a more precise diagnosis of pollen grain type based on changes in surface features. Moreover, conduct the lab activities especially during the Acetolysis process wisely to get a better result from pollen honey samples.

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APPENDICES