Pollen Analysis of Kelulut Honey Collected from Different Bee in Kedah

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DECLARATION

I hereby declare that the work embodies in this report is the result of my own research except individual citations and summaries that I have explained their sources.

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Analisis Debunga Madu Kelulut Dikumpul Daripada Pelbagai Lebah di Kedah

ABSTRAK

Madu adalah sejenis makanan berbentuk cecair semula jadi dan mempunyai rasa manis yang boleh dilihat dengan warna keemasan gelap. Lebah menghasilkan madu di dalam kantung madu. Warna dan rasa madu boleh ditentukan dari nektar bunga yang diperolehi. Pengeluaran madu boleh ditingkatkan jika tumbuhan yang terlibat dalam pengeluaran madu telah dikenal pasti. Kajian analisis debunga lebah madu kelulut di Kedah telah dijalankan untuk mengenal pasti asal usul botani debunga madu di Kedah. Analisis debunga ini melibatkan 16 sampel madu yang dikumpul dari pelbagai tempat di negeri Kedah. Analisis debunga dilakukan dengan menggunakan kaedah asetolisis dan pengenalpastian dilakukan secara mikroskopik. Daripada 16 sampel madu, 10 sampel madu dikenal pasti sebagai madu multifloral dan 6 sampel madu dikenal pasti sebagai madu unifloral. *Cocos nucifera, Sporobolus indicus,* dan *Elaeis guineensis* ialah debunga yang boleh ditemui dalam kebanyakan sampel. Analisis debunga atau melissopalinologi ialah kaedah yang penting untuk mengenal pasti asal usul botani dan geografi sampel madu.

Kata kunci: Lebah kelulut, asetolisis, analisis debunga, botani

Pollen Analysis of Kelulut Honey Collected from Different Bee in Kedah

ABSTRACT

Honey is a natural and sweet liquid food that can be seen in dark golden colour. Bees produced honey in their honey sacs. The colour and taste of honey can be determined from the flower which the nectar is obtained. The production of honey can be increased if the plant that involve in the production of honey is identified. This study of pollen analysis of kelulut honey bees in Kedah was carried out to identify the botanical origin of the honey pollen in Kedah. This pollen analysis involved 16 samples of honey collected from different places in Kedah state. The pollen analysis was performed using the acetolysis method and identification was done through microscopic characterization. The results show out of 16 honey samples, 10 samples were identified as multifloral honey and 6 samples were identified as unifloral honey. *Cocos nucifera, Sporobolus indicus*, and *Elaeis guineensis* are the pollens that can be found in most of the samples studied. Pollen analysis or melissopalynology is a valuable tool for the identification of the botanical and geographical origin of honey samples.

Keywords: Kelulut honey, acetolysis, pollen analysis, botanical

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Reference No		Page
g	Gram	4
ml	Milliliter	18
r.p.m	Revolution per minute	25
μm	Micrometre	37
PP	Predominant Pollen	28
SP	Secondary Pollen	28
IP	Important Minor Pollen	28
MP	Minor Pollen	28
SOP	Standart Operating Procedures	2
MPI	Marginal Propensity to Import	4
MBRDT	Malaysian Beekeping Research and Development Team	8
DOA	Department of Agriculture	8
YAS	Young Agroprenuer Scheme	10
ICBB	International Commission for Bee Botany	10
BOT	Balance of Trade	10
CRD	Complete Randomized Design	30
PT	Pinang Tunggal	19
KM	Kuala Muda	19

BP	Bukit Pinang	19
TM	Taman Mutiara	19
PD	Pendang	19
KB	Kepala Batas	19
KG	Karangan	19
KK	Kampung Keladi	19
SB	Kampung Sungai Buluh	19
JT	Jitra	19
LG	Langkawi	19
TM	Taman Siswa	19
PL	Kampung Padang Limau	19
BK	Kampung Batu Kembai	19

CHAPTER 1

Introduction

1.1 Definition of Honey

Honey is a sweet liquid food which is usually seen in dark golden colour. Honey is a natural resource for sugar that is produced by bees in their honey sacs. The definition of honey stipulates a pure product that does not allow for the addition of any other substance. The colour and taste of honey are determined by the flower from which the nectar is obtained. The inversion of the sucrose sugar in the nectar into fructose and glucose with the removal of some excess moisture will finally produce the honey. Honey is a protein-rich food that is commonly used in cooking, candies, and medical products. Honey contains 18% water and can granulate at temperatures ranging from 10 to 18 °C (Britannica, 2021). Other than sugars and proteins, honey also contains acids, vitamins, and minerals. Alkaloids, auterquinone glycosides, cardiac glycosides, flavonoids, and reducing compounds can be found in pure honey (Saeed et al., 2017).

Honey can be used to replace sugars in processed foods to reduce the detrimental and genotoxic consequences of mycotoxins and also improve gut microbiota (Ezz El-Arab et al., 2006). Honey, contain a high-quality nutrient and is also known as a functional food with a wide range of medical characteristics. Honey is the best medicine's catalyst and adjunct. Honey can be used to treat bruising and burns due to its antiseptic properties.

Honey is also important to the human body because it helps to increase appetite, good for voice, good for heart and vision, gives strength, and enhance memory. Honey can also help with diarrhoea since it reduces the duration of bacterial diarrhoea. Honey was essential to the ancients because it was the only sugar substitute available at the time, and it was also necessary for their medicine. It was used to produce fermented beer, mead, as well as wine and other alcoholic beverages. Honey is also mentioned in the Bible and Quran. In Sanskrit, honey is referred to as *Madhu*, which means sweet, pleasant, charming, flower nectar juice, and a sweet intoxicating beverage (Kavita, 2011).

1.2 Kelulut Bees or Stingless Bees

Kelulut honey is honey made by a small bee known as kelulut bees or *Trigona sp*. It is a member of the *Meliponidae* family (stingless bee) with a different life compared to the honey bee, *Apis Mellifera*. Their nests are comprised of wax, resin, and gum with the addition of mud collected by worker bees in some species. The non-stinging 'Lebah Kelulut' build their nests in existing cavities or hollowed-out parts of trees, hives, and structures (Mohd Zulkifli et al., 2018). Stingless bees can be cultivated in commercial farms with regulated environmental conditions or in houses in rural areas that follow standard operating procedures (SOP) according to their nesting behaviour. *Trigona sp.* is a *Meliponini* genus that is commonly found in tropical climates such as Malaysia, Northern Australia, Africa, Brazil, and Southeast Asian regions. In Malaysia, more than 38 stingless bee species have been found, however, only four of them are commercially

cultivated which are *Geniotrigona thoracica, Heterotrigona itama, Lepidotrigona terminata* and *Tetragonula leviceps* (Mohd Zulkifli et al., 2018).

Kelulut bee has a smaller body size than the honey bee. In general, stingless bees are easily distinguished from other bees. Kelulut bees are distinguished by their penicillium, lengthy setae on the hind tibia, and poor wing venation. Like other bees, the kelulut bee has a corbicula structure on the hind leg that is utilised to carry pollen (Michener, 2007). They live in a colony with a queen, a worker which is a sterile female, and a drone which are a male bee. Workers are in charge of constructing and protecting their nests, as well as acting as foragers to keep the reproduction rate and metabolism stable. Kelulut bees can produce more propolis that is important as a defence mechanism against bacteria, fungi, and viruses than honeybees (Simone-Finstrom & Spivak, 2010). Kelulut honey is kept in the cluster of small resin pots that is located in the nest while honey from honey bees are being kept in hexagonal-shaped combs (Kek et al., 2017). Kelulut honey is more diluted and has a sour taste and aroma.

1.3 Pollen Analysis of Honey

Pollen analysis, also known as Melissopalynological analysis, is a common method for determining the botanical and geographical background of honey. Melissopalynology is a branch of palynology those deals with microscopic analysis of bee honey, also known as pollen and spore research. Pollen data from all locally available honeys are synthesized in a pollen spectrum to obtain regional characterization.

Melissopalynology is derived from the Greek terms for "bee" and "honey," as well as "study of dust," which refers to "pollen." There are many other varieties of honey produced in Malaysia, including Tualang honey, Pineapple honey, Gelam honey, and Acacia honey (Biluca et al., 2014).

However, the technique's effectiveness is determined by the pollen sampler's abilities and the analyst's ability to interpret the findings. Honey has evolved into a significant commercial enterprise that offers sweetness to thousands of goods (Ponnuchamy et al., 2014). In the apiculture sector, pollen analysis is essential for adding value to honey and honey products. This knowledge can be utilised to develop a management strategy to keep the bee colony thriving and increase the quantity and quality of honey produced.

1.4 Pollen Count and Identification

Pollen counting is a method used to identify the origin of the honey and also the colour, smell, and taste of the honey. The pollen count method calculates the number of pollen grains per 10 g of honey and recognizes the most typical pollen grains contained in the honey sample. The test is frequently requested for honey that does not follow the Marginal Propensity to Import (MPI) concept of being monofloral or multifloral, and it is frequently used to classify the flowers that bees visit while collecting nectar. Pollen counts may be necessary for honey that is exported to some foreign countries. Pollen counts are difficult to interpret since it depends on the characteristics of the flowers or

plants involved and certain pollens may be over-represented or under-represented in honey. The frequency classes of pollen were classified as “Predominant pollen” (>45% of the total count), “Secondary pollen” (16-45%), “Important minor pollen” (3-15%), and Minor pollen (<3%) (Ohe et al., 2004).

Acetolysis is a process usually used to classify specific pollen grains. By spraying pollen grains with harsh chemicals, acetolysis extracts debris from the sample (Jones, 2014). This also removes some of the substance from the pollen grains' surfaces, revealing their skeletal features and making them easier to distinguish. After the sample has been washed several times to remove any unwanted contaminants, the pollen grains could be identified by the eye using a light microscope with high magnification. Depending on the process specifications, various laboratories can classify various types of pollen grains which ranging from 100 up to 500. Since each pollen must be identified by a professional individual eyes, the process takes longer if there are a greater number of pollen to distinguish. The percentage of total pollens found that come from various plant sources is recorded in the results. The cost of a pollen count is often proportional to the number of pollen grains contained in each sample.

1.5 Problem Statement

This research is conducted to identify the botanical origin of the kelulut honey collected from different locations in Kedah through pollen analysis. The reason for conducting this research at a particular region is because no pollen analysis of honey was

carried out before in these places. In Melissopalynology, the pollen found in the honey is used to determine the botanical origin of the honey. The microscopic analysis of pollen contained in honey is essential to determine the floral and botanical source that is important for honey standardization, honey geographical origin, and honeybee foraging ecology (Stephan & Vaughn, 2011). This research aids in the differentiation of multifloral and unifloral honey. Increased honey production necessitates a better understanding of the botanical sources of honey. This research will provide clear information about the origin of honey collected.

1.6 Hypothesis

The botanical origin of the honey can be identified through the analysis of pollen content of the honey.

1.7 Objectives

1. To analyse and identify the pollen contents of Kedah kelulut honey.
2. To classify the type of honey based on their botanical origin collected from various locations in Kedah.

1.8 Scope of the Study

The focus of this study is to carry out pollen analysis by identifying the characteristics of honey collected and analysing the properties of the honey pollen by using the Acetolysis method (Mushtaq et al., 2015). The pollen from the honey sample was viewed under a microscope to identify the pollen type, morphology, and to conduct the pollen count. This research is also important to characterize the honey collected from different locations in Kedah. By the end of the experiment, the botanical origin of the pollen and the pollen spectra will be determined through the percentage of the abundance of pollen from different plant species.

1.9 Significance of the Study

The significance of the study is to determine the botanical origin of the honey pollen. As we know, Melissopalynology is used to determine the quality and validity of honey. Pollen is always present in flower nectar in varying amounts and this pollen can be found in the honey liquid (Massimo et al., 2009). It is possible to track the botanical species gathered by identifying these pollen, estimating the percentage of the pollen present, and eventually identifying elements contained. It is also possible to track down the origins of a certain kind of honey. The pollen composition of honey can be influenced by a variety of variables such as the morphological characteristics of flowers and pollen and the operations performed on flower nectar and honey in succession. This research is

also essential to avoid honey fraud and mislabelling. When evidencing statements related to a specific source for the sample, information obtained from the analysis is useful. Unifloral honey made from a single source of plant is more expensive than honey made from a variety of plant sources (Stefan, 2016). Honey is also priced differently depending on the location from which it comes.

CHAPTER 2

LITERATURE REVIEW

2.1 Apiculture in Malaysia

Apiculture is a scientific method of rearing and managing honeybees known as beekeeping. Traditional beekeeping in Malaysia has been practised since the Malacca Sultanate and it is believed that beekeeping started a very long time ago (Wan Iryani, 2016). Honey hunters who are the villagers harvested honey directly from beehives. Honey is usually harvested in the middle of the night by using traditional apparatus such as a bamboo ladder, a cow-bone knife, and a cow-skin container. Honey is harvested once per year between January and April.

In 1970s, modern beekeeping started in Malaysia. It began with a set-up of an apiary which is located in Johor by using commercial bees known as *Apis mellifera* that was imported from Taiwan. Malaysian Beekeeping Research and Development Team (MBRDT) was established in 1981 that function to develop and create systematic beekeeping in Malaysia. In 1988, the Department of Agriculture (DOA) established a national apiary centre at Parit Botak which functions to design, develop, and organise training in modern beekeeping. Modern beekeeping involves domesticated bees such as *A. mellifera, A. cerana,* and some species of stingless bees known as *Geniotrigona thoracica, Heterotrigona itama, Lepidotrigona terminate*, and *Tetragonula leaviceps.* By

using more developed apparatus in the beekeeping industry, it helps to increase the development of apiculture for rapid growth (Mardan, 2008).

The apiculture industry in Malaysia are facing many hurdles for almost 10 years. In 2009, honey production started to increase and RM 5.26 million was recorded with a positive balance of trade (BOT). The rapid advancement of the beekeeping sector in Malaysia has been spectacular from 2010 until now. After 10 years, the sector is currently attracting significant interest from a variety of agencies. In 2013, Young Agropreneur Scheme (YAS) was launched as an initiative to train and develop new agriculture projects particularly among young beekeepers in Malaysia to supply pure honey to the local market to achieve a high-income nation. Malaysian farmers might earn RM 5000 per month through the development of agro-entrepreneurs in beekeeping (Ismail, 2014). Simultaneously, stingless beekeeping was growing rapidly due to its ease of setup and expansion for commercial purposes.

2.2 Melissopalynology of Honey

The earliest research on pollen analysis was done in 1895 by Pfister and continued by Zander (1935, 1937, 1941, 1949, 1951) which led to an analytical approach (Omur Gencay & Kadriye, 2018). In 1978, International Commission for Bee Botany (ICBB) proposed and published a method for melissopalynology. ICBB method is a well-established method that have been used in most European laboratories involved in honey analysis. This method is deemed suitable for practical purposes especially in verifying the pollen spectrum whether it fulfills the stated botanical and geographical

characteristics. The requirement for unifying, adapting, and analysing this approach was debated at the International Honey Commission of Apimondia meetings after many laboratories incorporated some modest alterations to the original ICBB procedure in their everyday work.

A working group was formed to find out the details of the method, eliminate some of the unpredictability caused by sample preparation and the number of grains counted, and determine the method's precision parameters through ring trials (repeatability and reproducibility). A method with more specific instruction was developed to assess the honey pollen spectrum based on relative frequencies (qualitative melissopalynological study). The reliability of the procedure was tested in a ring experiment conducted in 1999 and the accurate parameters were derived after some more discussions, contributions, and modifications. A process for determining the absolute numbers of plant elements in honey (quantitative melissopalynological analysis) was finalised in 2003 and another ring trial was conducted to determine its precision parameters (Ohe et al., 2004).

2.3 Types of Honey

Bee pollen is the product of worker honey bees agglutinating flower pollens with nectar, honey, and salivary substances and collecting it at the hive door. There are two forms of pollen produced by bees. The first category refers to the pollen's water content. The substance was obtained in its natural state, with a water content of 20 to 30% (Campos et al., 2008). To prevent bacterial and mould infection, the pollen should be stored in the freezer. Desiccated bee pollen is described as a product that has been dried

at a temperature of less than 42 °C and has a water content of less than 6% (Melo & Almeida-Muradian, 2011). A drying process is needed because bee pollen has a high water content in its structure which triggers fermentation and rapid deterioration. Since moisture content influences enzyme activity and microbiological viability, thus, the shelf life of food through water activity and moisture content can be used for biocontrol in the preservation and commercialization of the product.

The second approach is based on the floral source's material. Monofloral and multifloral bee pollen are the two types of floral sources. Honey bees make monofloral honey from nectar collected from a variety of flower origins. Honey bees, on the other hand, make monofloral honey from the nectar of a single flower species. The main taxon must account for at least 80% of the total (Campos et al., 2008). In honey cultures, some monofloral honey variations are valued for their sugar content. The two major sugars present in honey are glucose and fructose. Manuka honey, which comes from the flowers of the Manuka bush, is an example of monofloral honey. Multifloral bee pollen is made by honey bees that using the nectar from a variety of flower species and include a variety of taxa. Multifloral honey usually lack of their pure properties of all of their nectar sources at the production stage. These honey varieties, on the other hand, reflect the qualities of several flowers working together. One of the most appealing aspects of multifloral honey varieties is their singularity. An example of multifloral honey is Buckwheat honey.

2.4 Importance of Honey

2.4.1 Remedy for Stomach Disorders

Using scientifically experimental research, many researchers have confirmed the traditional uses of honey. Honey is usually used to cure and prevent gastrointestinal infections caused by bacteria such as gastritis, duodenitis, and stomach ulcers. The earliest stage in the bacterial infections development in the gastrointestinal tract is the attachment of bacteria to mucosal epithelial cells (Arawwawala & Hewageegana, 2017). Honey has the ability to prevent bacterial adhesion via several pathways which are non-specific mechanical suppression due to the honey coating the bacteria, altering bacterial electrostatic charge or hydrophobicity, or killing bacteria due to the antibacterial properties in honey (Nasuti et al., 2006). Perfusion of the stomach with isotonic honey resulted in a significant reduction of the area of the lesions generated by ethanol, which was utilised to assess natural honey's gastric cytoprotective characteristics. Natural honey has also been suggested to have curative effects for the healing of antral ulcers and to be used as a substitute for sucralfate in the treatment of peptic ulcer disease. Honey is also used as a treatment for diarrhoea and gastroenteritis at 5% (v/v) concentration (Gharzouli et al., 2002).

2.4.2 Antimicrobial Activity

Antimicrobial activity is critical in the treatment of infections, particularly when the body's immune response is inadequate to eliminate the infection. In other words, it

has demonstrated potent antimicrobial activity against pathogenic and non-pathogenic microorganisms, including those that have gained resistance to a variety of antibiotics. Honey is known as a supersaturated sugar product, which means that the sugars have a strong affinity with the water molecules and resulting in an inadequate amount of water for the microorganisms including bacteria and yeast to develop. In the end, the microorganisms become dehydrated and die.

The natural acidity of honey prevents the development of a variety of pathogens. Many pathogens have a pH range between 4.0 and 4.5. When honey is diluted, their antimicrobial activity from hydrogen peroxide which is formed by the enzyme glucose-oxidase which oxidize the glucose (Temaru et al., 2007). When the hydrogen peroxide breaks down, it will produce highly reactive free radicals, which react with the bacteria and destroy them. In most situations, heat or the presence of catalase will easily dissolve the peroxide activity in honey.

2.4.3 A Good Source of Antioxidant

Honey contains the same amount of antioxidants as fruits and vegetables. Antioxidants are important because it helps to prevent cell damage caused by the free radicals. Free radicals have been linked to the ageing process as well as the development of chronic diseases like cancer and heart disease. Honey works as an antioxidant by preventing the development of free radicals that is catalysed by metal ions. Honey's flavonoids and other polyphenols can deposit these metal ions like copper and iron in complexes and immediately eliminate the creation of free radicals (Makawi et al., 2009).

2.4.4 Honey as A Wound Treatment Agent

Organic unprocessed honey from various sources are used to treat a wide range of wounds all over the world. Honey can also be used as an active ingredient in medicines such as ointments for mild burns and wounds (Krishnakumar et al., 2020). Honey cleanses wounds, promotes tissue healing, and decreases inflammation, and honey-impregnated patches serve as a non-adhesive tissue dressing. Honey aids in the regeneration of newly produced tissue, which is an important phase in the wound-healing process. The wound environment is acidified, which encourages macrophage activity, restricts bacterial development, and neutralises ammonia created by bacterial metabolisms which could harm tissues cell (Yaghoobi et al., 2013). However, the honey's acidic pH inhibits protease activity which could inactivate tissue growth hormones and degrade plasma fibronectin and collagen matrix which are required for fibroblast activity and tissue re-epithelialization (Winter, 2017). The lower pH in the wound bed allows more oxygen to be derived from haemoglobin in the blood. Furthermore, all of honey's beneficial components such as sugars, amino acids, vitamins, and trace elements promote cell growth and the formation of healing tissues.

2.4.5 Antidiabetic Benefits in Honey

Honey consumption in diabetes was linked to a lower glycemic index than glucose or sucrose consumption in regular diabetes. Honey tends to minimize the consumption of digested food due to its low glycemic index. Honey caused a slightly lower rise in blood glucose levels in diabetics than dextrose. In normal and hyperlipidaemia individuals, it

also reduced blood lipids, homocysteine levels, and C-reactive protein levels (Krishnakumar et al., 2020). Honey has been shown to promote insulin release, lower blood glucose levels, elevate haemoglobin concentration, and increase lipid profile in previous studies (Erejuwa et al., 2012). According to some research, Manuka honey functions synergistically with numerous antibiotics by lowering the amounts required to limit bacterial growth or reversing previously acquired antibiotic resistance. These findings point to the possibility of using honey-antibiotic combination therapy.

2.5 Sensory, Physical, and Chemical Properties of Honey

Colour and flavour are important sensory properties in determining honey quality. They are affected by a variety of elements such as geographical, botanical, and seasonal conditions. Colour is the most variable property of honey and it is extremely important as a sign of its origin and quality. Honey's colour ranges from extremely pale yellow to amber, dark reddish amber, and nearly black (Escuredo et al., 2013). Honey that is mass-produced and mixed with several types of honey can be uniformly coloured. The colour of honey can be different due to the flower origin, the duration between nectar collecting and honey harvesting, and production factors such as heating, temperature, storage time, and pollen grains and shape. The colour and the taste of honey can also be affected by the mineral content which causes the honey to have a darker colour and stronger taste. Honey can be seen in a few conditions such as fluid, viscous, partly or totally crystallized form (Engin Gündoğdu et al., 2019).

The pH for honey ranging from 3.2 to 4.5. Honey's water activity is vital in preventing spoiling, ranges between 0.5-0.65 (Gleiter et al., 2006). The honey flavour is determined by volatile substances, which might vary depending on the season and geographical origin. The scent of the honey is influenced by the quantity and type of acids and amino acids present. Another one of honey's features is viscosity, which is considered one of the most crucial factors. This trait is particularly essential for beekeepers and honey processors, as rheological behaviour is critical for extending the shelf life of honey and enabling safe handling, packing, and processing.

CHAPTER 3

Materials and Methods

3.1 Materials

Table 3.1: List of chemicals for Acetolysis

Chemicals	Amount (ml)
Acetic Anhydride	45.0
Glacial Acetic Acid	1.0
Sulphuric Acid	5.0

Table 3.2: List of chemicals for preparation of Glycerine Jelly

Chemical	Amount
Distilled Water	87.5 ml
Gelatine Powder	25.0 g
Glycerine	75.0 ml
Phenol Crystals	3.5 g
Safranin Powder	0.1 g

3.2 List of apparatus and quantity

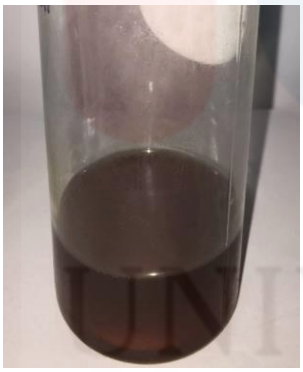
Table 3.3: List of apparatus and quantity

Apparatus	**Quantity**
Beaker	5 container
Centrifuge	1 set
Compound Microscope with Camera, Leica DM 2500	1 set
Cover Slip	16 pieces
Falcon Tube (15 ml)	16 pieces
Hot Plate	1 set
Microcentifuge Tubes (2 ml)	16 pieces
Micropipette	1 set
Microscope Slides	16 pieces
Petri Dish	16 pieces
Spatula	1 pieces
Thermometer	1 set
Vortex	1 set
Water Bath	1 set

3.3 Honey Sample Collection

16 honey sample were collected from different location in Kedah which were from Pinang Tunggal (PT), Kuala Muda (KM), Bukit Pinang (BP), Taman Mutiara (TM), Pendang (PD), Kampung Kepala Titi (KT), Pekan Pendang (PP), Kelapa Btatas (KB), Karangan (KG), Kampung Keladi (KK), Kampung Sungai Buluh (SB), Jitra (JT), Langkawi (LG), Taman Siswa (TS), Kampung Padang Limau (PL), and Kampung Batu Kembai (BK). The type of pollen and their amounts from the samples were examined.

Table 3.4: Honey sample and description

Diagram	Description
	**Honey Sample 1** Place: Pinang Tunggal (PT) Honey Colour: Dark Amber Date of Collection: 18/09/2021
	**Honey Sample 2** Place: Kuala Muda (KM) Honey Colour: Amber Date of Collection: 22/09/2021

	**Honey Sample 3** Place: Bukit Pinang (BP) Honey Colour: Dark Amber Date of Collection: 21/09/2021
	**Honey Sample 4** Place: Taman Mutiara (TM) Honey Colour: Amber Date of Collection: 22/09/2021
	**Honey Sample 5** Place: Pendang (PD) Honey Colour: Light Amber Date of Collection: 15/09/2021
	**Honey Sample 6** Place: Kampung Kepala Titi (KT) Honey Colour: Light Amber Date of Collection: 25/09/2021

	**Honey Sample 7** Place: Pekan Pendang (PP) Honey Colour: Light Amber Date of Collection: 15/09/2021
	**Honey Sample 8** Place: Kepala Batas (KB) Honey Colour: Light Amber Date of Collection: 21/09/2021
	**Honey Sample 9** Place: Karangan (KG) Honey Colour: Amber Date of Collection: 19/09/2021
	**Honey Sample 10** Place: Kampung Keladi (KK) Honey Colour: Light Amber Date of collection: 25/09/2021

	**Honey Sample 11** Place: Kampung Sungai Buluh (SB) Honey Colour: Light Amber Date of Collection: 19/09/2021
	**Honey Sample 12** Place: Jitra (JT) Honey Colour: Light Amber Date of Collection: 21/09/2021
	**Honey Sample 13** Place: Langkawi (LG) Honey Colour: Light Amber Date of Collection: 4/10/2021
	**Honey Sample 14** Place: Taman Siswa (TS) Honey Colour: Light Amber Date of Collection: 21/09/2021

	**Honey Sample 15** Place: Kampung Padang Limau (PL) Honey Colour: Extra Light Amber Date of Collection: 19/09/2021
	**Honey Sample 16** Place: Kampung Baru Kembai (BK) Honey Colour: Extra Light Amber Date of Collection: 26/09/2021

3.4 Location of The Study

3.4.1 Honey sample collected in differents regions in Kedah.

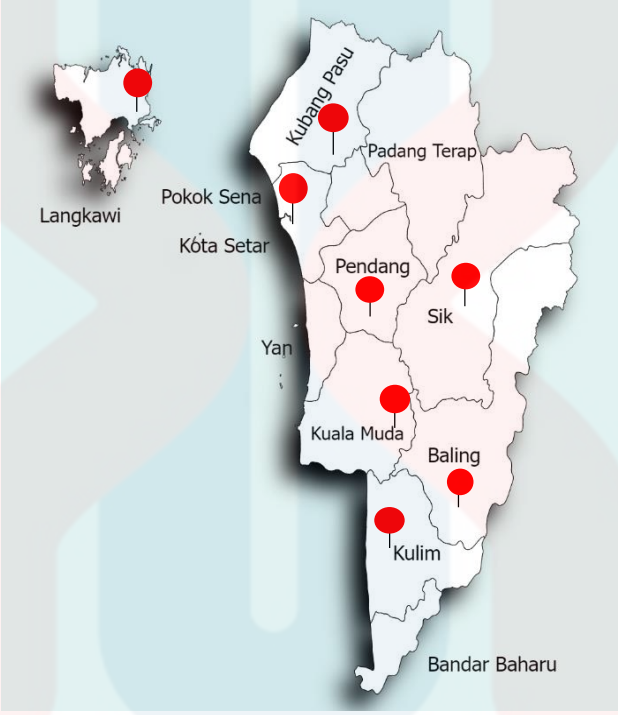


Figure 3.1: Location of sample honey collected in Kedah

(Source: Google map)

3.5 Sample Analysis

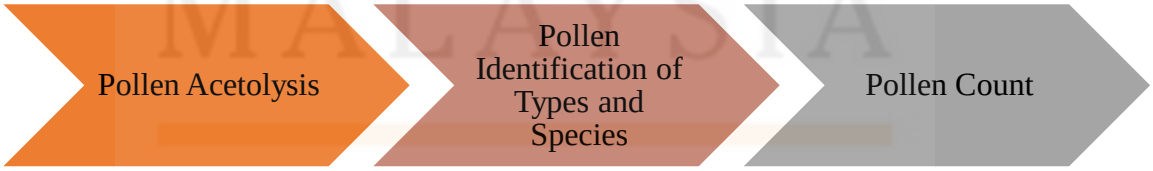


Figure 3.2: Flow of sample analysis

3.6 Methods

3.6.1 Pollen Acetolysis

The acetolysis method used in this pollen analysis was according to Louveaux *et al.*, (1970). Acetolysis was one of the best procedures to recover pollen because it dissolved any tissue and lipids and also removed debris from the sample and the pollen grains make it easier to stain, capture, and identify (Jones, 2014).

In this experiment, 5 ml of Kelulut honey sample was mixed with 10 ml of 70% alcohol and was agitated manually by using a vortex to form a homogenous mixture. The sample was centrifuged at 5000 r.p.m (revolutions per minute) for 10 minutes. A clear separation of the supernatant and pellet was retained. 1 ml of glacial acetic acid was added to the residues. Then, the sample was transferred into a microcentrifuge tube and centrifuged at 5000 r.p.m for 10 minutes. After 10 minutes, the supernatant was removed. The remaining residues were mixed with 0.5 ml of acetolysis mixture and boiled on a water bath for 20 minutes. Centrifugation was done after the boiling at 5000 r.p.m for 10 minutes and the supernatant was removed. The residues that contained pollen material was washed with 0.5 ml of glacial acetic acid, centrifuged, and decanted. The pollen that remained on the residues was mounted on glycerine jelly and was transferred to a slide. The slide was observed under a microscope by using a compound microscope with camera, Leica DM 2500. Acetolysis was a procedure to recover pollen from a large number of pollinators by removing the lipids and debris on and around the pollen grains that allowing the diagnostic properties of the pollen grains to be seen more clearly. These characteristics help to give an accurate pollen identification (Jones, 2014).

3.6.1.1 Flowchart of Acetolysis Method

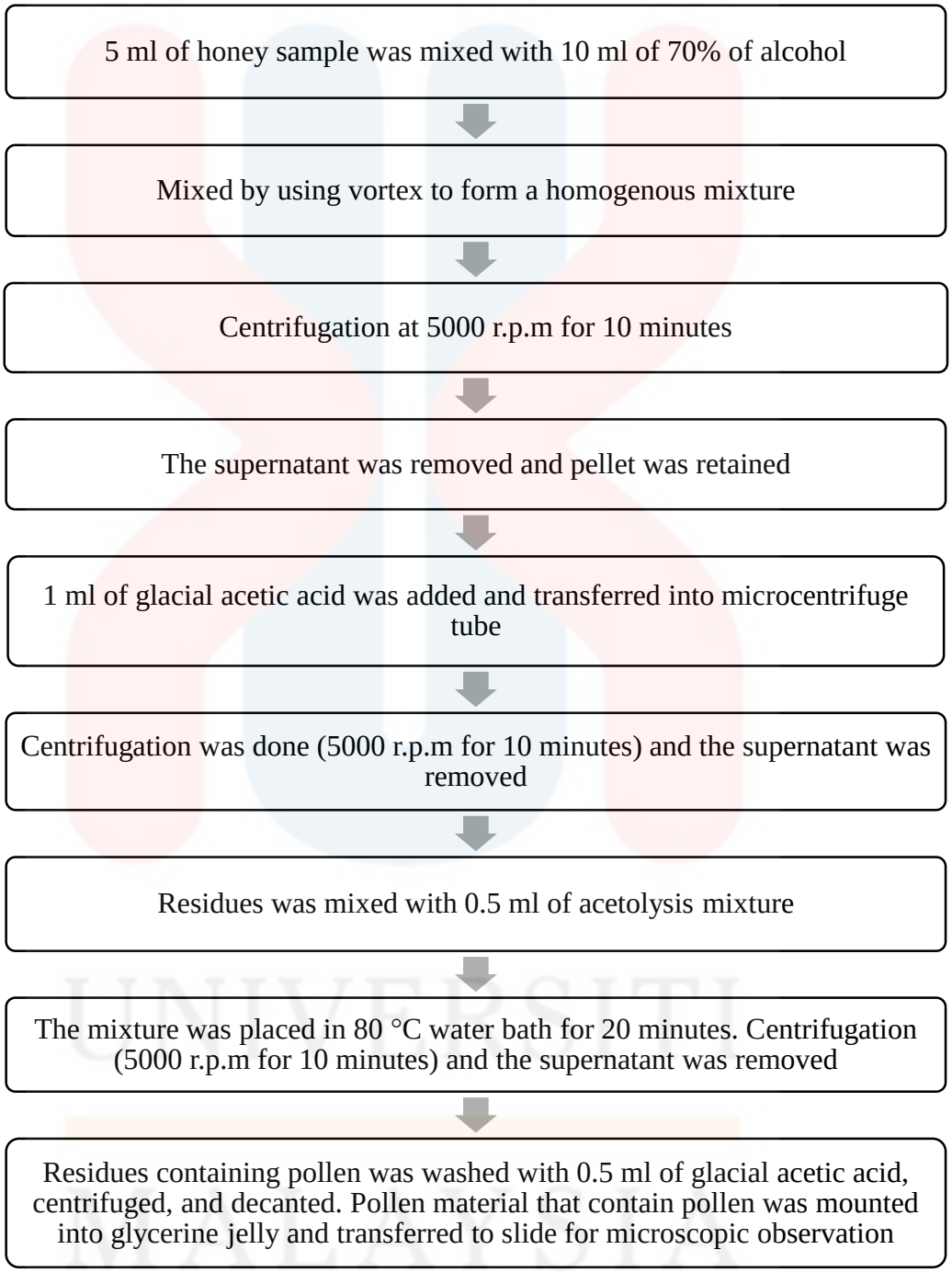


Figure 3.3: Flowchart of Acetolysis Method

3.6.2 Preparation of Glycerine Jelly

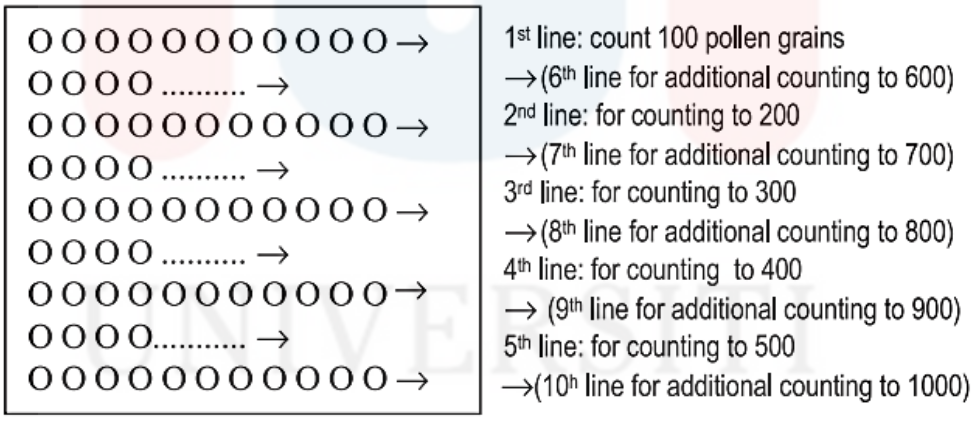
25 g of gelatin powder was dissolved in 87.5 ml of distilled water. The mixture was stirred continuously until the mixture was boiled and became homogenous. 75 ml of glycerine was added into the boiling mixture and was stirred continuously by using a glass rod. 0.1 g of safranin powder was added into the mixture to stain the jelly into pinkish colour for the observation process under the microscope. During the stirring, 3.5 g of phenol crystal was added. Phenol crystal was hazardous and the addition was done in the fume hood. The mixture was stirred continuously. The boiling was stopped when the jelly started turning into a transparent mixture. The hot glycerine jelly was filtered by using a muslin cloth and poured into a petri dish and let it solidify. The petri dish was put in the refrigerator for further solidification. The glycerine jelly prepared was used to mount the pollen material for microscopic observation of the pollen morphology. The preparation of glycerine jelly was according to Kisser's method because it increases the visibility of the pollen morphology and enables quick drying (Holanda et al., 2019).

3.6.3 Preparation of Pollen Slides for Microscopic Observation

A small amount of glycerine jelly was mixed with the pollen material from the pallet obtained at the bottom of the microcentrifuge tube. The glycerine jelly was melted so that it will spread smoothly throughout the microscope slide (Jones, 2014). The pollen from the microcentrifuge tube was mounted on the glass slide covered with a cover slip. The pollen sample was viewed under a compound microscope with a camera.

3.6.4 Pollen Count

Pollen grains were identified and counted in groups of 100, following the parallel intermediate lines evenly distributed from one side of the cover slip to the other until 500 grains were calculated. If the relative frequencies were not stabilised or the count of 500 pollen grains were insufficient for the interpretation, the count was continued to 1000 following another 5 parallel lines situated between the first 5. The percentages of each pollen type were used to characterize the pollen. The percentage of pollen can be grouped into predominant pollen (PP) with a percentage more than 45%, secondary pollen (SP) with a percentage from 16 to 45%, important minor pollen (IP) with a percentage 3 to 15%, and minor pollen (MP) with percentage 1 to 2% (Jones, 2014).



Cover slip 22 x 22 mm

(() = 1 field of vision)

Figure 3.4: Matrix for counting pollen grains that guarantees a homogeneous examination of the slide

(O = a whole microscopic field of view)

Source: (Ohe et al., 2004)

The following formula were used to obtain the pollen count per slide:

Square coverslip = 50 views per side

Area of coverslip = L x L

= 50 x 50

= 2 500

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

Where N represent the number of pollen counted in a slide

$$\text{Abundance (\%)} = \frac{\text{Total number of pollen of a particular species}}{\text{Total number of all observed pollen}} \text{ x } 100$$

3.6.5 Pollen Identification

Acetolysis method was used to identify the pollen grains. Acetolysis process degrades the tissue cells, organic debris, proteins, lipids, and carbohydrates on the surface of the pollen grain (Jones, 2014). This makes it easier to stain, photograph, and identify the pollen grains. The acetolysis solution was a mixture of 5 ml of concentrated sulfuric acid and 45 ml of acetic anhydride. Acetolysis was conducted under a fume hood since the mixture was corrosive and react violently with water. Extra caution was advised when handling and discarding the acetolysis mixture.

3.6.6 Experimental Design

In this research, Complete Randomized Design (CRD) was used to analyse the data of pollen after the percentage abundance of pollen was calculated and the pollen type was identified. The CRD method was done by using Microsoft Excel Data Sheet.

CHAPTER 4

RESULTS AND DISCUSSION

Pollen analysis of honey or melissopalynology had been done in this research for 16 samples of honey collected from 16 different locations in Kedah state. The locations involved were Pinang Tunggal (PT), Kuala Muda (KM), Bukit Pinang (BP), Taman Mutiara (TM), Pendang (PD), Kampung Kepala Titi (KT), Pekan Pendang (PP), Kelapa Btatas (KB), Karangan (KG), Kampung Keladi (KK), Kampung Sungai Buluh (SB), Jitra (JT), Langkawi (LG), Taman Siswa (TS), Kampung Padang Limau (PL), and Kampung Batu Kembai (BK). The method used for this honey pollen analysis was the Acetolysis method which was used to prepare the honey sample while pollen count and identification were used to identify the botanical origin of the honey and the honey spectra. The pollen was counted and identified according to the method of melissopalynology proposed by (Ohe et al., 2004). Melissopalynological study of 16 honey samples collected from different locations showed the presence of both unifloral and multifloral honeys.

Table 4.1 showed that 10 samples of honey were identified as multifloral honey which were from PT, KT, PP, KB, KG, KK, SB, TS, PL, and BK. Other 6 honey samples were identified as unifloral honey which were from KM, BP, TM, PD, JT, and LG. From table 4.1, honey collected from SB had the highest number of pollen counted which were 2227. The least pollen count found in the honey sample came from TS with 149 pollen counted. If more pollen type or pollen content of honey was found, it showed that the honey was rich with the source of nectar collection (Rosdi et al., 2021).

Table 4.2 showed that the honey sample from PT was a multifloral type of honey because none of the plant species found getting >45% of pollen abundance. The highest percentage of abundance that can be seen in this sample was from Cucurbitaceae family, *Cucumis sativus* with 40.77%. There was no predominant pollen found in all six plant species in this sample. In table 4.3, *Amaranthus viridis* from Amaranthaceae family had the highest percentage of abundance which was 57.75% and describe the honey from KM as unifloral honey. Table 4.4, depicted the honey from BP as unifloral honey since *Cassia biflora* from the family Fabaceae had 66.50% of abundance.

For the sample from TM (Table 4.5), there was a plant species with 80.79% of abundance which was *Sporobolus indicus* from Poaceae family that represent the honey type as unifloral honey. Table 4.6, showed that the honey sample from PD was unifloral honey since *Brassica chinensis* had a percentage of abundance 83.73%. In table 4.7, there was no predominant pollen found in the honey sample from KT. The highest percentage of abundance was 31.24% came from the Cucurbitaceae family, *Cucumis sativus* and the least abundance was *Albizia lebbeck* from the Fabaceae family.

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

Areas of Origin	Total No. of Pollen	No. of Pollen Types	Honey Spectra
Pinang Tunggal (PT)	233	6	Multifloral
Kuala Muda (KM)	213	7	Unifloral
Bukit Pinang (BP)	388	7	Unifloral
Taman Mutiara (TM)	864	5	Unifloral
Pendang (PD)	252	5	Unifloral
Kampung Kepala Titi (KT)	1242	7	Multifloral
Pekan Pendang (PP)	930	6	Multifloral
Kepala Batas (KB)	1953	6	Multifloral
Karangan (KG)	237	7	Multifloral
Kampung Keladi (KK)	380	9	Multifloral
Kampung Sungai Buluh (SB)	2227	13	Multifloral
Jitra (JT)	702	7	Unifloral
Langkawi (LG)	781	8	Unifloral
Taman Siswa (TS)	149	10	Multifloral
Kampung Padang Limau (PL)	317	7	Multifloral
Kampung Batu Kembai (BK)	342	9	Multifloral

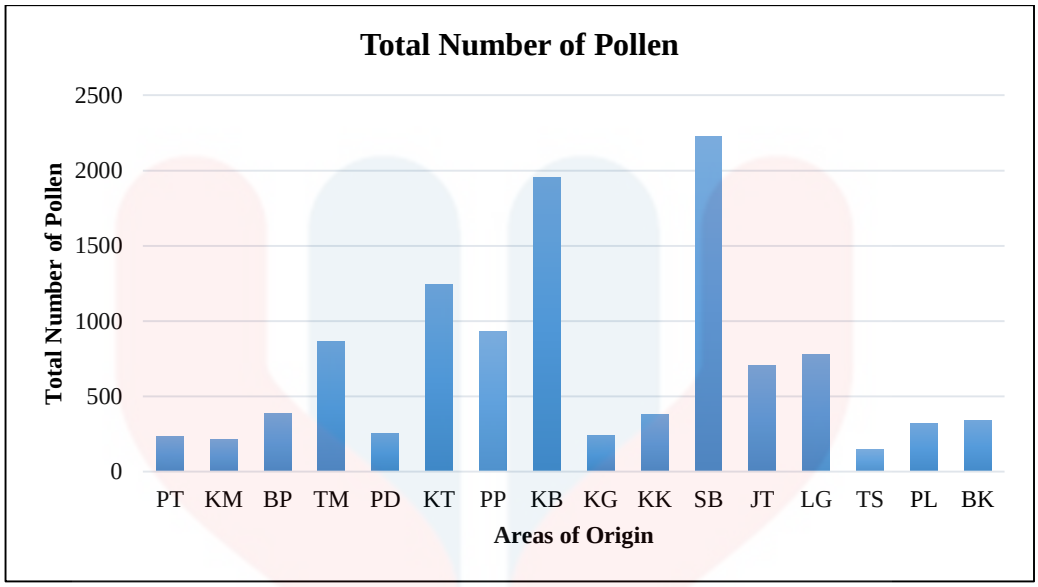


Figure 4.1: Total number of pollen honey samples according to areas of origin

In table 4.8, it showed that the sample from PP was a multifloral type of honey because there was no predominant pollen found. The highest percentage of abundance was *Holcus mollis* from the Poaceae family with 37.63% and the least was *Jacaranda obtusifolia* from the family Bignoniaceae with 4.95% of abundance. For table 4.9, the most abundance was *Sporobolus indicus* from Poaceae family which was 36.25% and this sample was known as multifloral honey since none of them got >45%. Sample from KG (Table 4.10) was known as multifloral honey type since there was no predominant pollen found and *Desmodium adscendens* was the most abundance pollen.

Table 4.11 and table 4.12 revealed that there was no existence of predominant pollen for KK and SB samples due to their low percentage of abundance of pollen types in the honey sample. Table 4.13 depicted that the honey sample from JT was unifloral type of honey since *Zea mays* has the highest percentage of abundance which was

78.92%. For table 4.14, *Sporobolus indicus* from Poaceae family was the most abundance with a percentage of 46.09% and showed that the honey sample from LG was unifloral type of honey.

In table 4.15, there were 10 types of pollen and the most abundance was *Melastoma malabathricum* while the rarely occurs pollen belongs to 3 species which were *Acacia auriculiformis, Cocos nucifera*, and *Commelina diffusa*. Table 4.16 revealed that there was no predominant pollen found in the PL sample because of their low percentage of abundance of pollen types in the honey sample. The last sample (Table 4.17), showed that the sample from BK was a multifloral type of honey since none of the pollen types found has predominant pollen >45% and the most abundance was *Sporobolus indicus* from the Poaceae family with 28.07%.

4.2 Honey sample from Pinang Tunggal, Sungai Petani (Multifloral)

Table 4.2: The number of pollen and percentage of pollen types in the honey sample from Pinang Tunggal (PT).

Plant Species	Family	Total No. of Pollen	Percentage of Abundance (%)
Annona squamosa	Annonaceae	13	5.60
Capsicum frutescens	Solanaceae	25	10.73
Cucumis sativus	Cucurbitaceae	95	40.77
Melastoma malabathricum	Melastomataceae	6	2.58
Veitchia merrillii	Arecaceae	18	7.73
Zea mays	Poaceae	76	32.62

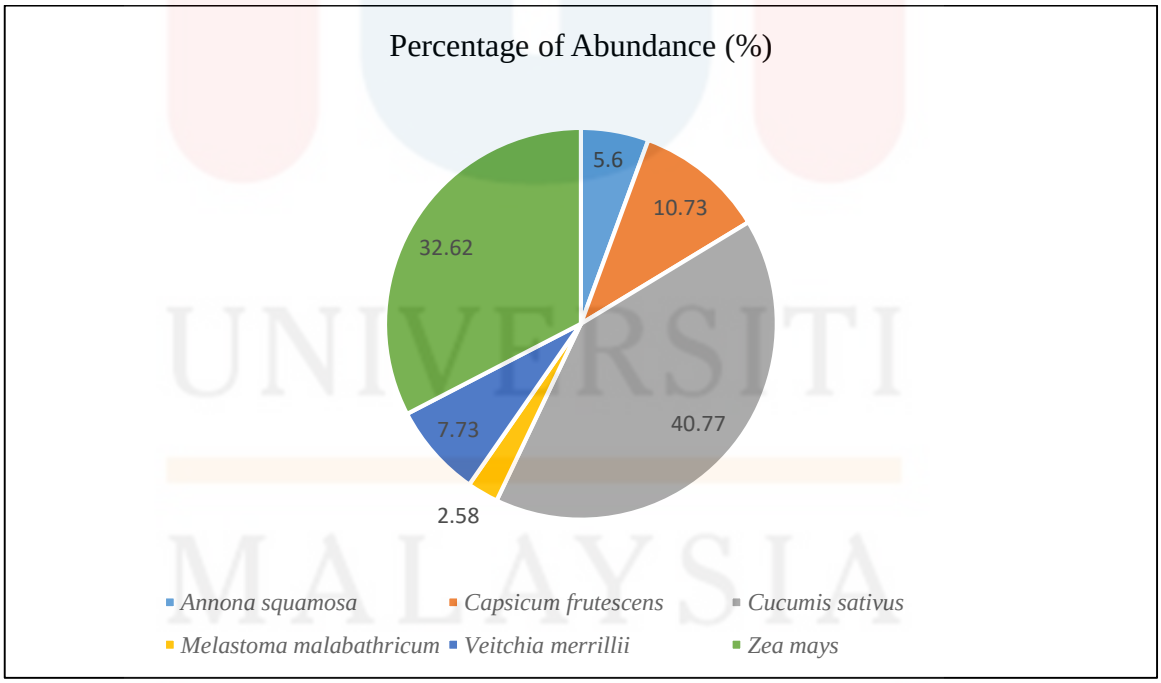


Figure 4.2: Pollen spectrum of honey sample from Pinang Tunggal, Sungai Petani

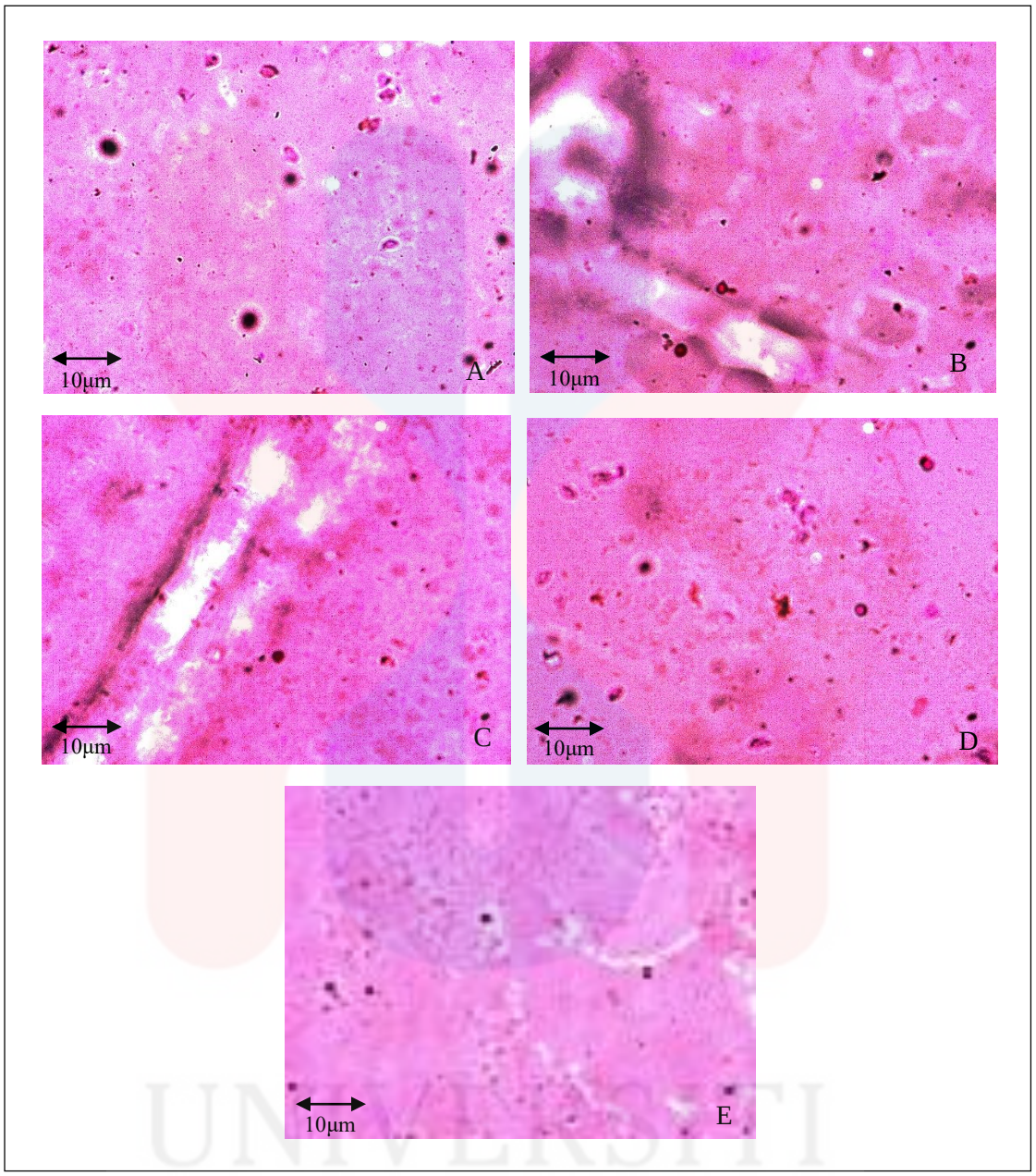


Figure 4.2 (a): Microscopic overviews of pollen density in honey sample from Pinang Tunggal under 10x magnification. Overviews of A- Right edge of cover slip (top); B- Left edge of cover slip (top); C- Right edge of cover slip (bottom); D- Left edge of cover slip (bottom) and E- centre of cover slip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

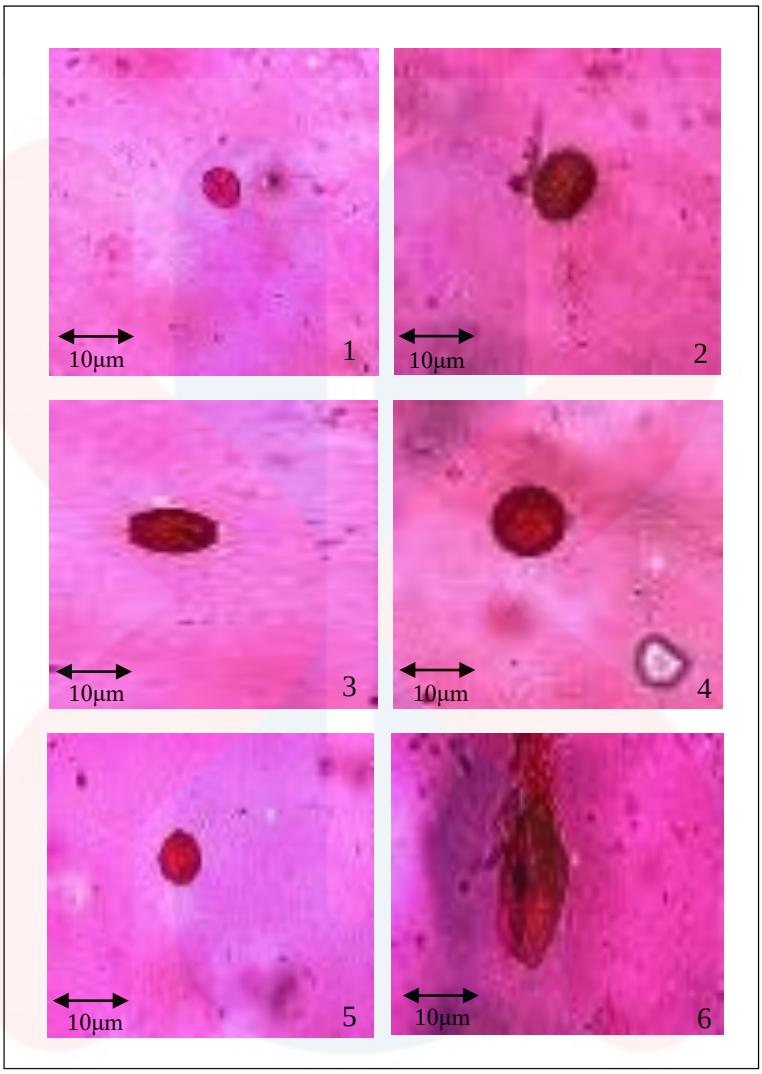


Figure 4.2 (b): Morphology of different pollen honey sample from Pinang Tunggal under 40x magnification. 1- *Annona squamosa* (Annonaceae); 2- *Melastoma malabathricum* (Melastomataceae); 3- *Cucumis sativus* (Cucurbitaceae); 4- *Zea mays* (Poaceae); 5- *Capsicum frutescens* (Solanaceae) and 6- *Veitchia merrillii* (Arecaceae)

In the honey sample from Pinang Tunggal, there were different pollen types from different plant species present which were *Annona squamosa* (Annonaceae), *Melastoma malabathricum* (Melastomataceae), *Cucumis sativus* (Cucurbitaceae), *Zea mays* (Poaceae), *Capsicum frutescens* (Solanaceae), and *Veitchia merrillii* (Arecaceae).

This honey sample was included in multifloral type of honey because there was no predominant pollen found. The most abundance pollen came from *Cucumis sativus* which was commonly known as a cucumber with 40.77% of abundance which referred to secondary pollen types. *Zea mays*, corn also had been identified as secondary pollen type with 32.62% of pollen abundance. There were 3 types of important minor pollen types found which were *Capsicum frutescens* (tabasco pepper), *Veitchia merrillii* (Manila palm), and *Annona squamosa* (custard apple) while *Melastoma malabathricum* or known as 'Senduduk' in Malay was found as a minor pollen type. Minor pollen type refers to frequency classes of pollen that was <3% of pollen abundance. The relative proportions of pollen were important because it gives information about the source of nectar and pollen where the stingless bee collect (Ulusoy & Kolayli, 2013).

4.3 Honey sample from Kuala Muda, Sungai Petani (Unifloral)

Table 4.3: The number of pollen and percentage of pollen types in the honey sample from Kuala Muda (KM).

Plant Species	Family	Total No. of Pollen	Percentage of Abundance (%)
Amaranthus viridis	Amaranthaceae	123	57.75
Chlorophytum comosum	Asparagaceae	9	4.23
Cocos nucifera	Arecaceae	11	5.16
Elaeis guineensis	Arecaceae	18	8.45
Hibiscus trionum	Malvaceae	1	0.47
Melilotus suaveolens	Fabaceae	28	13.15
Vaccinium bracteatum	Ericaceae	23	10.80

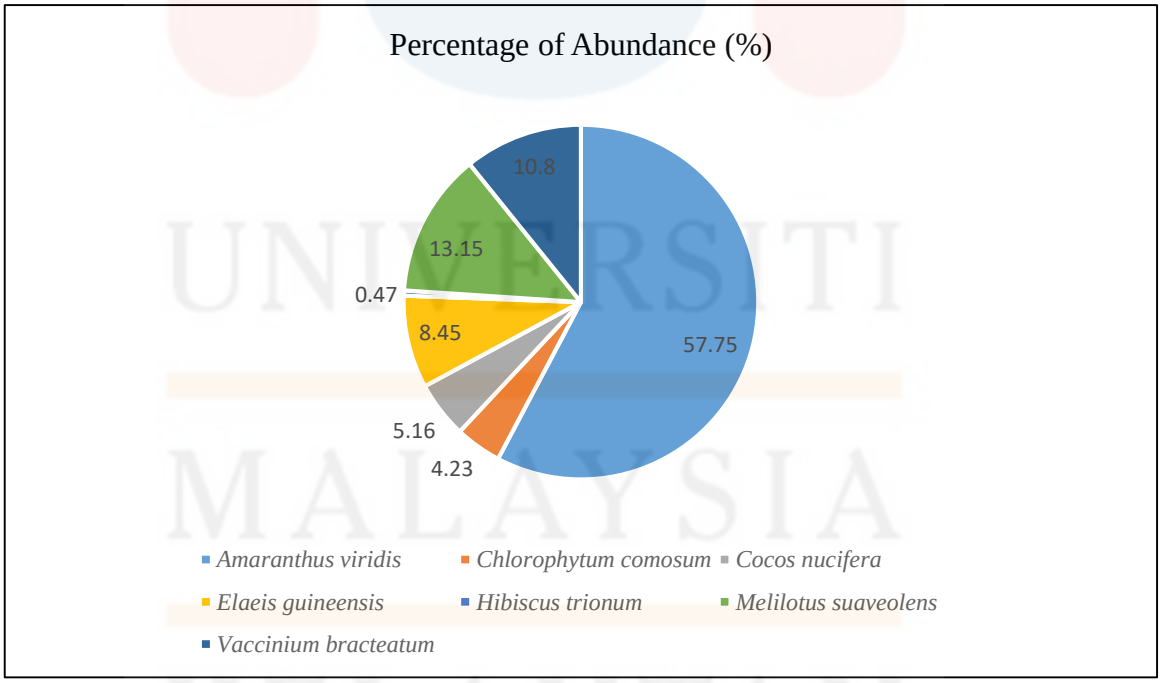


Figure 4.3: Pollen spectrum of honey sample from Kuala Muda, Sungai Petani

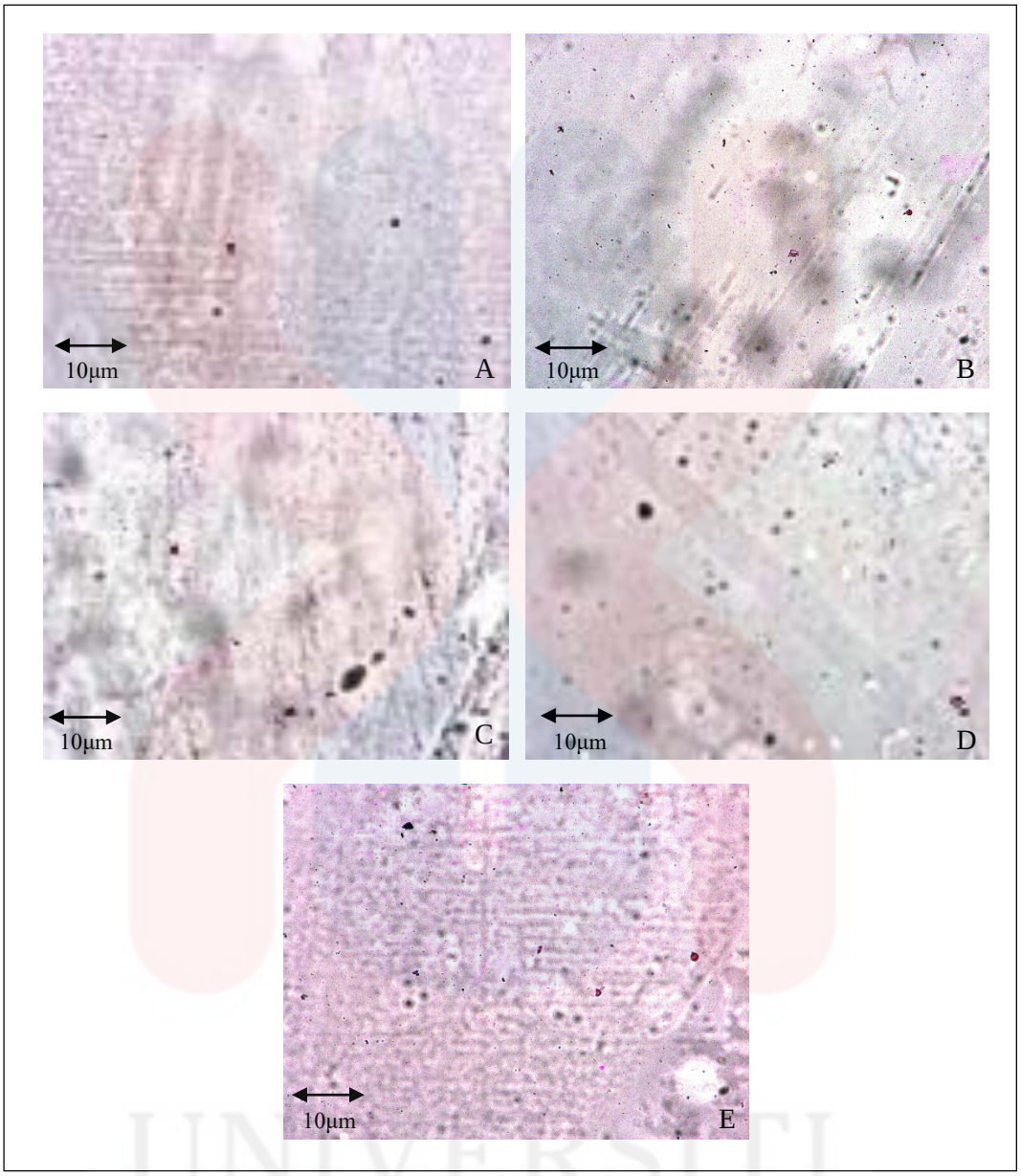


Figure 4.3 (a): Microscopic overviews of pollen density in honey sample from Kuala Muda under 10x magnification. Overviews of A- Right edge of cover slip (top); B- Left edge of cover slip (top); C- Right edge of cover slip (bottom); D- Left edge of cover slip (bottom) and E- centre of cover slip (middle)

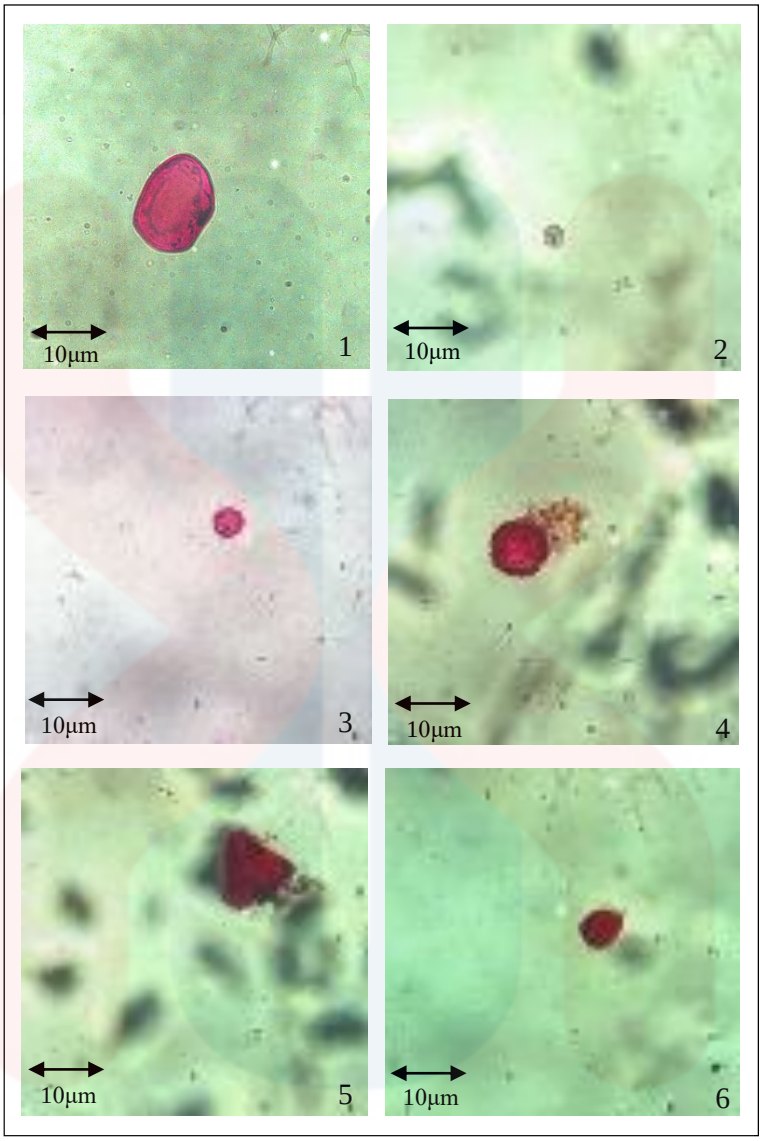


Figure 4.3 (b): Morphology of different pollen honey sample from Kuala Muda under 40x magnification. 1- *Cocos nucifera* (Arecaceae); 2- *Vaccinium bracteatum* (Ericaceae); 3- *Amaranthus viridis* (Amaranthaceae); 4- *Chlorophytum comosum* (Asparagaceae); 5- *Elaeis guineensis* (Arecaceae) and 6- *Melilotus suaveolens* (Fabaceae)

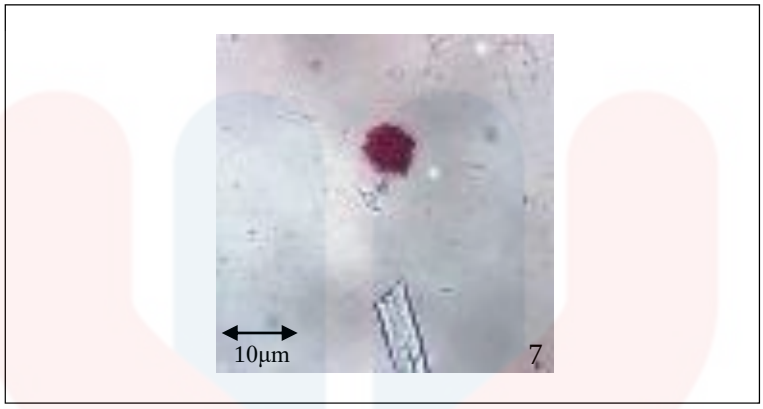


Figure 4.3 (c): Morphology of different pollen honey sample from Kuala Muda under 40x magnification. 7-*Hibiscus trionum* (Malvaceae)

7 different types of pollen were found from Kuala Muda samples which were *Cocos nucifera* (Arecaceae), *Vaccinium bracteatum* (Ericaceae), *Amaranthus viridis* (Amaranthaceae), *Chlorophytum comosum* (Asparagaceae), *Elaeis guineensis* (Arecaceae), *Melilotus suaveolens* (Fabaceae), and *Hibiscus trionum* (Malvaceae). This honey sample was classified as a unifloral honey type since the predominant pollen exceeds 45%. Pollen of a particular plant species was said to be predominant if its occurrence in the honey sample was more than 45% of the total pollen count. The predominant pollen in the Kuala Muda sample was *Amaranthus viridis* from the family Amaranthacea which accounted up to 57.75% of pollen abundance. *Amaranthus viridis* or known as green amaranthus was an annual herbaceous plant that can be distinguished by the wrinkled fruits and its small green flower that radiated around the stem which was acute and less than 1 mm long (Reyad-ul-Ferdous, 2015).

Melilotus suaveolens and *Vaccinium bracteatum* were identified as important minor pollen types with 13.15% and 10.80% of pollen abundance respectively. *Melilotus suaveolens* common name was sweet clover while *Vaccinium bracteatum* was known as Asiatic bilberry. 3 plant species were identified as minor pollen types which were *Chlorophytum comosum* (spider plant), *Cocos nucifera* (coconut), and *Elaeis guineensis* (oil palm). Pollen from *Hibiscus trionum* (venice mallow) was also present in the Kuala Muda sample. Honey from Kuala Muda can be called green amaranthus honey due to the abundance of pollen from this type of plant species. The information on pollen found was important in many areas since it can help to increase the yield of useful crops which the stingless bees foraged by keeping them close to the plant species (Jones, 2014). The relative abundance of pollen indicates that green amaranthus species was the most important source of nectar and pollen in the Kuala Muda area.

4.4 Honey sample from Bukit Pinang, Alor Setar (Unifloral)

Table 4.4: The number of pollen and percentage of pollen types in the honey sample from Bukit Pinang (BP).

Plant Species	Family	Total No. of Pollen	Percentage of Abundance (%)
Azadirachta indica	Meliaceae	18	4.64
Beckwithia glacialis	Ranunculaceae	1	0.26
Cassia biflora	Fabaceae	258	66.50
Myrica gale	Myricaceae	97	25.00
Nasturtium officinale	Brassicaceae	1	0.26
Nymphaea alba	Nymphaeaceae	4	1.03
Veitchia merrillii	Arecaceae	9	2.32

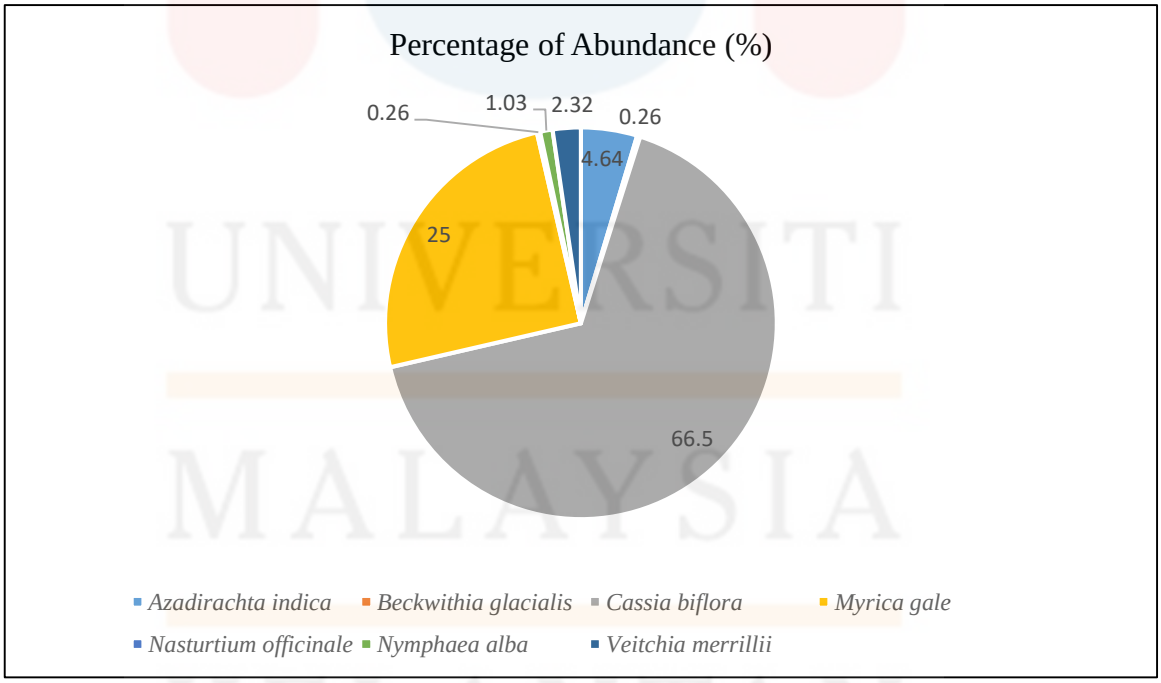


Figure 4.4: Pollen spectrum of honey sample from Bukit Pinang, Alor Setar

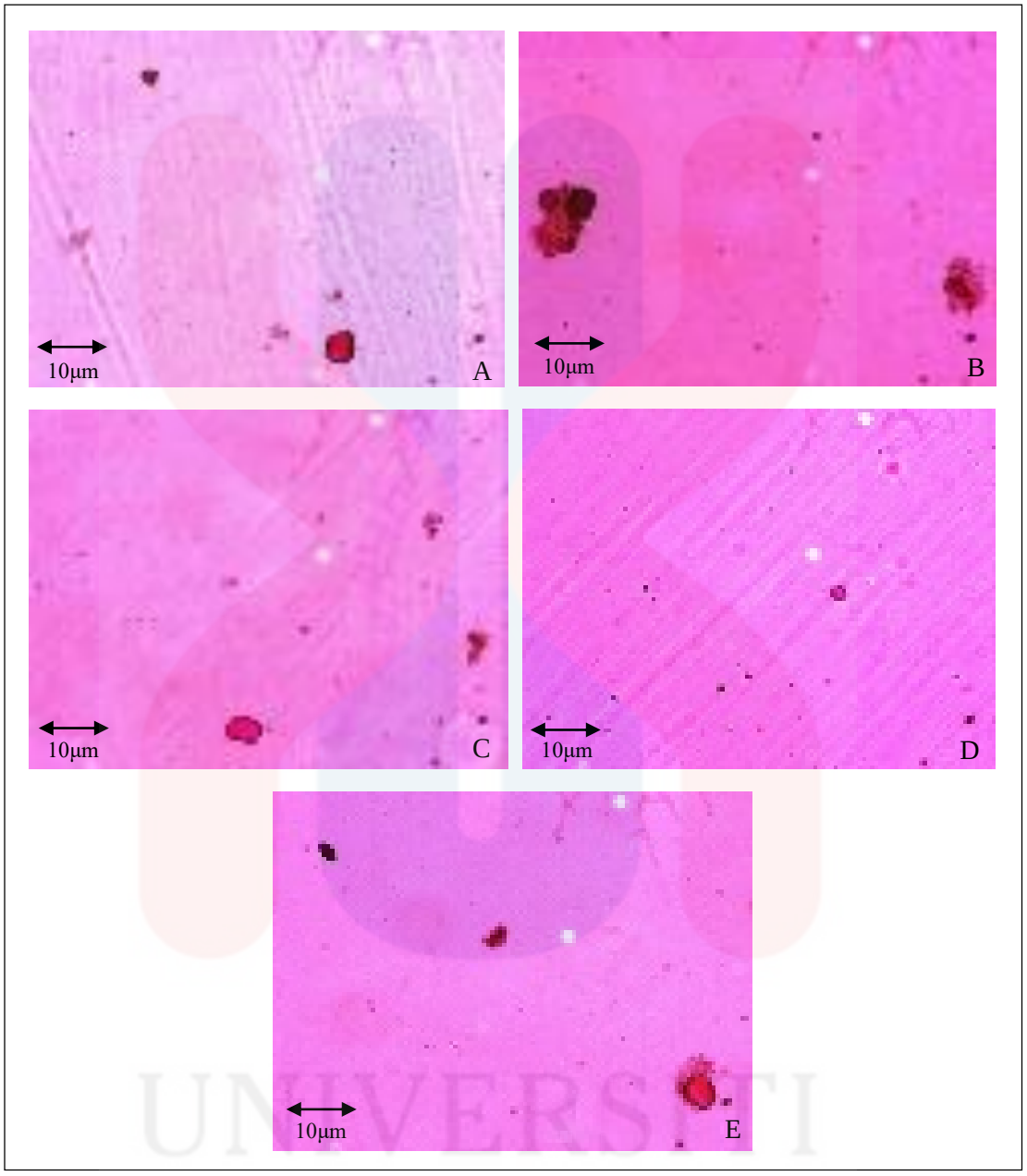


Figure 4.4 (a): Microscopic overviews of pollen density in honey sample from Bukit Pinang under 10x magnification. Overviews of A- Right edge of cover slip (top); B- Left edge of cover slip (top); C- Right edge of cover slip (bottom); D- Left edge of cover slip (bottom) and E- centre of cover slip (middle)

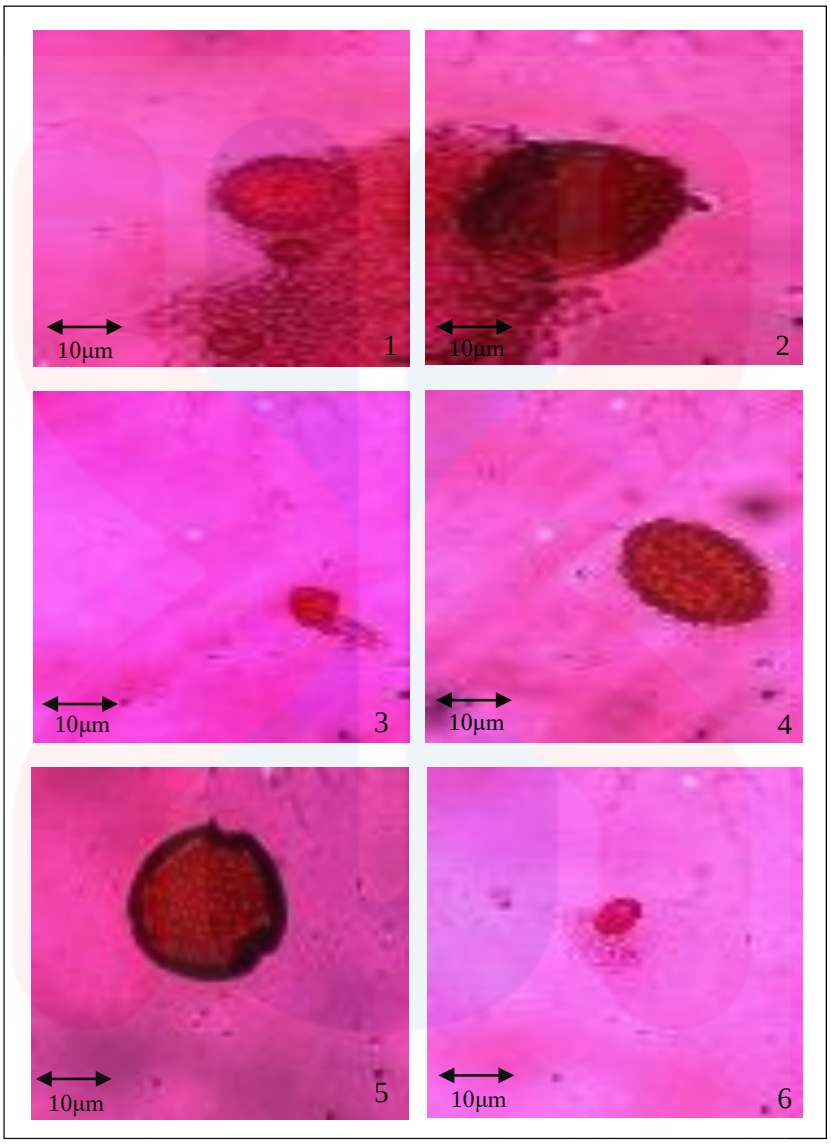


Figure 4.4 (b): Morphology of different pollen honey sample from Bukit Pinang under 40x magnification. 1- *Nasturtium officinale* (Brassicaceae); 2- *Veitchia merrillii* (Arecaceae); 3- *Myrica gale* (Myricaceae); 4- *Nymphaea alba* (Nymphaeaceae); 5- *Cassia biflora* (Leguminosae) and 6- *Azadirachta indica* (Meliaceae)

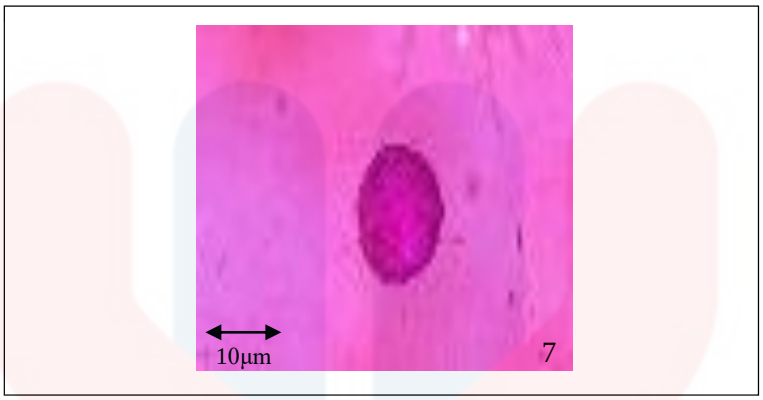


Figure 4.4 (c): Morphology of different pollen honey sample from Bukit Pinang under 40x magnification. 7- *Beckwithia glacialis* (Ranunculaceae)

Honey sample from Bukit Pinang consists of 7 different types of pollen which were *Nasturtium officinale* (Brassicaceae), *Veitchia merrillii* (Arecaceae), *Myrica gale* (Myricaceae), *Nymphaea alba* (Nymphaeaceae), *Cassia biflora* (Leguminosae), *Azadirachta indica* (Meliaceae), and *Beckwithia glacialis* (Ranunculaceae). Predominant pollen for this sample was *Cassia biflora* plant species that accounted up to 66.50% of the total pollen abundance. It was called desert cassia which was included in the shrub category with feathery branches and arching canopy. It has a monoecious yellow flowers which attracts pollinators such as bees and butterflies.

Myrica gale was identified as secondary pollen type with 25.00% of total pollen abundance while *Azadirachta indica* was found as an important minor pollen type with 4.64% pollen counted. Other pollen that were present in Bukit Pinang sample were *Beckwithia glacialis* (0.26%), *Nasturtium officinale* (0.26%), *Nymphaea alba* (1.03%), and *Veitchia merrillii* (2.32%). The occurrence of predominant pollen showed that the

honey sample from Bukit Pinang was a unifloral type. This proved that Kelulut bees preferred to forage pollen and nectar from the *Cassia biflora* plant that can be found around Bukit Pinang areas. Pollen and nectar are collected by bees based on the accessibility to the botanical sources that suits their foraging ranges which can be influenced by environmental and seasonal conditions (Fechner et al., 2016).

4.5 Honey sample from Taman Mutiara, Sungai Petani (Unifloral)

Table 4.5: The number of pollen and percentage of pollen types in the honey sample from Taman Mutiara (TM).

Plant Species	Family	Total No. of Pollen	Percentage of Abundance (%)
Cissus rubiginosa	Vitaceae	88	10.19
Pterocarpus indicus	Fabaceae	56	6.48
Scurrula ferruginea	Loranthaceae	1	0.12
Sporobolus indicus	Poaceae	698	80.79
Veitchia merrillii	Arecaceae	21	2.43

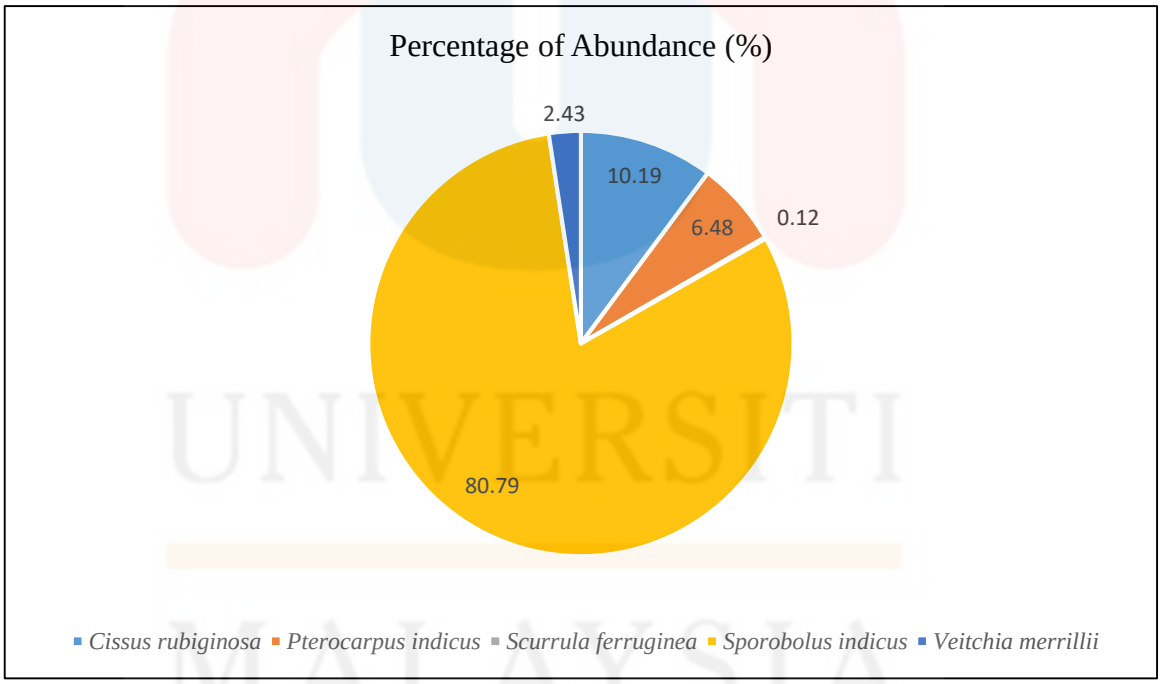


Figure 4.5: Pollen spectrum of honey sample from Taman Mutiara, Sungai Petani

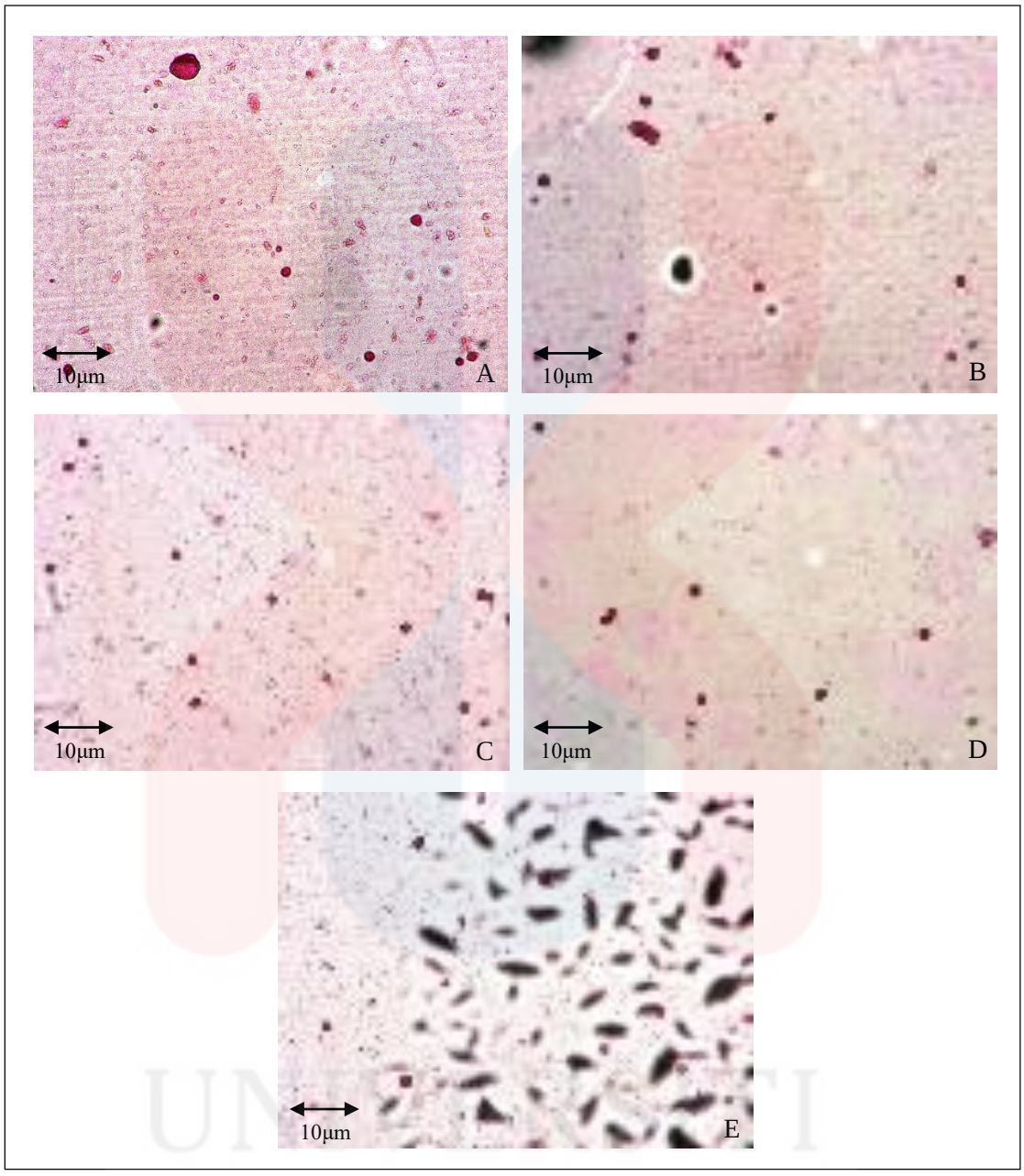


Figure 4.5 (a): Microscopic overviews of pollen density in honey sample from Taman Mutiara under 10x magnification. Overviews of A- Right edge of cover slip (top); B- Left edge of cover slip (top); C- Right edge of cover slip (bottom); D- Left edge of cover slip (bottom) and E- centre of cover slip (middle)

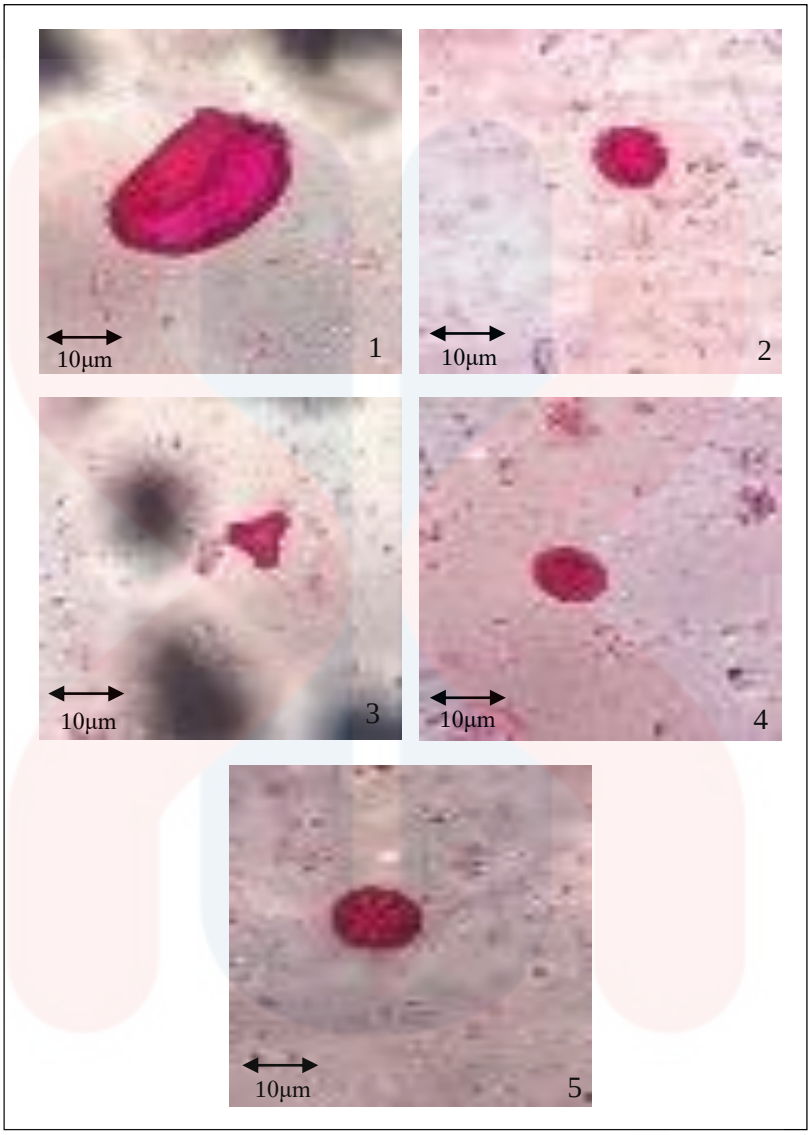


Figure 4.5 (b): Morphology of different pollen honey sample from Taman Mutiara under 40x magnification. 1- *Veitchia merrillii* (Arecaceae); 2- *Pterocarpus indicus* (Fabaceae); 3- *Scurrula ferruginea* (Loranthaceae); 4- *Cissus rubiginosa* (Vitaceae) and 5- *Sporobolus indicus* (Poaceae)

The table showed the results obtained for the honey sample from Taman Mutiara. The pollen that can be identified were from *Veitchia merrillii* (Arecaceae), *Pterocarpus indicus* (Fabaceae), *Scurrula ferruginea* (Loranthaceae), *Cissus rubiginosa* (Vitaceae),

and *Sporobolus indicus* (Poaceae). The highest percentage of abundance was from the family Poaceae which was *Sporobolus indicus* with 80.79% of pollen counted. *Sporobolus indicus* was a grass species known as smut grass which can be found in many regions. Smut grass was a perennial grass with basal leaves, green spike-like flowers, and usually grew during warm seasons (Sellers et al., 2020).

The important minor pollen were *Cissus rubiginosa*, family Vitaceae and *Pterocarpus indicus*, family Fabaceae with 10.19% and 6.48% of pollen abundance respectively. Minor pollen type also presents which was *Veitchia merrillii* species with 2.43% of pollen abundance. Pollen from *Scurrula ferruginea* species also was present in this sample. The honey sample for Taman Mutiara was identified as unifloral type due to the occurrence of predominant pollen. This emphasises the value of pollen analysis in understanding the botanical and geographical origins of honey. Stingless bee honey had a higher pollen content, which could be owing to the presence of stingless bees which predate the stinging honey bee (Ramli et al., 2017).

4.6 Honey sample from Pendang (Unifloral)

Table 4.6: The number of pollen and percentage of pollen types in the honey sample from Pendang.

Plant Species	Family	Total No. of Pollen	Percentage of Abundance (%)
Brassica chinensis	Brassicaceae	211	83.73
Cyperus rotundus	Cyperaceae	5	1.98
Mimosa pudica	Fabaceae	3	1.19
Narcissus pseudonarcissus	Amaryllidaceae	8	3.17
Psidium guajava	Myrtaceae	25	9.92

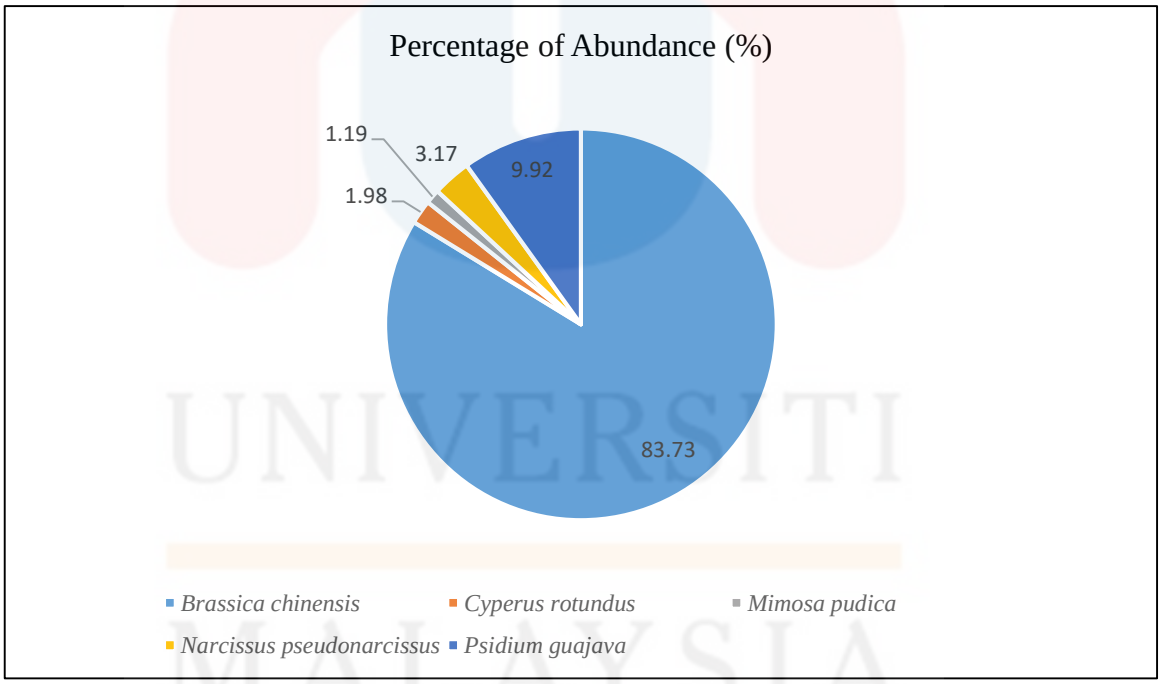


Figure 4.6: Pollen spectrum of honey sample from Pendang

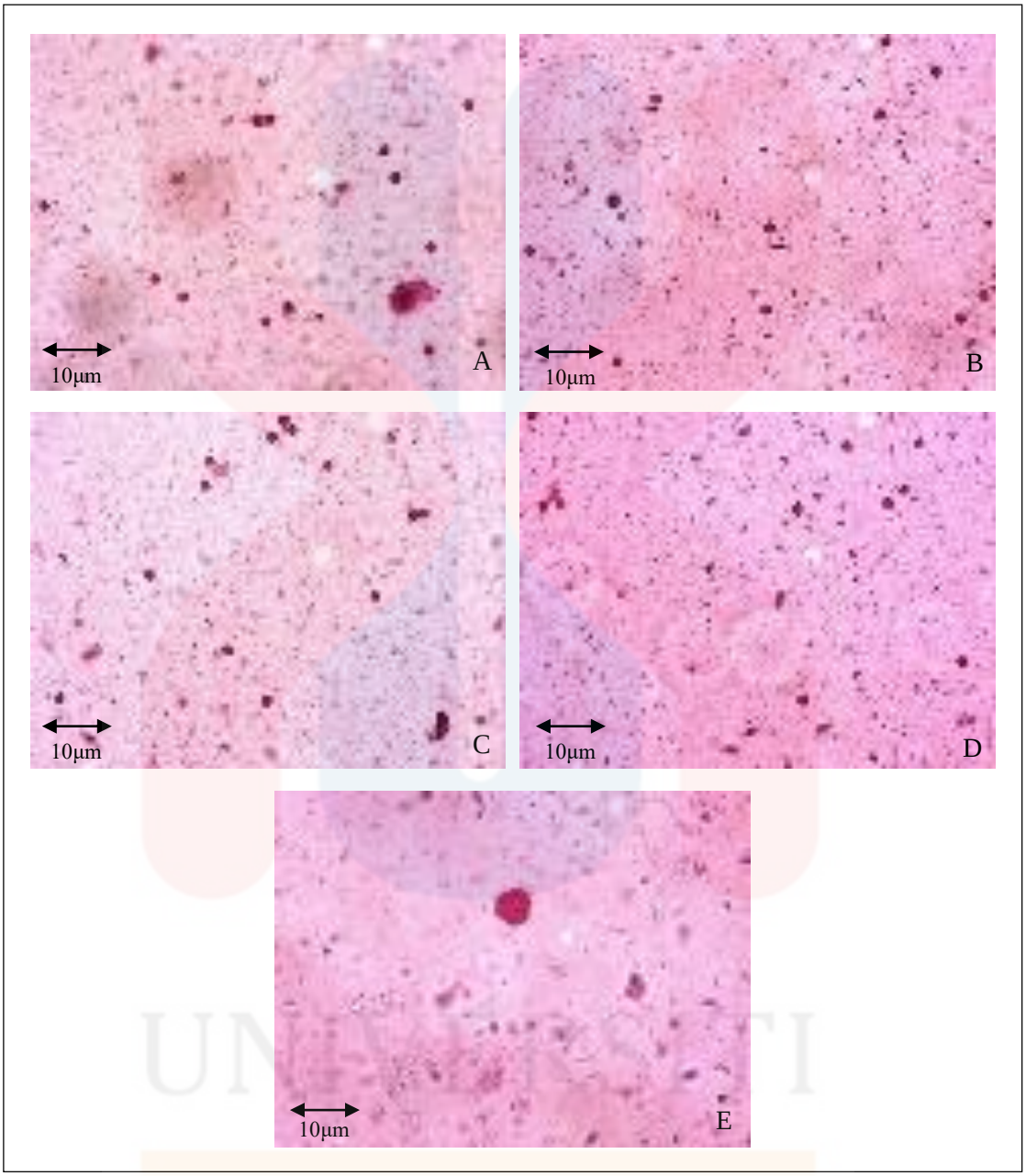


Figure 4.6 (a): Microscopic overviews of pollen density in honey sample from Pendang under 10x magnification. Overviews of A- Right edge of cover slip (top); B- Left edge of cover slip (top); C- Right edge of cover slip (bottom); D- Left edge of cover slip (bottom) and E- centre of cover slip (middle)

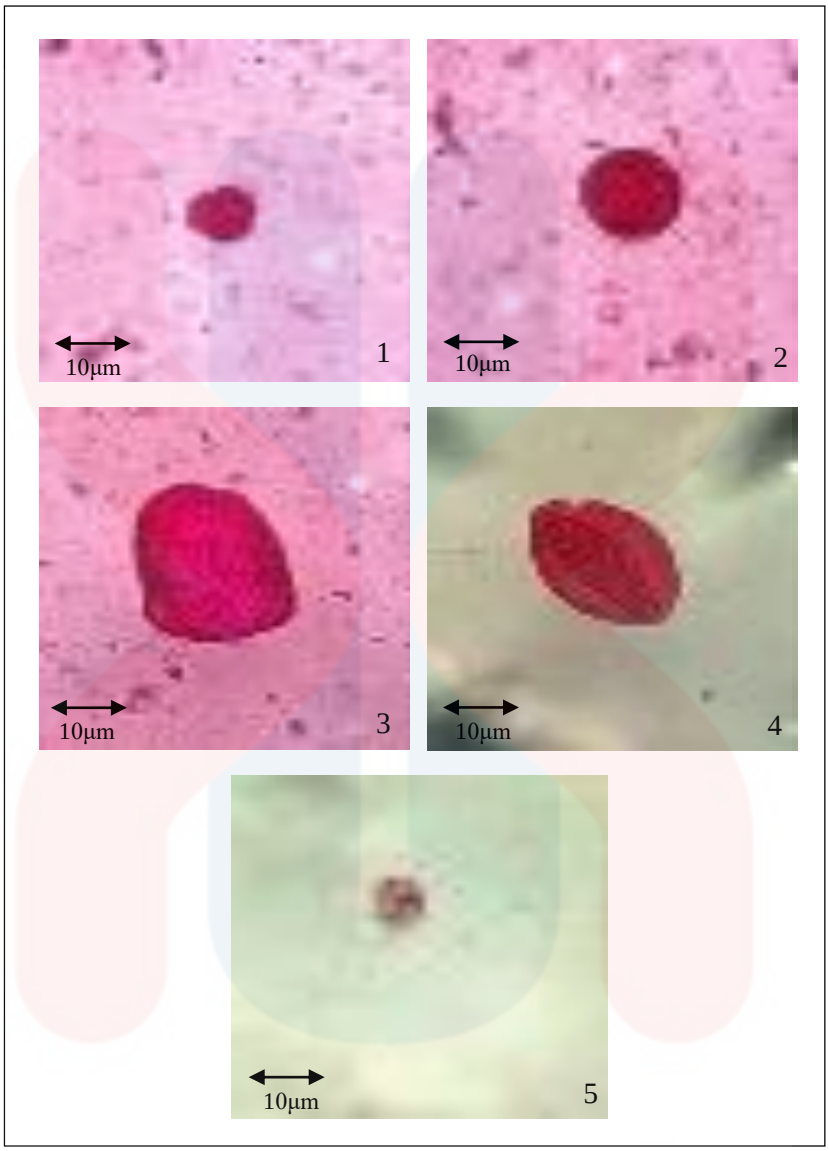


Figure 4.6 (b): Morphology of different pollen honey sample from Pendang under 40x magnification. 1- *Psidium guajava* (Myrtaceae); 2- *Brassica chinensis* (Brassicaceae); 3- *Cyperus rotundus* (Cyperaceae); 4- *Narcissus pseudonarcissus* (Amaryllidaceae) and 5- *Mimosa pudica* (Fabaceae)

There were 5 different types of pollen that were successfully identified from honey collected in Pendang which were *Psidium guajava* (Myrtaceae), *Brassica chinensis* (Brassicaceae), *Cyperus rotundus* (Cyperaceae), *Narcissus pseudonarcissus*

(Amaryllidaceae), and *Mimosa pudica* (Fabaceae). The highest percentage of abundance came from *Brassica chinensis* which was in family Brassicaceae with 83.73%. *Narcissus pseudonarcissus* and *Psidium guajava* were categorized in important minor pollen types with 3.17% and 9.92% respectively while *Cyperus rotundus* and *Mimosa pudica* were categorized in minor pollen type with 1.98% and 1.19% respectively. The presence of predominant pollen proved that honey from Pendang was unifloral type of honey.

Brassica chinensis was known as Chinese cabbage or pak choi. It was known as annual herb species that have thick and soft stems, green petiole, produce small yellow flowers, and was harvested as green vegetables. It is widely cultivated in the Philippines, Malaysia, Indonesia, and Thailand (Thumdee & B. Poonlarp, 2018). *Narcissus pseudonarcissus* was commonly known as wild daffodil and *Psidium guajava* was called guava or 'Jambu Batu' in Malay. *Cyperus rotundus* common name was nutgrass and *Mimosa pudica* was known as touch-me-not plant or 'Rumput Simalu' in Malay.

4.7 Honey sample from Kampung Kepala Titi, Sedim, Kulim (Multifloral)

Table 4.7: The number of pollen and percentage of pollen types in the honey sample from Kampung Kepala Titi (KT).

Plant Species	Family	Total No. of Pollen	Percentage of Abundance (%)
Albizia lebbeck	Fabaceae	25	2.01
Cocos nucifera	Arecaceae	71	5.72
Cucumis sativus	Cucurbitaceae	388	31.24
Elaeis guineensis	Arecaceae	69	5.56
Euphorbia neriifolia	Euphorbiaceae	32	2.58
Nephelium lappaceum	Sapindaceae	371	29.87
Tetrastigma hookeri	Vitaceae	286	23.03

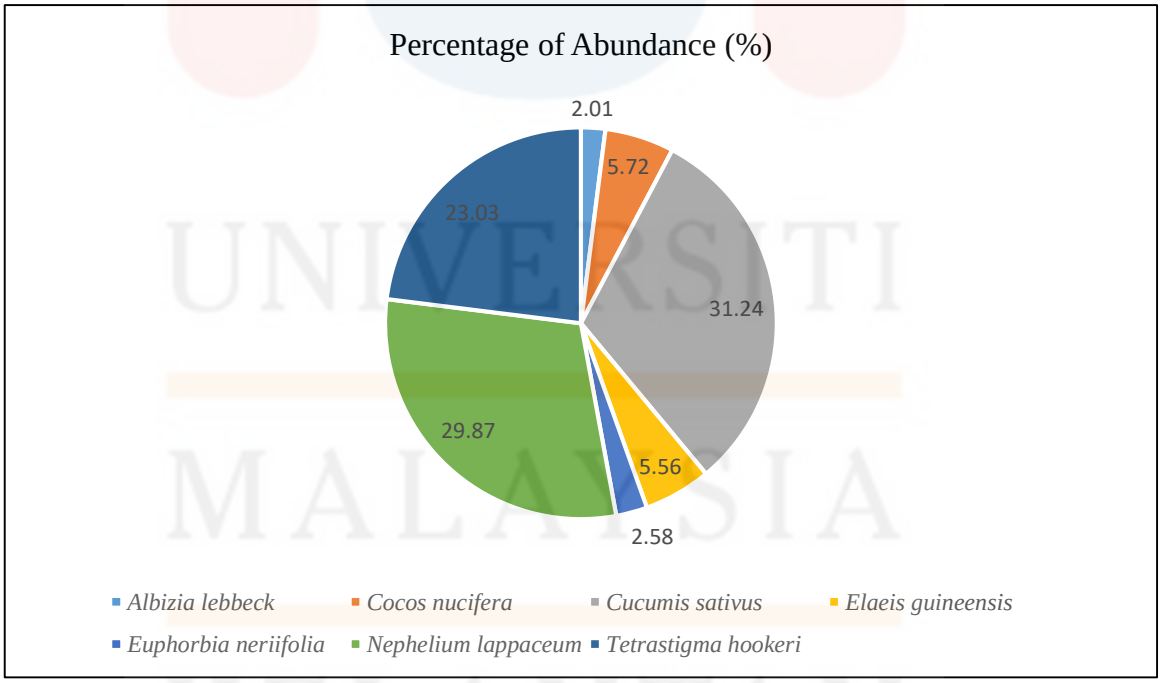


Figure 4.7: Pollen spectrum of honey sample from Kampung Kepala Titi, Sedim, Kulim

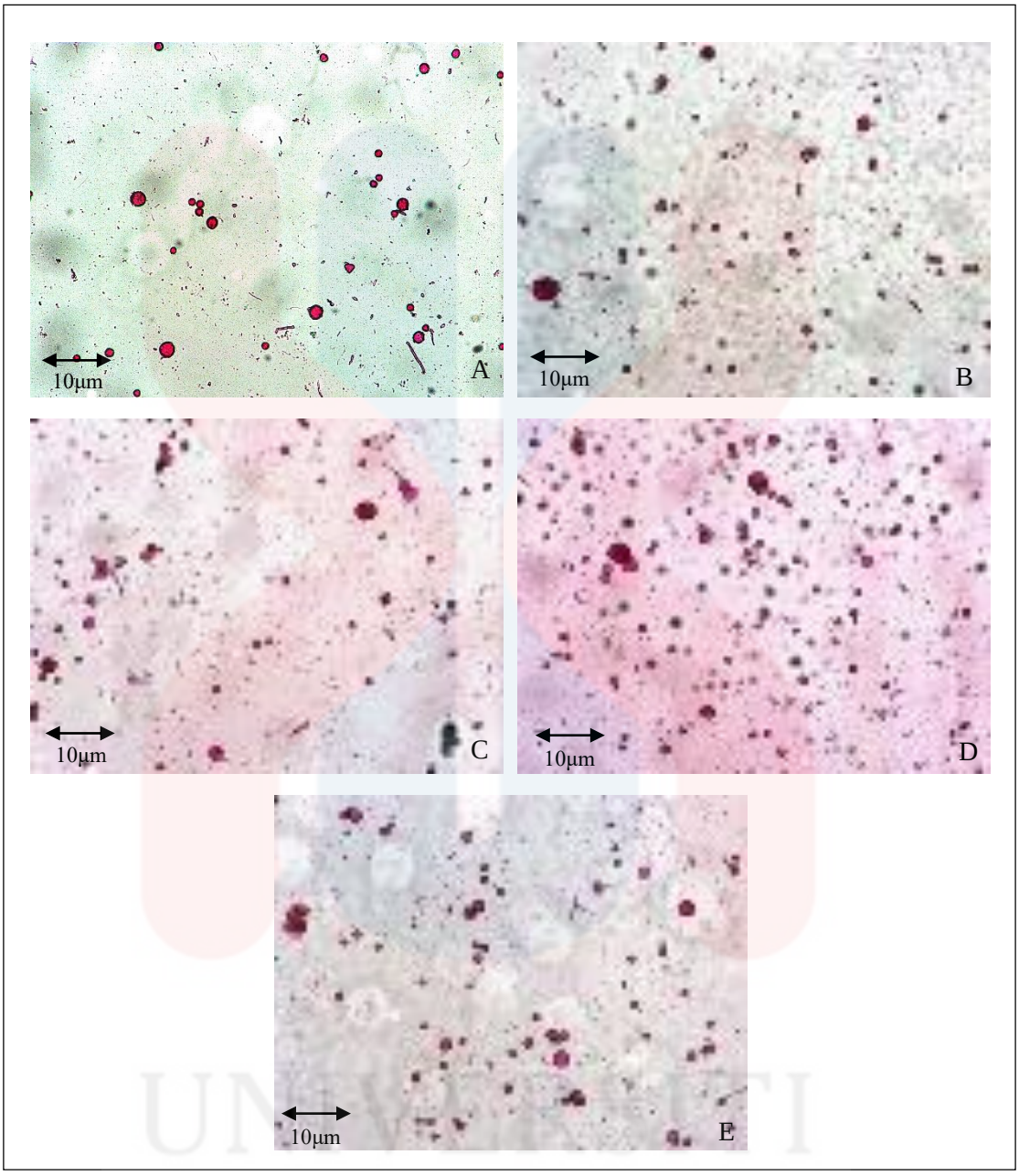


Figure 4.7 (a): Microscopic overviews of pollen density in honey sample from Kampung Kepala Titi, Sedim under 10x magnification. Overviews of A- Right edge of cover slip (top); B- Left edge of cover slip (top); C- Right edge of cover slip (bottom); D- Left edge of cover slip (bottom) and E- centre of cover slip (middle)

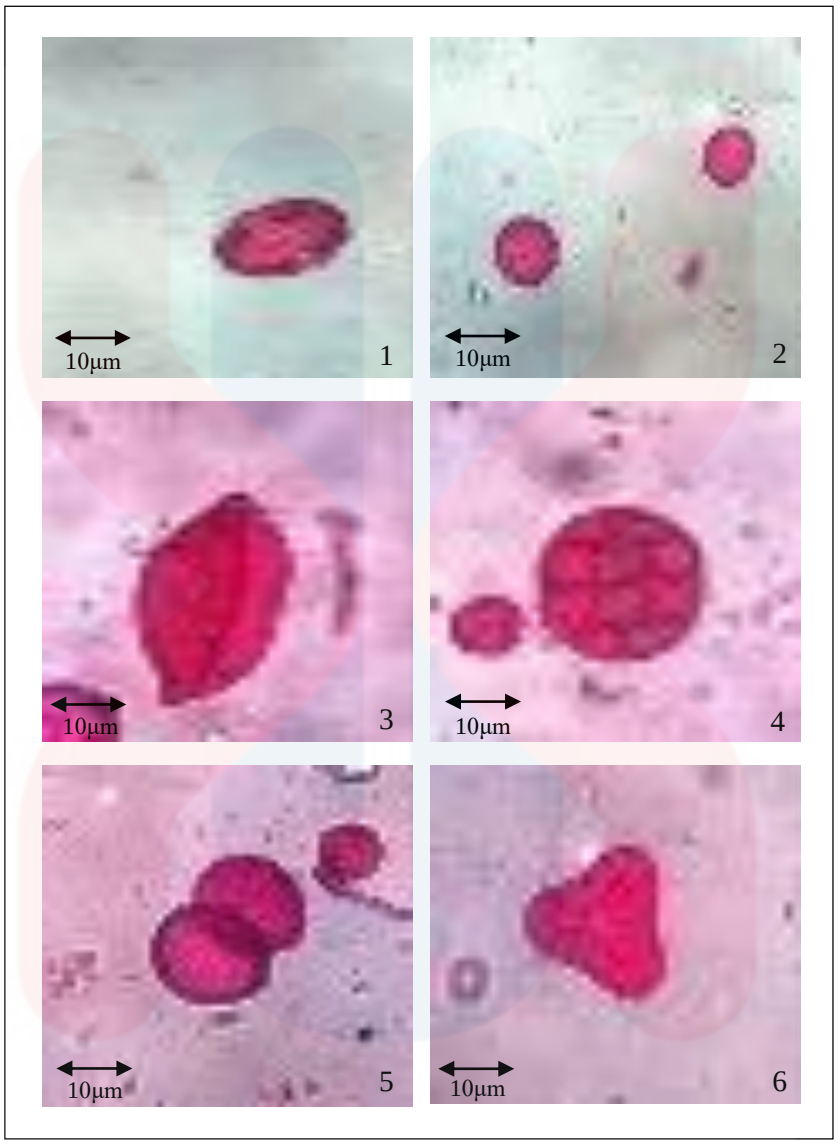


Figure 4.7 (b): Morphology of different pollen honey sample from Kampung Kepala Titi, Sedim under 40x magnification. 1- *Tetrastigma hookeri* (Vitaceae); 2- *Cucumis sativus* (Cucurbitaceae); 3- *Cocos nucifera* (Arecaceae); 4- *Albizia lebbeck* (Fabaceae); 5- *Euphorbia neriifolia* (Euphorbiaceae) and 6- *Elaeis guineensis* (Arecaceae)

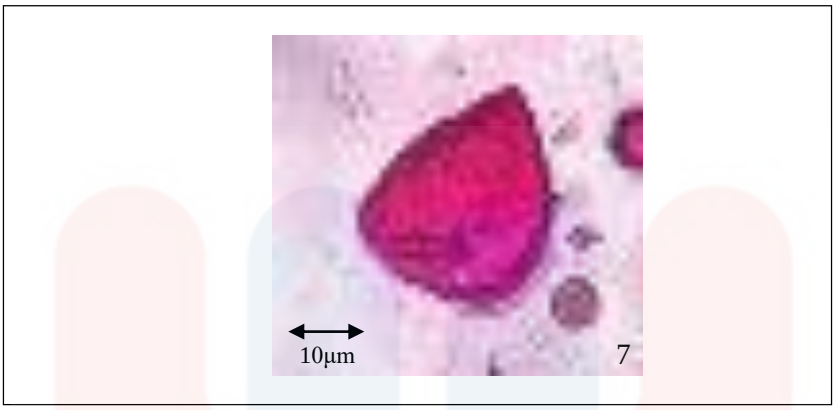


Figure 4.7 (c): Morphology of different pollen honey sample from Kampung Kepala Titi, Sedim under 40x magnification. 7- *Nephelium lappaceum* (Sapindaceae)

7 different types of pollen species were identified in the Kampung Kepala Titi sample which were *Tetrastigma hookeri* from the Vitaceae family, *Cucumis sativus* from the Cucurbitaceae family, *Cocos nucifera* from the Arecaceae family, *Albizia lebbeck* from the Fabaceae family, *Euphorbia neriifolia* from the Euphorbiaceae family, *Elaeis guineensis* from the Arecaceae family, and *Nephelium lappaceum* from the Sapindaceae family. This sample was identified as a multifloral type of honey since there were no species that contain pollen accounted up to 45% of abundance.

The highest percentage of pollen abundance came from *Cucumis sativus* (cucumber) with 31.24% and followed by *Nephelium lappaceum* (rambutan) with 29.87% and *Tetrastigma hookeri* (akar papan) with 23.03%. These 3 species were included in the secondary pollen type group. 2 species were identified as important minor pollen types which were *Cocos nucifera* that was known as coconut with 5.72% and *Elaeis guineensis* that was known as oil palm with 5.56%. *Albizia lebbeck* (siris tree) and *Euphorbia neriifolia* (sesudu) were identified as minor pollen types with 2.01% and 2.58% of

abundance respectively. Pollinating a flower was not the same as collecting nectar or pollen from it. Before it can be said with certainty that bees were pollinators, more research must be done. This should include extensive embryological analysis, as some fruits for example rambutan was produced without pollination (Rincón-Rabanales et al., 2014).

4.8 Honey sample from Pekan Pendang, Pendang (Multifloral)

Table 4.8: The number of pollen and percentage of pollen types in the honey sample from Pendang (PD).

Plant Species	Family	Total No. of Pollen	Percentage of Abundance (%)
Cocos nucifera	Arecaceae	294	31.61
Jacaranda obtusifolia	Bignoniaceae	46	4.95
Eremospatha haullevilleana	Arecaceae	65	6.99
Holcus mollis	Poaceae	350	37.63
Melastoma malabathricum	Melastomatacea	84	9.03
Avicennia alba	Acanthaceae	91	9.78

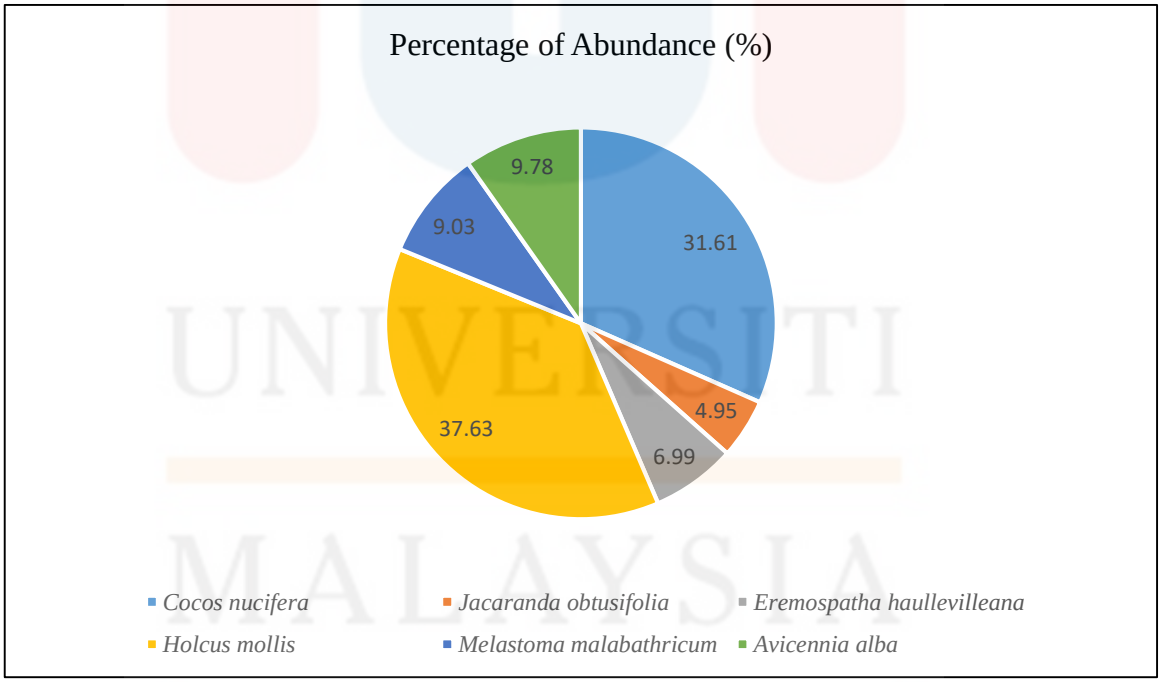


Figure 4.8: Pollen spectrum of honey sample from Pekan Pendang, Pendang

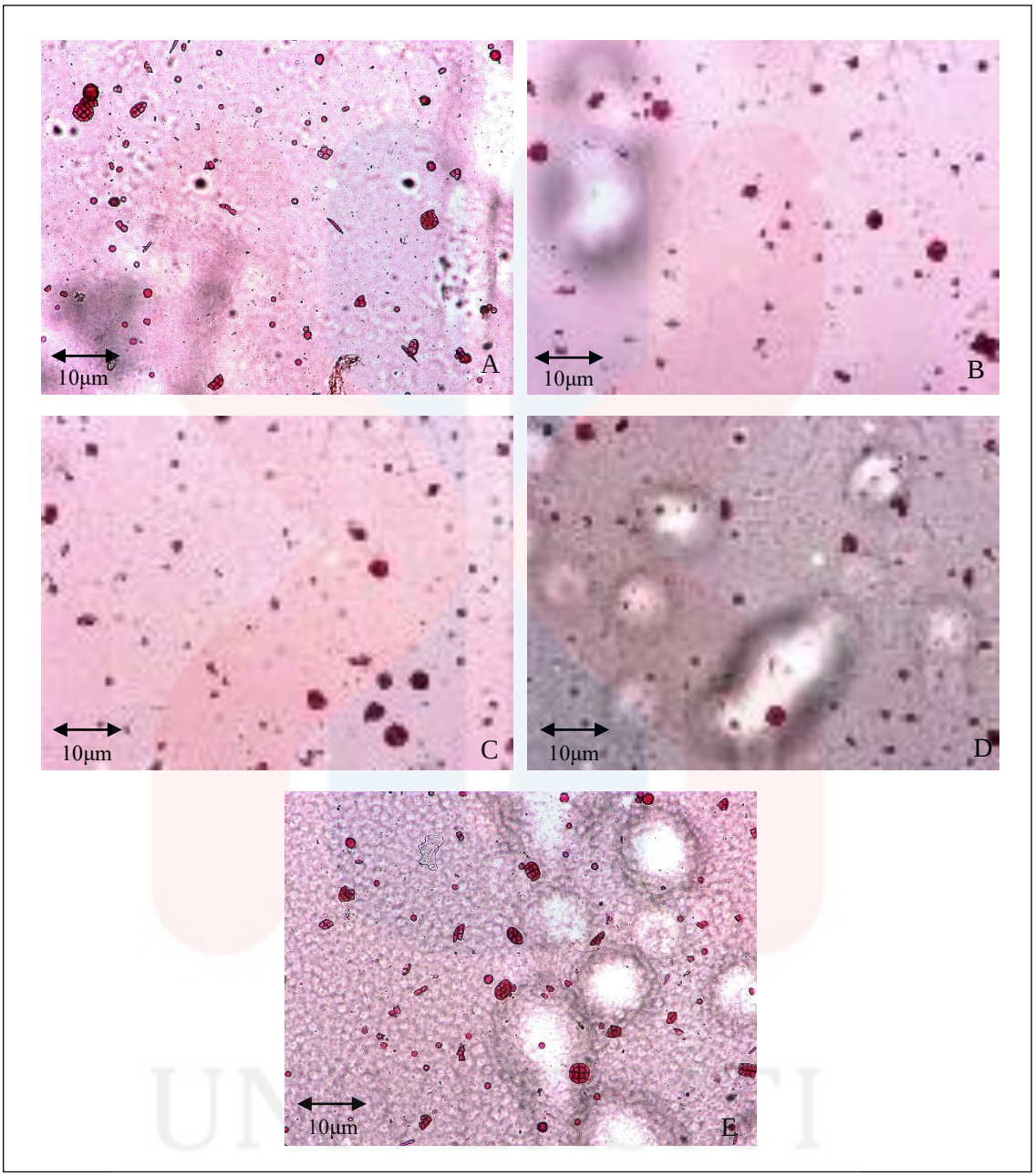


Figure 4.8 (a): Microscopic overviews of pollen density in honey sample from Pekan Pendang under 10x magnification. Overviews of A- Right edge of cover slip (top); B- Left edge of cover slip (top); C- Right edge of cover slip (bottom); D- Left edge of cover slip (bottom) and E- centre of cover slip (middle)

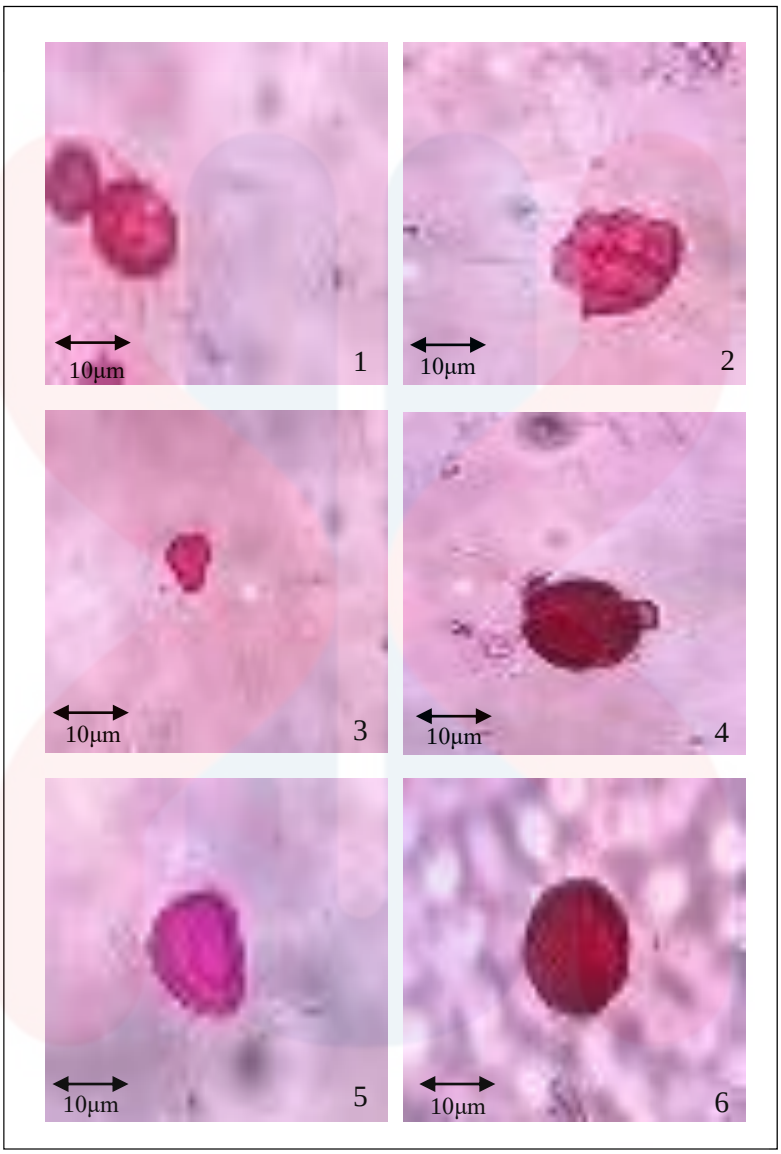


Figure 4.8 (b): Morphology of different pollen honey sample from Pekan Pendang under 40x magnification. 1- *Melastoma malabathricum* (Melastomatacea); 2- *Cocos nucifera* (Arecaceae); 3- *Avicennia alba* (Acanthaceae); 4- *Jacaranda obtusifolia* (Bignoniaceae); 5- *Eremospatha haullevilleana* (Arecaceae) and 6- *Holcus mollis* (Poaceae)

From the honey sample collected from Pekan Pendang, 6 different types of pollen species were discovered. Among the types of pollen species found were *Melastoma malabathricum* (Melastomatacea), *Cocos nucifera* (Arecaceae), *Osmunda javanica* (Osmundaceae), *Cordia aurantiaca* (Boraginaceae), *Eremospatha haullevilleana* (Arecaceae), and *Holcus mollis* (Poaceae).

There was no predominant pollen species had been discovered in this sample, while the most abundant pollen in this honey sample was *Holcus mollis* which accounted up to 37.63% of total pollen counted. *Holcus mollis* was known as velvet grass in general. The pollen grain morphology for *Holcus mollis* were monads, with a single pore-type aperture, with annulus and operculum, and have an outline that was circular, sub-circular, and ovalate (Morgado et al., 2015). Then followed by *Cocos nucifera* which accounted up to 31.61% of total pollen counted. It was a scientific name for coconut trees. *Cocos nucifera* was a good honey plant since it was a major source of forage for bees (Majid et al., 2020). *Cocos* nectar also contains high sugar concentration and was one of the most important markers to distinguish Malaysian honey from imported honey (K. S. Raghunandan & S. Basavarajappa, 2011). The presence of a great variety of pollen in this honey sample showed the multiflorality of the honey from this area.

Important minor pollen type plant species were also identified which were *Jacaranda obtusifolia* (4.95%), *Eremospatha haullevilleana* (6.99%), *Melastoma malabathricum* (9.03%), and *Avicennia alba* (9.78%). *Jacaranda obtusifolia* was known as ‘Jambul Merak’ locally. It was usually planted as an ornamental tree with a large, odourless, pale lilac-blue flower and was intermittent throughout the year. *Eremospatha*

haullevilleana was a scientific name for rattan species which was usually found in closed-canopy forests and open areas. *Melastoma malabathricum* was known as 'Senduduk' for local people and *Avicennia alba* was known as 'Api-api' which was a common mangrove tree. *Avicennia alba* can be found in the coastal area in India, Singapore, Malaysia, Indonesia, and also can be found in Australia and Papua New Guinea (Ranjan et al., 2015).

4.9 Honey sample from Kepala Batas (Multifloral)

Table 4.9: The number of pollen and percentage of pollen types in the honey sample from Kepala Batas (KB).

Plant Species	Family	Total No. of Pollen	Percentage of Abundance (%)
Allium cepa	Amaryllidaceae	78	3.99
Ananas comosus	Bromeliaceae	45	2.30
Jacaranda obtusifolia	Bignoniaceae	48	2.46
Cucumis melo	Cucurbitaceae	511	26.16
Sporobolus indicus	Poaceae	708	36.25
Zea mays	Gramineae	563	28.83

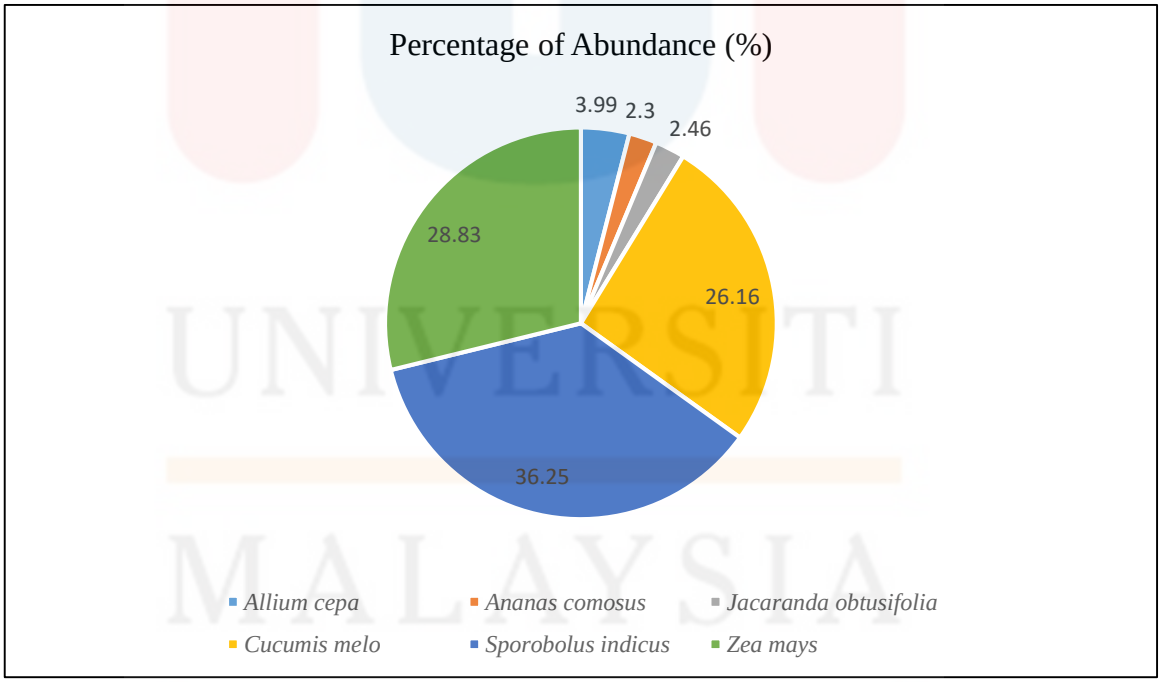


Figure 4.9: Pollen spectrum of honey sample from Kepala Batas

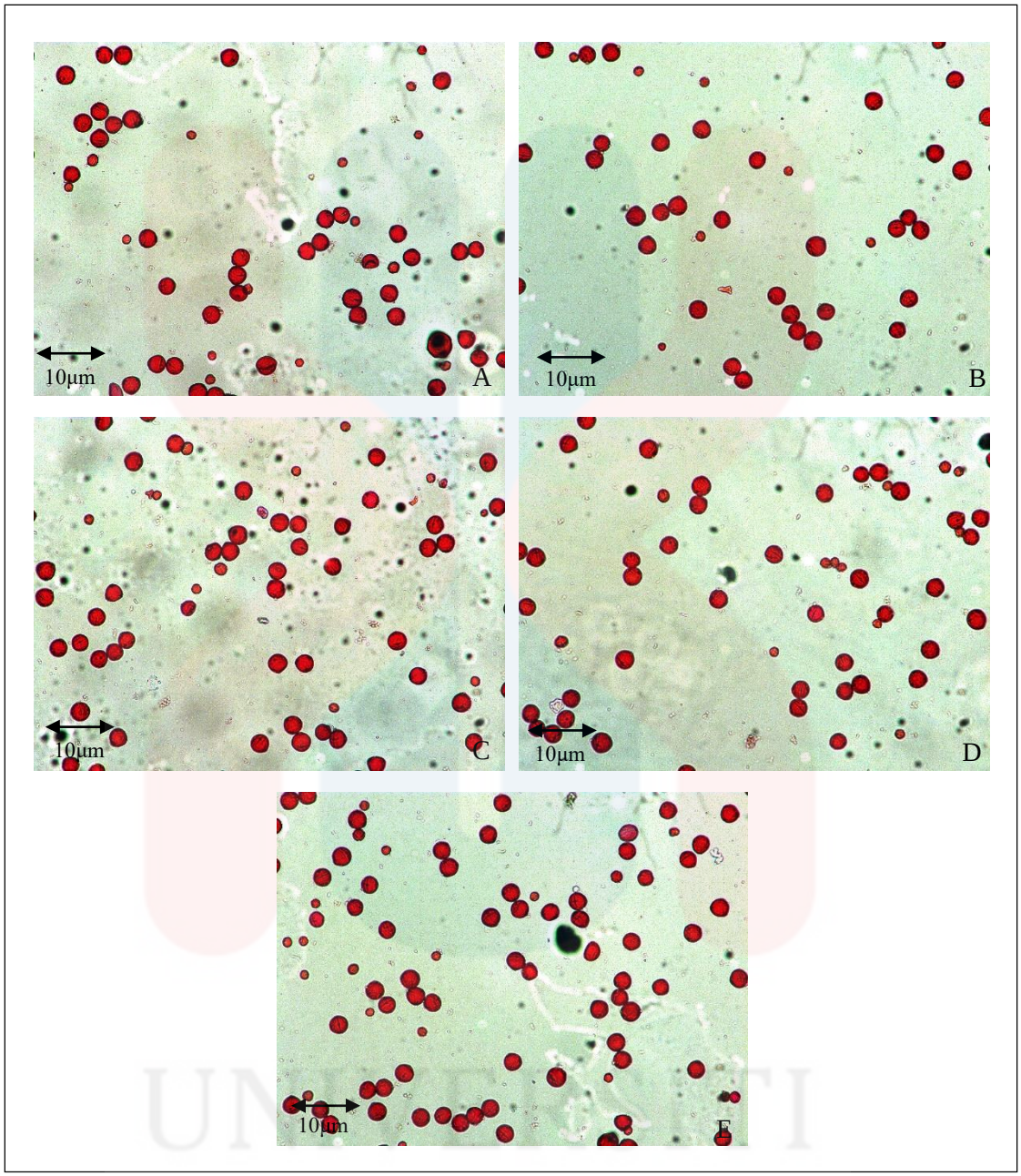


Figure 4.9 (a): Microscopic overviews of pollen density in honey sample from Kepala Batas under 10x magnification. Overviews of A- Right edge of cover slip (top); B- Left edge of cover slip (top); C- Right edge of cover slip (bottom); D- Left edge of cover slip (bottom) and E- centre of cover slip (middle)

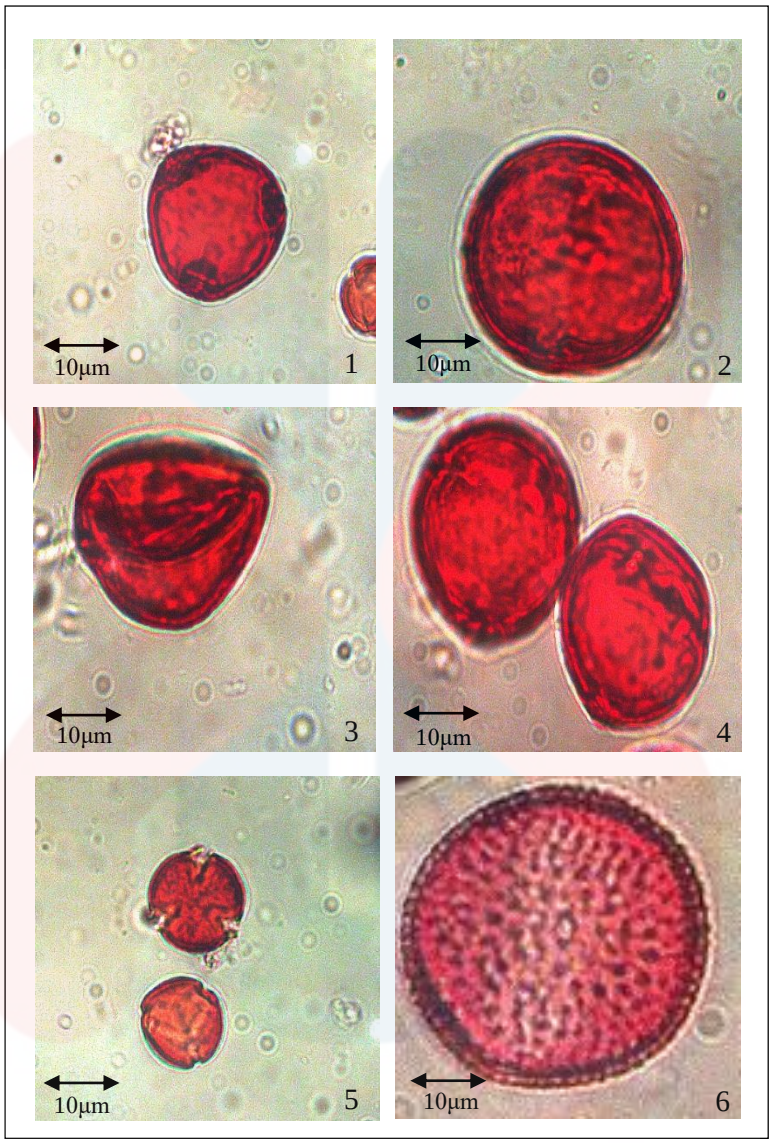


Figure 4.9 (b): Morphology of different pollen honey sample from Kepala Batas under 40x magnification. 1- *Cucumis melo* (Cucurbitaceae); 2- *Zea mays* (Gramineae); 3- *Allium cepa* (Amaryllidaceae); 4- *Sporobolus indicus* (Poaceae); 5- *Jacaranda obtusifolia* (Bignoniaceae) and 6- *Ananas comosus* (Bromeliaceae)

From the results obtained in the pollen analysis of the honey sample from Kepala Batas, 6 different types of pollen had been successfully identified. Among the types of pollen present in the sample were *Cucumis melo* (Cucurbitaceae), *Zea mays* (Gramineae), *Allium cepa* (Amaryllidaceae), *Sporobolus indicus* (Poaceae), *Jacaranda obtusifolia* (Bignoniaceae), and *Ananas comosus* (Bromeliaceae). From the analysis, no predominant pollen was found in this sample and conclude that this sample was a multifloral type of honey.

The most abundance pollen was from *Sporobolus indicus* plant species accounting up to 36.25% of the pollen count, followed by *Zea mays* with 28.83% of pollen abundance and *Cucumis melo* with 26.16%. These 3 pollen plant species were included in the secondary pollen type. *Cucumis melo* was a scientific name for muskmelon. It was an important vegetable that contain a lot of nutrient values such as protein, minerals, Vitamin A, and Vitamin K (Baktemur et al., 2013). *Allium cepa* were identified as important minor pollen types with 3.99% of pollen abundance. Other pollen that were present in this sample were from *Ananas comosus* and *Jacaranda obtusifolia* with 2.30% and 2.46% of pollen abundance respectively. *Ananas comosus* was a scientific name for pineapple. It was a terrestrial herb with 0.75 to 1.5 meters of height (Shiwoto et.al, 2021). The pineapple flower was an inflorescence that grows in an acropetal pattern from the apical meristem. Only insects or specialist birds can access the flower since the inflorescence was made up of 100 to 200 lowers arranged in a narrow compact tubular form (Jones, 2014).

4.10 Honey Sample from Karangan, Kulim (Multifloral)

Table 4.10: The number of pollen and percentage of pollen types in the honey sample from Karangan (KG).

Plant Species	Family	Total No. of Pollen	Percentage of Abundance (%)
Acacia auriculiformis	Fabaceae	2	0.84
Amaranthus lividus	Amaranthaceae	9	3.80
Carduus nutans	Asteraceae	5	2.11
Cocos nucifera	Arecaceae	31	13.08
Desmodium adscendens	Fabaceae	86	36.29
Psidium guajava	Myrtaceae	21	8.86
Veitchia merrillii	Arecaceae	83	35.02

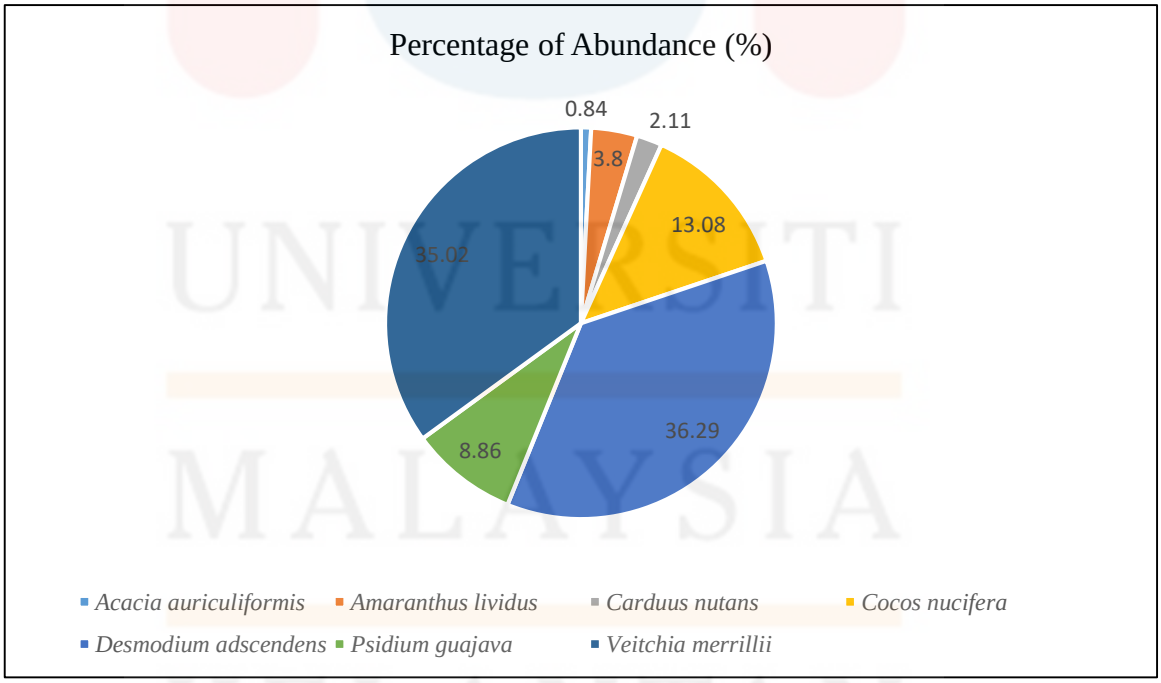


Figure 4.10: Pollen spectrum of honey sample from Karangan, Kulim

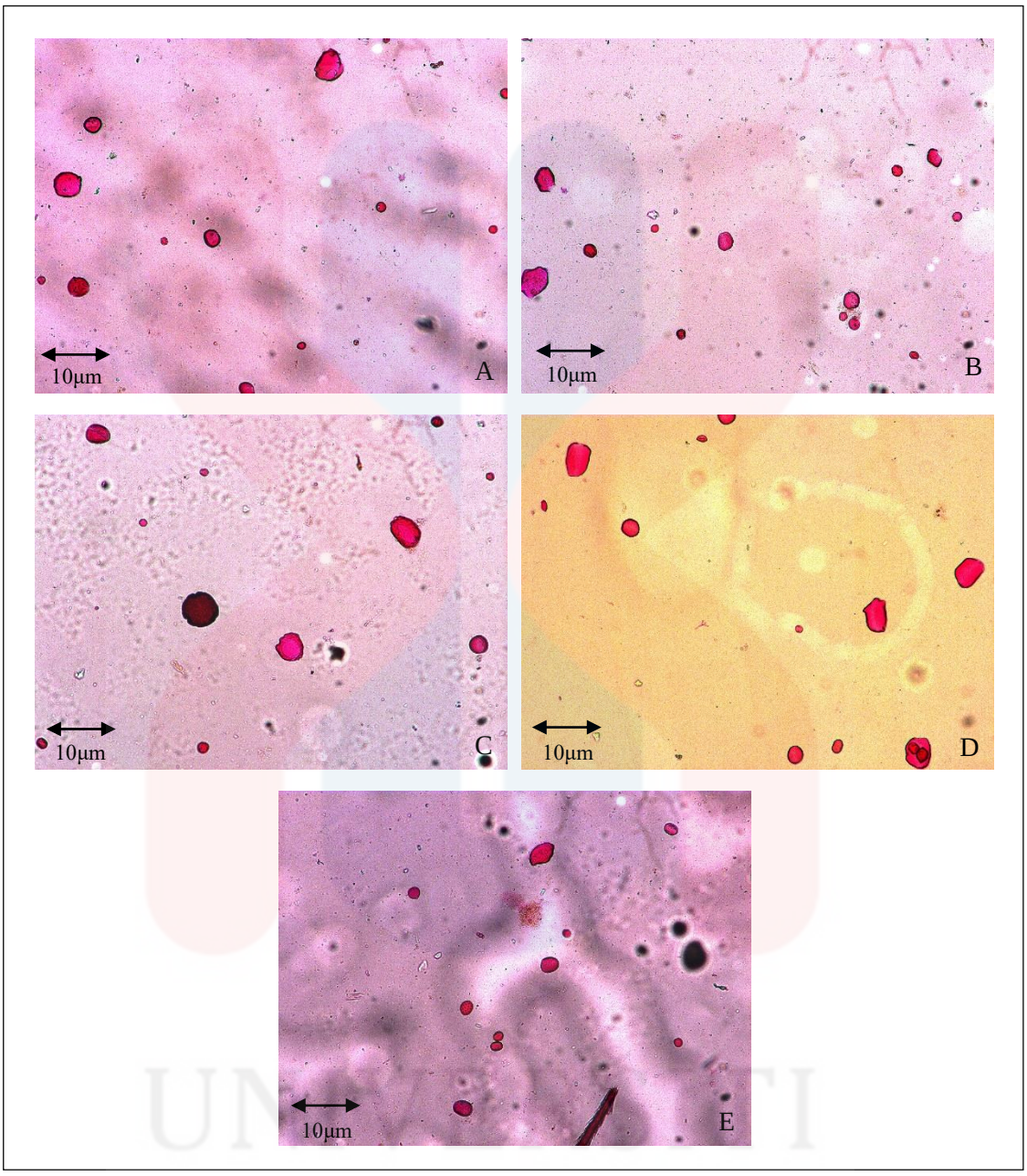


Figure 4.10 (a): Microscopic overviews of pollen density in honey sample from Karangan under 10x magnification. Overviews of A- Right edge of cover slip (top); B- Left edge of cover slip (top); C- Right edge of cover slip (bottom); D- Left edge of cover slip (bottom) and E- centre of cover slip (middle)

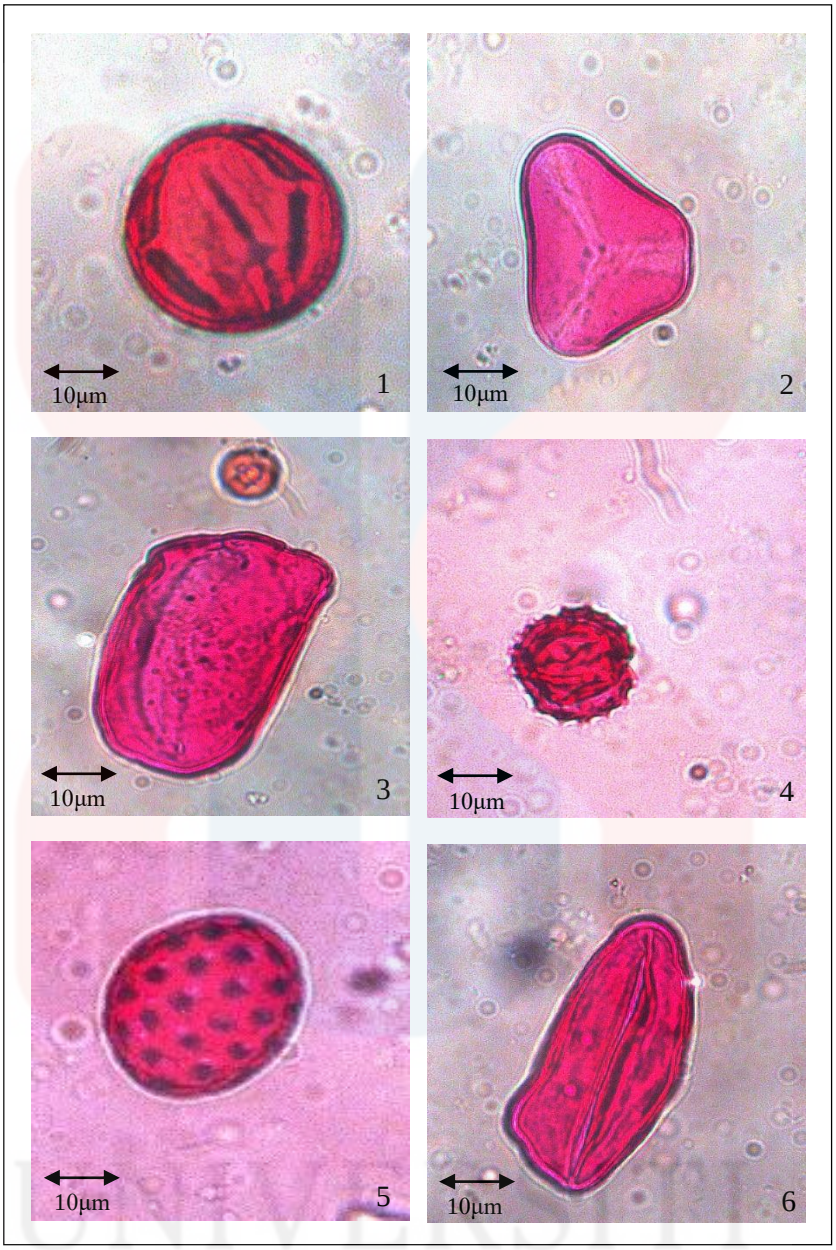


Figure 4.10 (b): Morphology of different pollen honey sample from Karangan under 40x magnification. 1- *Desmodium adscendens* (Fabaceae); 2- *Psidium guajava* (Myrtaceae); 3- *Veitchia merrillii* (Arecaceae); 4- *Carduus nutans* (Asteraceae); 5- *Amaranthus lividus* (Amaranthaceae) and 6- *Cocos nucifera* (Arecaceae)

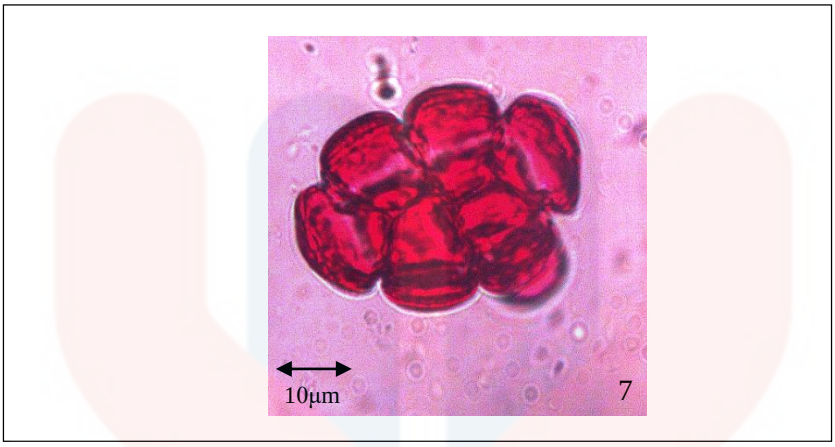


Figure 4.10 (c): Morphology of different pollen honey sample from Karangan under 40x magnification. 7- *Acacia auriculiformis* (Fabaceae)

From the samples collected in Karangan, there were 237 pollen grains found came from different plant species which were *Desmodium adscendens* from family Fabaceae, *Psidium guajava* from family Myrtaceae, *Veitchia merrillii* from family Arecaceae, *Carduus nutans* from family Asteraceae, *Amaranthus lividus* from family Amaranthaceae, *Cocos nucifera* from family Arecaceae, and *Acacia auriculiformis* from family Fabaceae. This honey sample was a multifloral honey type because none of the plant species contain pollen exceed 45%.

The highest pollen counted came from secondary pollen type which was *Desmodium adscendens* species or known as tick clover with pollen accounted up to 36.29% and followed by *Veitchia merrillii* with 35.02% of pollen abundance. 3 plant species were discovered as important minor pollen types which were *Amaranthus lividus* which was known as livid pigweed with 3.80%, *Cocos nucifera* with 13.08%, and

Psidium guajava with 8.86% of pollen abundance. Other pollen that were identified were *Acacia auriculiformis* and *Carduus nutans* with 0.84% and 2.11% of pollen abundance respectively.

A complete understanding of mutualism between bees and available plant taxa in a specific region and also particular seasons was required to enhance the beekeeping sector (Bhusari et al., 2005). The detected taxon was not only significant for the growth of beekeeping in the area but also important for economic crops. The knowledge about the floral condition of the location where honey was produced and the identification of geographical origin based on the pollen was crucial for the beekeeping industry (Mamatha et al., 2018).

4.11 Honey sample from Kampung Keladi, Kulim (Multifloral)

Table 4.11: The number of pollen and percentage of pollen types in the honey sample from Kampung Keladi (KK).

Plant Species	Family	Total No. of Pollen	Percentage of Abundance (%)
Amaranthus lividus	Amaranthaceae	7	1.84
Jacaranda obtusifolia	Bignoniaceae	27	7.11
Juniperus chinensis	Cupressaceae	18	4.74
Melilotus suaveolens	Fabaceae	67	17.63
Psidium guajava	Myrtaceae	61	16.05
Scurrula ferruginea	Loranthaceae	2	0.53
Sporobolus indicus	Poaceae	155	40.79
Tridax procumbens	Asteraceae	3	0.79
Veitchia merrillii	Arecaceae	40	10.53

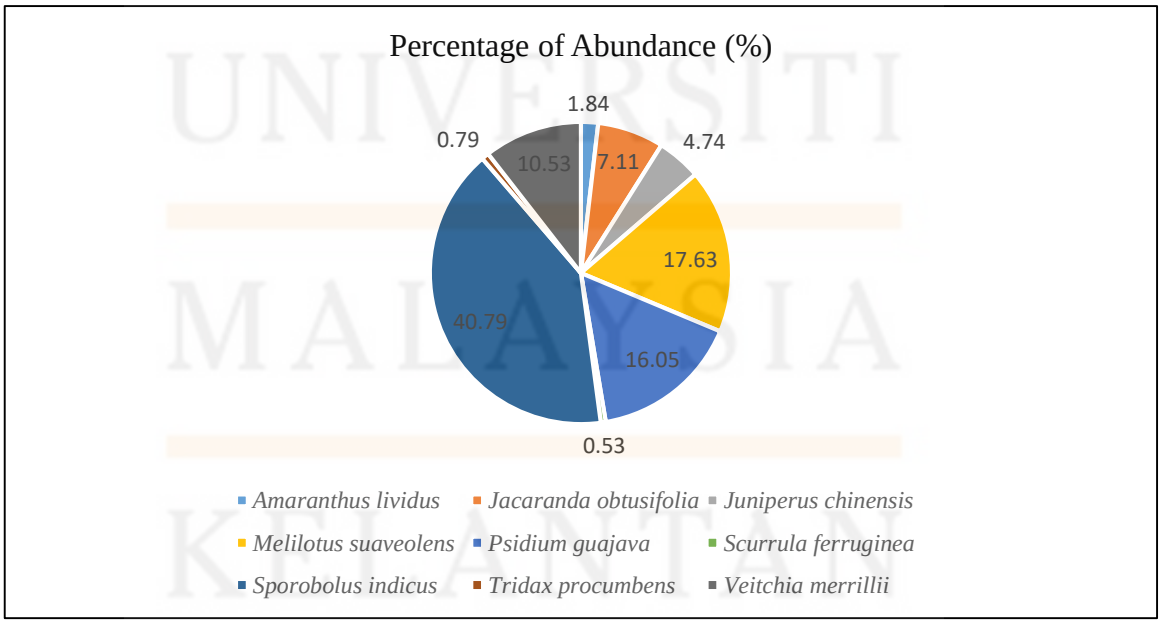


Figure 4.11: Pollen spectrum of honey sample from Kampung Keladi, Kulim

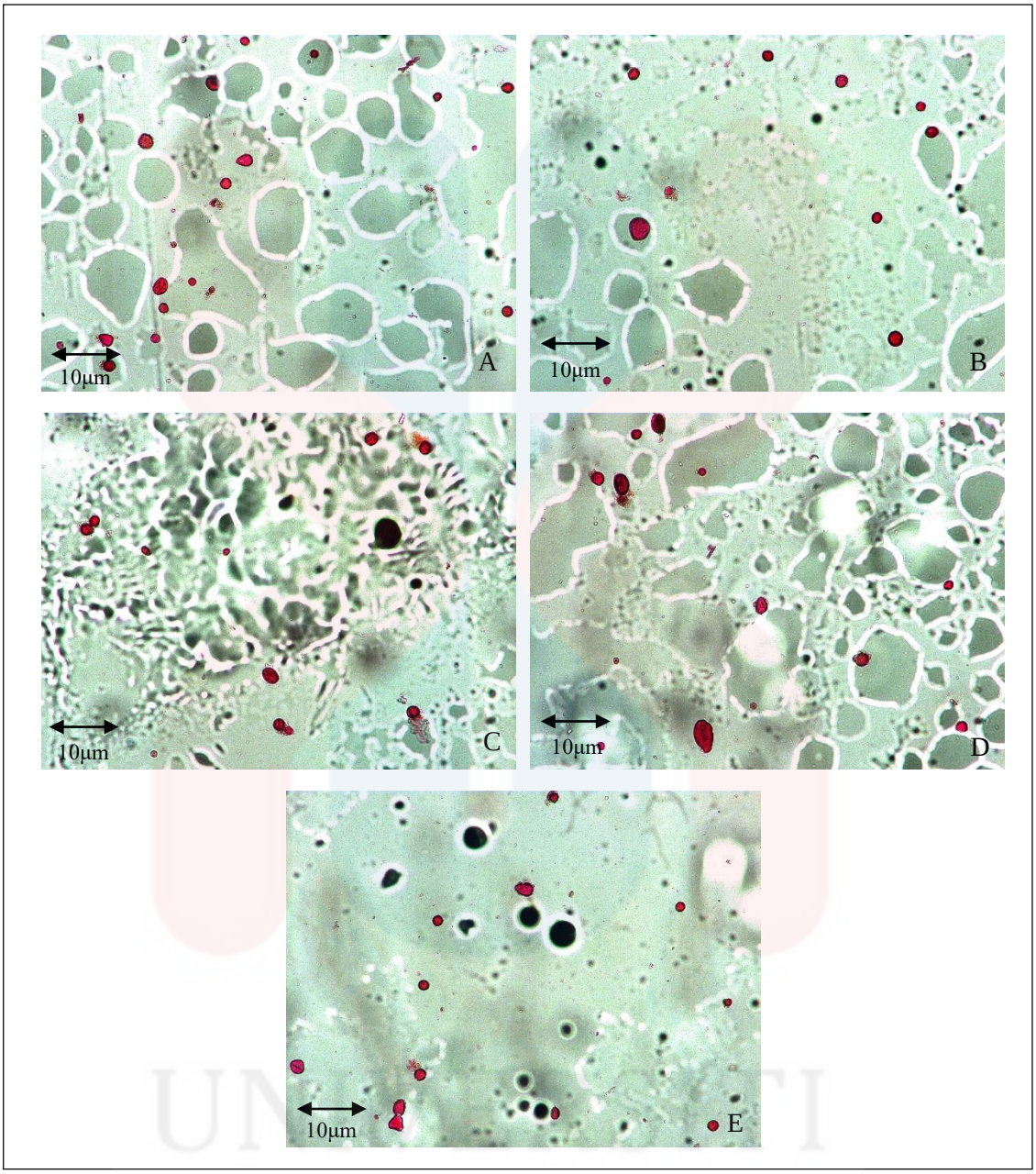


Figure 4.11 (a): Microscopic overviews of pollen density in honey sample from Kampung Keladi under 10x magnification. Overviews of A- Right edge of cover slip (top); B- Left edge of cover slip (top); C- Right edge of cover slip (bottom); D- Left edge of cover slip (bottom) and E- centre of cover slip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

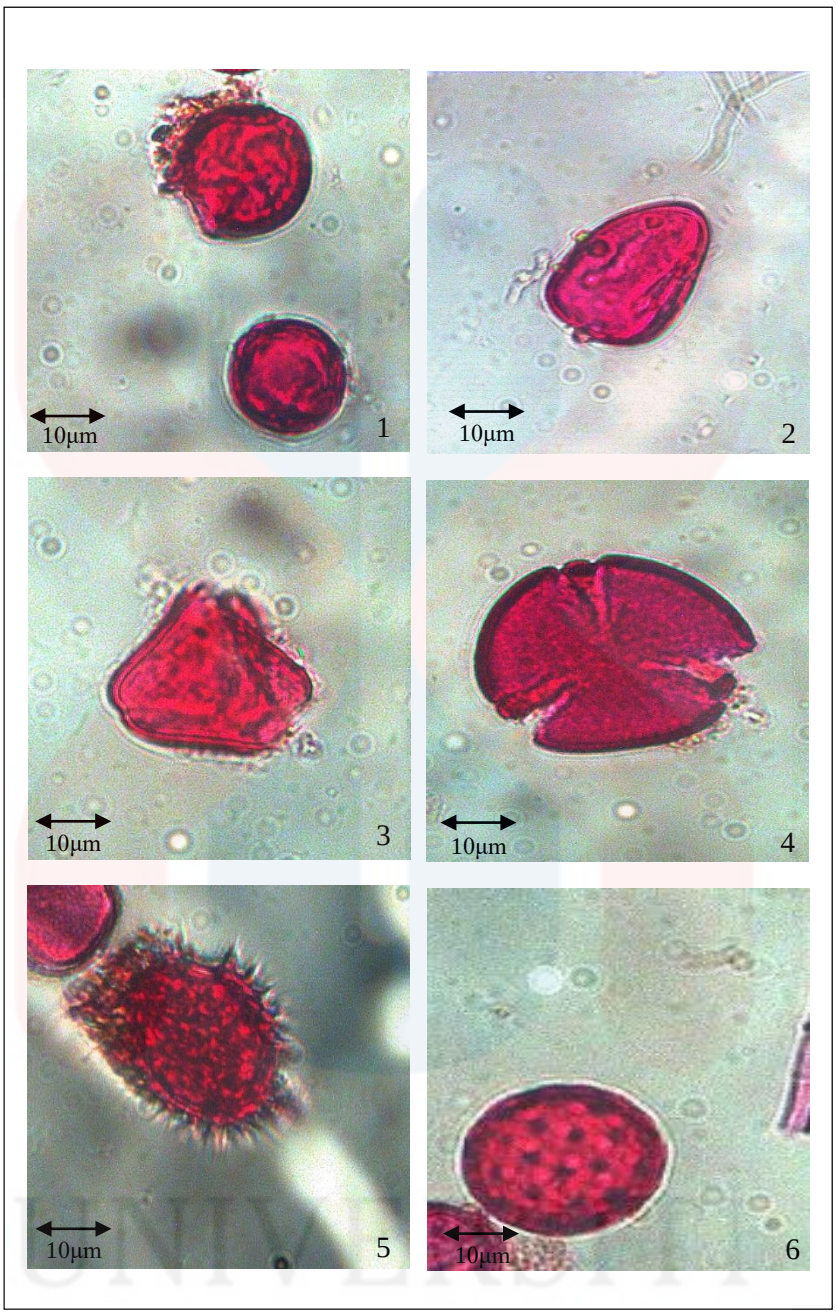


Figure 4.11 (b): Morphology of different pollen honey sample from Kampung Keladi under 40x magnification. 1- *Sporobolus indicus* (Poaceae); 2- *Melilotus suaveolens* (Fabaceae); 3- *Psidium guajava* (Myrtaceae); 4- *Jacaranda obtusifolia* (Bignoniaceae); 5- *Tridax procumbens* (Asteraceae) and 6- *Amaranthus lividus* (Amaranthaceae)

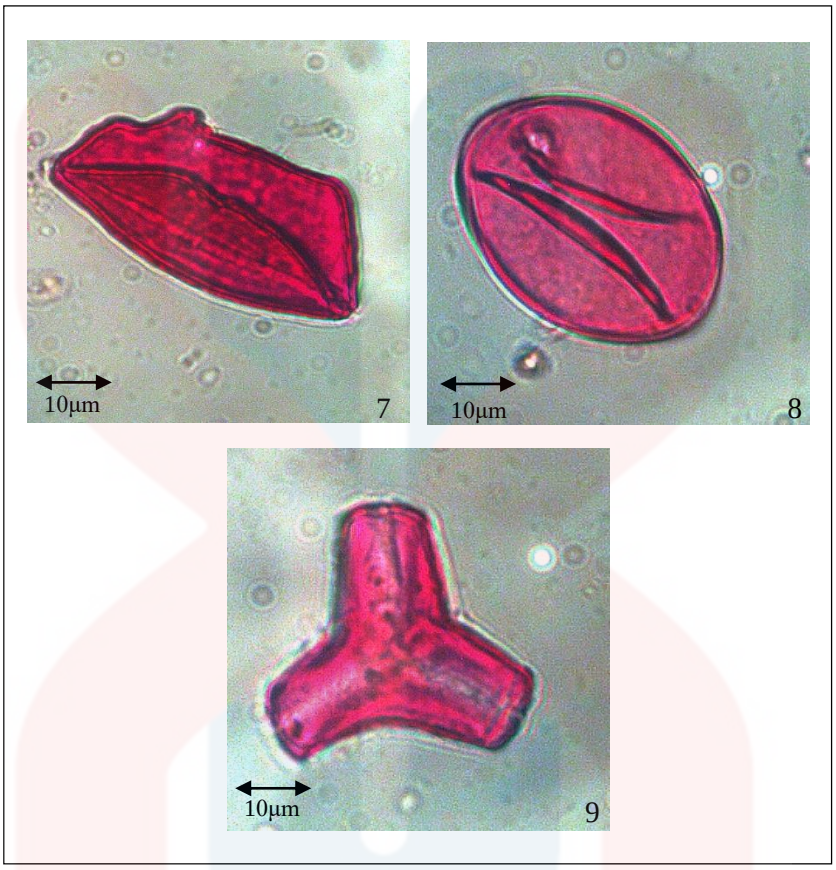


Figure 4.11 (c): Morphology of different pollen honey sample from Kampung Keladi under 40x magnification. 7- *Veitchia merrillii* (Arecaceae); 8- *Juniperus chinensis* (Cupressaceae) and 9- *Scurrula ferruginea* (Loranthaceae)

From the honey sample collected in Kampung Keladi, 9 different types of pollen were present. Among the different species of pollen found were *Sporobolus indicus* (Poaceae), *Melilotus suaveolens* (Fabaceae), *Psidium guajava* (Myrtaceae), *Jacaranda obtusifolia* (Bignoniaceae), *Tridax procumbens* (Asteraceae), *Amaranthus lividus* (Amaranthaceae), *Veitchia merrillii* (Arecaceae), *Juniperus chinensis* (Cupressaceae), and *Scurrula ferruginea* (Loranthaceae). However, there was no predominant pollen found in this sample and the highest percentage of pollen abundance was from *Sporobolus*

indicus with 40.79% which was referred as secondary pollen type. Other sample that were recognized as secondary pollen type were *Melilotus suaveolens* and *Psidium guajava* with 17.63% and 16.05% of pollen abundance respectively.

Important minor pollen for this sample were *Jacaranda obtusifolia* with 7.11%, *Juniperus chinensis* with 4.74%, and *Veitchia merrillii* with 10.53% of pollen abundance. Minor pollen types of plant species were *Amaranthus lividus* (1.84%), *Scurrula ferruginea* (0.53), and *Tridax procumbens* (0.79%). *Tridax procumbens* was a scientific name for coat buttons or 'Kanching Baju' in Malay. This plant species was an annual or perennial herbaceous weed that was usually found in crops lands, lawns, and roadside. Its flowers are tubular yellow in colour with hairs. The plant has two types of flowers which were ray florets and disc florets (P. Meena et al., 2016). The morphology of pollen for this plant species was polypantoporate, prolate-spheroida with pointed ends and short spines, thin wall and pores that were densely situated (Ekeke et al., 2016). Pollen was important to identify the plant involved in honey production. Most plant species can be identified to genus through the combination of the pollen size, shape, and surface structure.

4.12 Honey sample from Kampung Sungai Buluh, Sedim, Kulim (Multifloral)

Table 4.12: The number of pollen and percentage of pollen types in the honey sample from Kampung Sungai Buluh (SB).

Plant Species	Family	Total No. of Pollen	Percentage of Abundance (%)
Acacia auriculiformis	Fabaceae	15	0.67
Anacardium occidentale	Anacardiaceae	167	7.50
Averrhoa carambola	Oxalidaceae	55	2.47
Cocos nucifera	Arecaceae	189	8.49
Cucumis sativus	Cucurbitaceae	716	32.15
Delonix regia	Fabaceae	18	0.81
Elaeis guineensis	Arecaceae	116	5.21
Eugenia malaccensis	Myrtaceae	158	7.09
Juniperus chinensis	Cupressaceae	1	0.04
Melilotus suaveolens	Fabaceae	103	4.63
Nypa fruticans	Arecaceae	3	0.13
Rhizophora mucronata	Rhizophoraceae	75	3.37
Zea mays	Poaceae	611	27.44

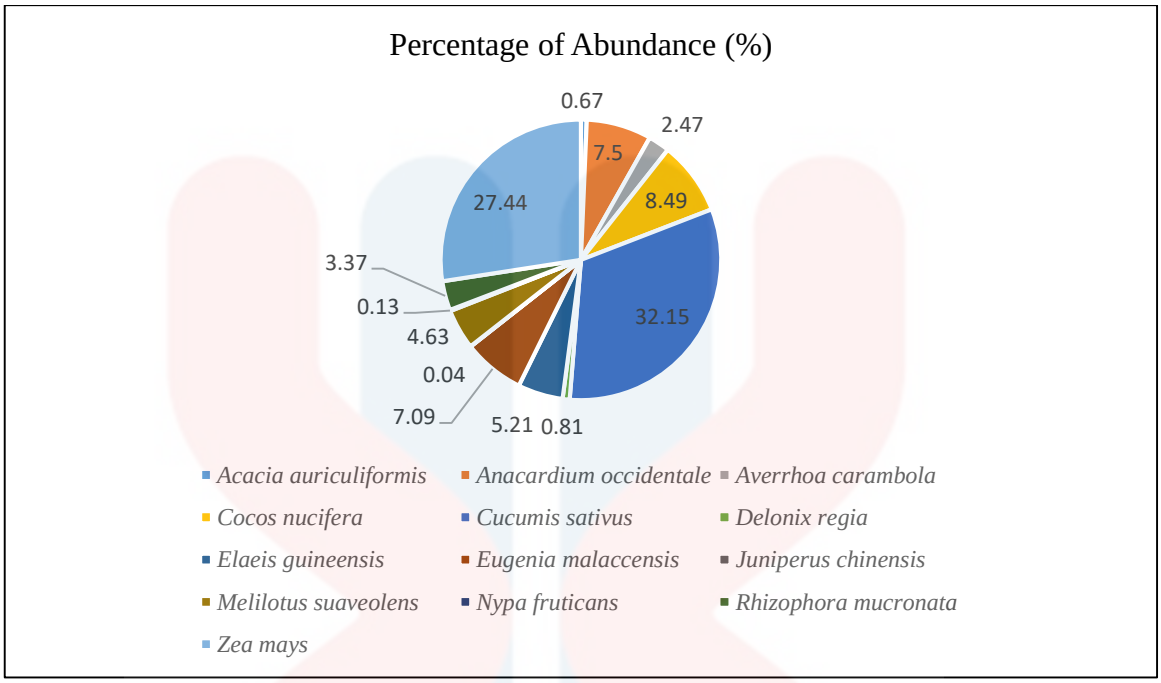


Figure 4.12: Pollen spectrum of honey sample from Kampung Sungai Buluh, Sedim, Kulim

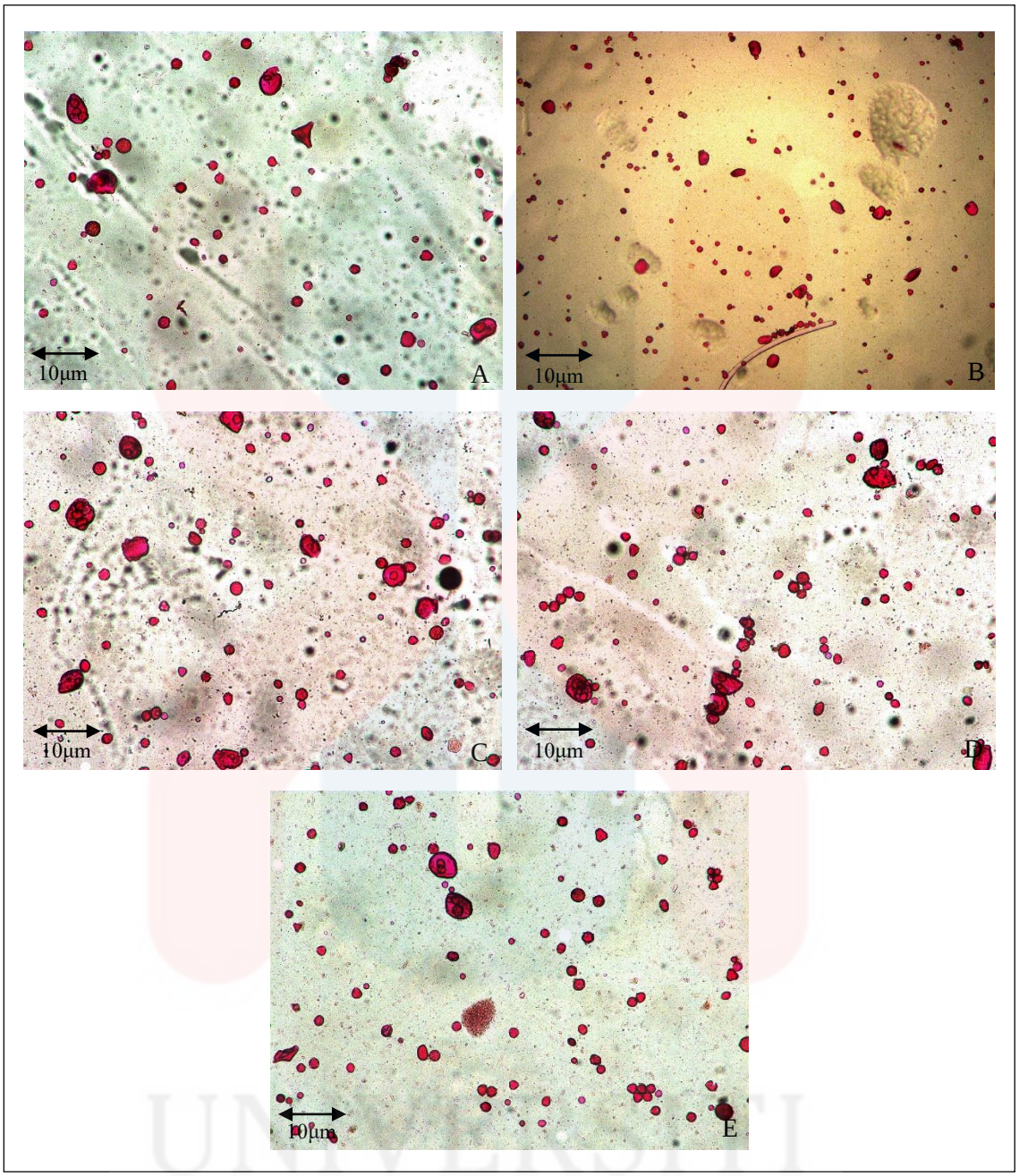


Figure 4.12 (a): Microscopic overviews of pollen density in honey sample from Kampung Sungai Buluh under 10x magnification. Overviews of A- Right edge of cover slip (top); B- Left edge of cover slip (top); C- Right edge of cover slip (bottom); D- Left edge of cover slip (bottom) and E- centre of cover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

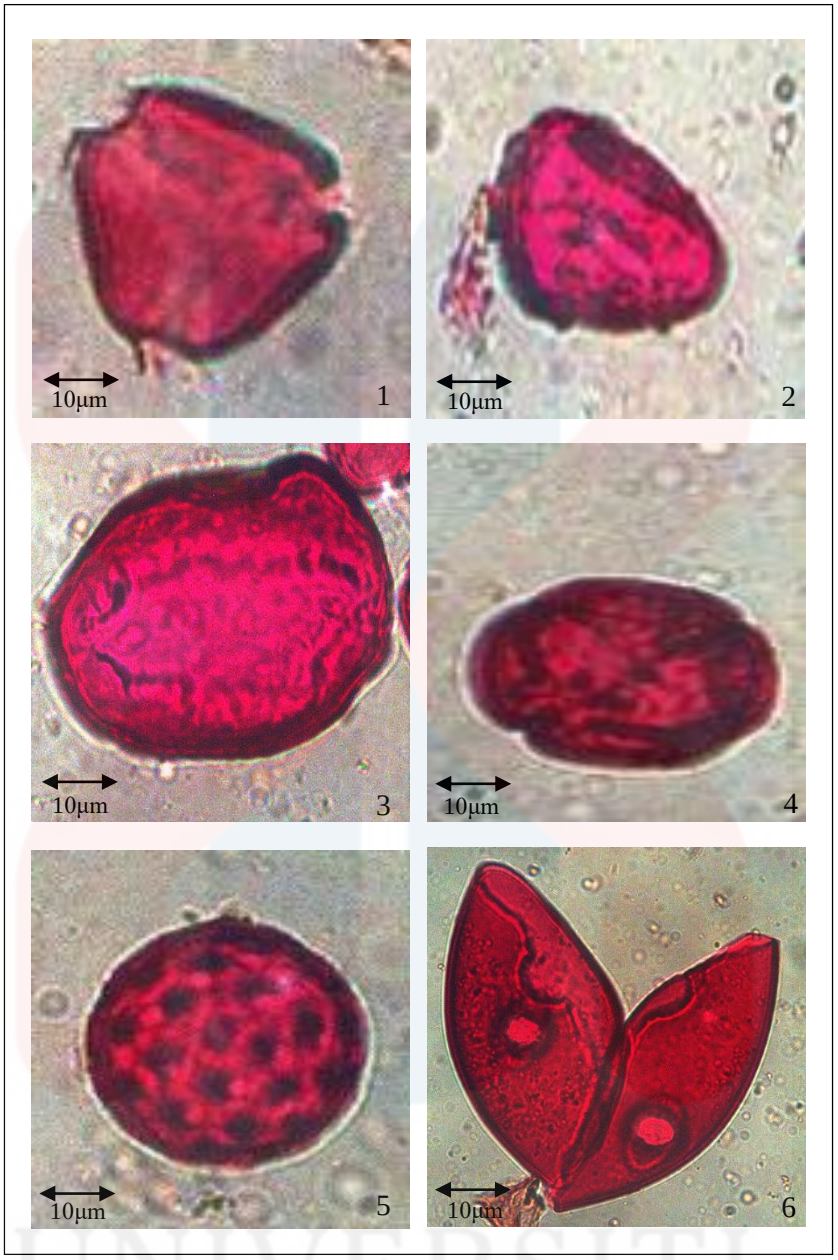


Figure 4.12 (b): Morphology of different pollen honey sample from Kampung Sungai Buluh under 40x magnification. 1- *Eugenia malaccensis* (Myrtaceae); 2- *Melilotus suaveolens* (Fabaceae); 3- *Cocos nucifera* (Arecaceae); 4- *Anacardium occidentale* (Anacardiaceae); 5- *Acacia auriculiformis* (Fabaceae) and 6- *Juniperus chinensis* (Cupressaceae)

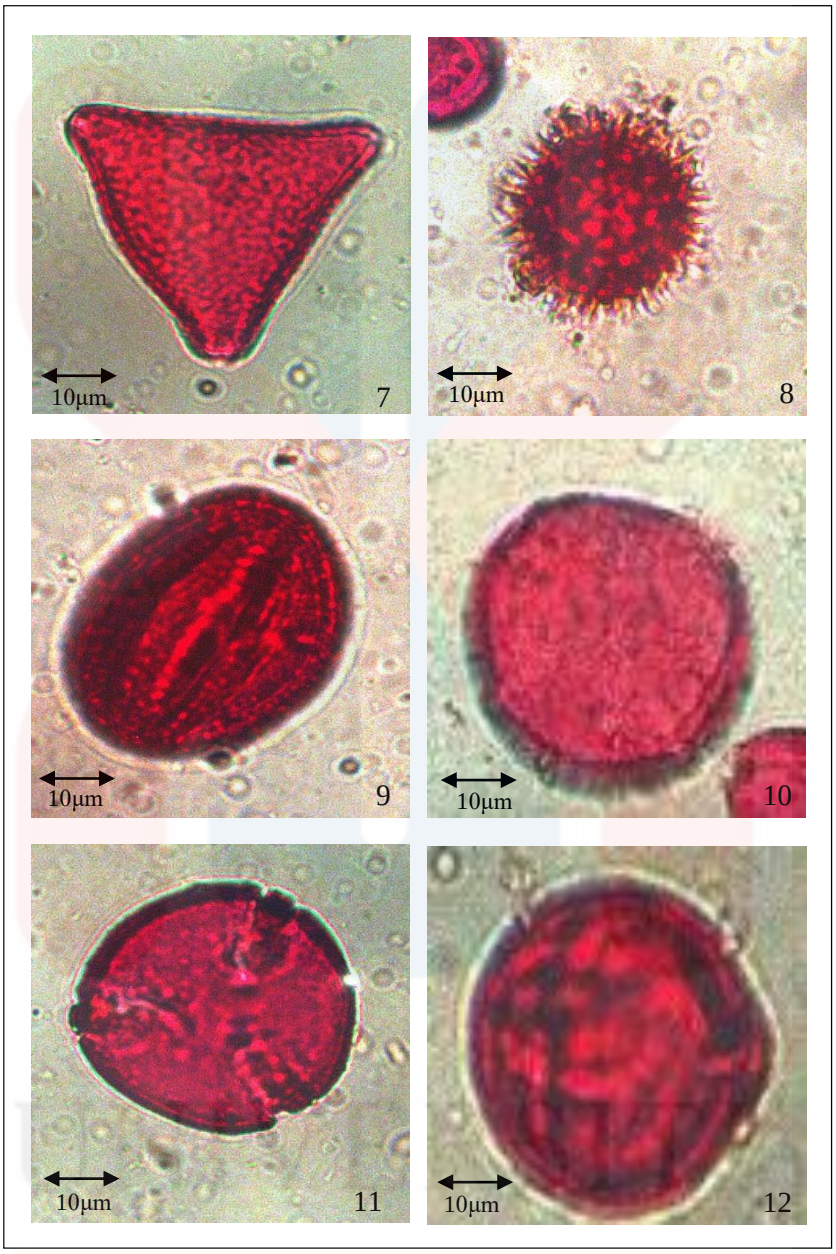


Figure 4.12 (c): Morphology of different pollen honey sample from Kampung Sungai Buluh under 40x magnification. 7- *Elaeis guineensis* (Arecaceae); 8- *Nypa fruticans* (Arecaceae); 9- *Rhizophora mucronata* (Rhizophoraceae); 10- *Delonix regia* (Fabaceae); 11- *Averrhoa carambola* (Oxalidaceae) and 12- *Cucumis sativus* (Cucurbitaceae)

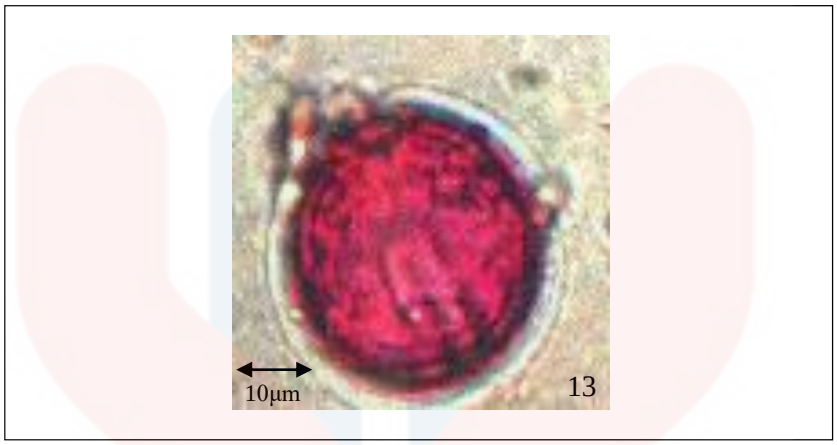


Figure 4.12 (d): Morphology of different pollen honey sample from Kampung Sungai Buluh under 40x magnification. 13- *Zea mays* (Poaceae)

From the sample collected in Kampung Sungai Buluh, 13 types of different pollen from different plant species were successfully identified. The pollen found were *Eugenia malaccensis* (Myrtaceae), *Melilotus suaveolens* (Fabaceae), *Cocos nucifera* (Arecaceae), *Anacardium occidentale* (Anacardiaceae), *Acacia auriculiformis* (Fabaceae), *Juniperus chinensis* (Cupressaceae), *Elaeis guineensis* (Arecaceae), *Nypa fruticans* (Arecaceae), *Rhizophora mucronata* (Rhizophoraceae), *Delonix regia* (Fabaceae), *Averrhoa carambola* (Oxalidaceae), *Cucumis sativus* (Cucurbitaceae), and *Zea mays* (Poaceae). This honey sample belongs to multifloral type honey since there was no species that contained predominant pollen. The highest percentage of pollen abundance came from secondary pollen types which were *Cucumis sativus* and *Zea mays* with 32.15% and 27.44% of pollen abundance respectively.

Anacardium occidentale, cashew nut (7.50%), *Cocos nucifera*, coconut (8.49%), *Elaeis guineensis*, oil palm (5.21%), *Eugenia malaccensis*, chermai Belanda (7.09%), *Melilotus suaveolens*, sweet clover (4.63%), *and Rhizophora mucronata*, mangrove (3.37%) were species of important minor pollen type in this sample that belongs to different families. Besides that, other species were minor pollen type observed which were *Acacia auriculiformis*, earleaf acacia (0.67%), *Averrhoa carambola*, star fruit (2.47%), *Delonix regia*, flame-of-the-forest (0.81%), *Juniperus chinensis*, Chinese juniper (0.04%), and *Nypa fruticans*, nipah palm (0.13%). This honey sample contains the most important marker of pollen which were *Cocos nucifera* and *Elaeis guineensis* that usually used to differentiate between Malaysian honey and imported honey (Rosdi et al., 2016). Pollen grains generated by different species have varying appearances and different forms of pollen were used to signify plant nectar sources used by bees in the production of honey (Shukla & Kumar, 2020).

4.13 Honey sample from Jitra (Unifloral)

Table 4.13: The number of pollen and percentage of pollen types in the honey sample from Jitra (JT).

Plant Species	Family	Total No. of Pollen	Percentage of Abundance (%)
Areca catechu	Arecaceae	10	1.42
Elaeis guineensis	Arecaceae	57	8.12
Erythrina orientalis	Fabaceae	6	0.85
Melilotus suaveolens	Fabaceae	13	1.85
Tecoma stans	Bignoniaceae	33	4.70
Veitchia merrillii	Arecaceae	29	4.13
Zea mays	Poaceae	554	78.92

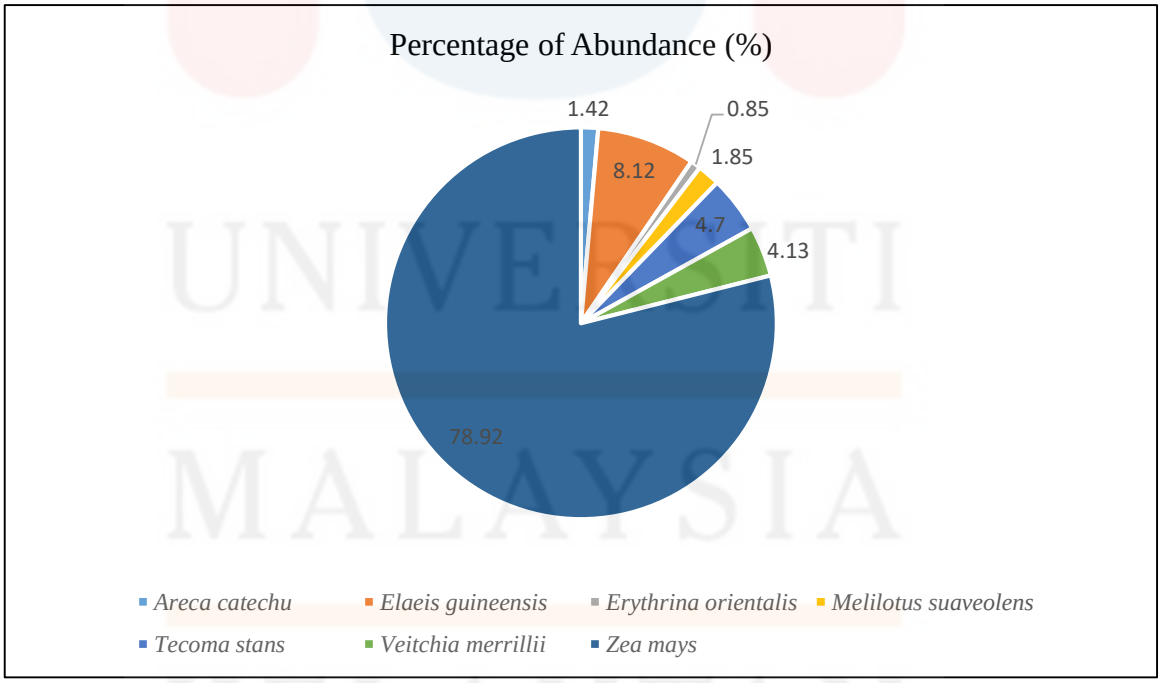


Figure 4.13: Pollen spectrum of honey sample from Jitra

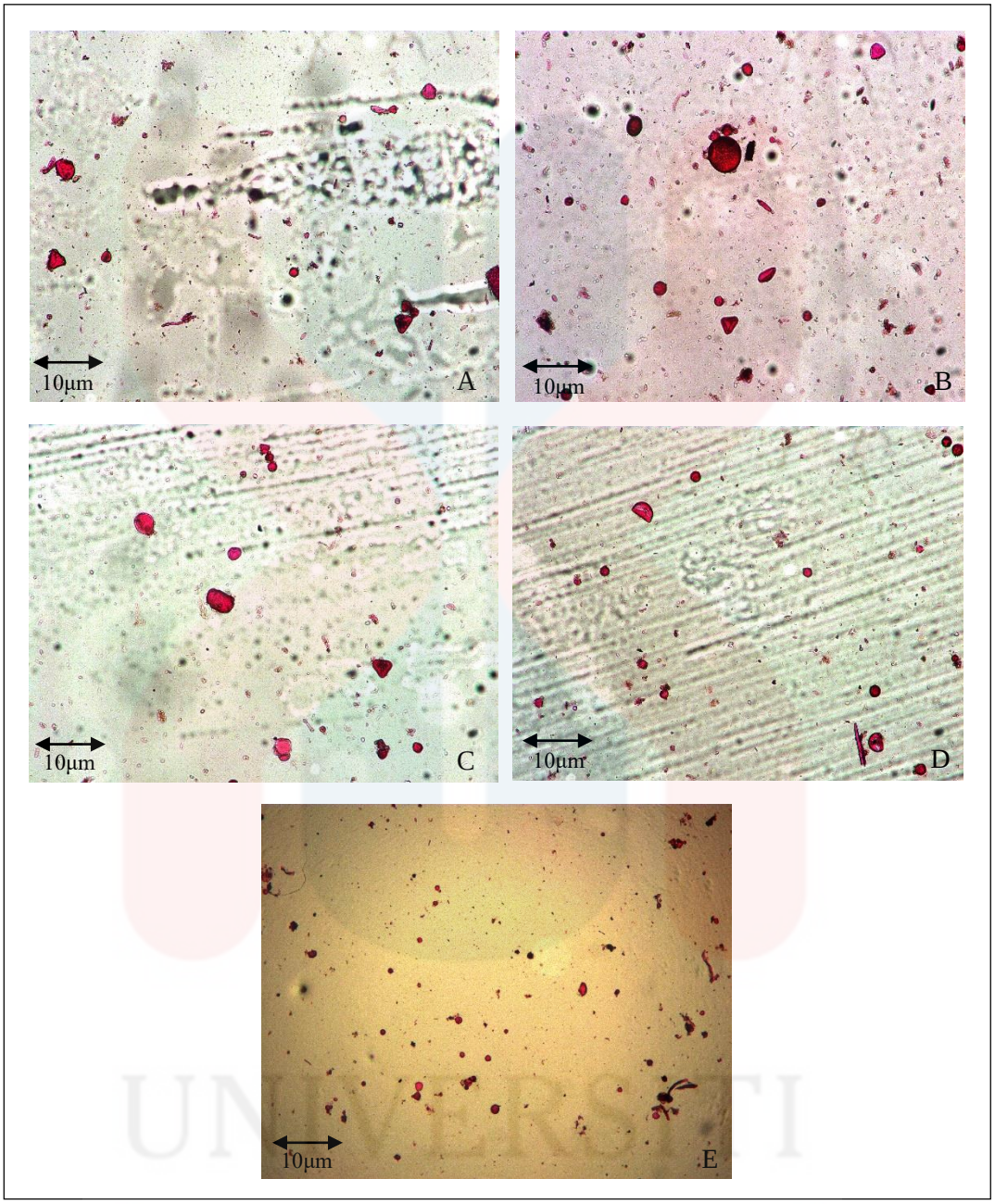


Figure 4.13 (a): Microscopic overviews of pollen density in honey sample from Jitra under 10x magnification. Overviews of A- Right edge of cover slip (top); B- Left edge of cover slip (top); C- Right edge of cover slip (bottom); D- Left edge of cover slip (bottom) and E- centre of cover slip (middle)

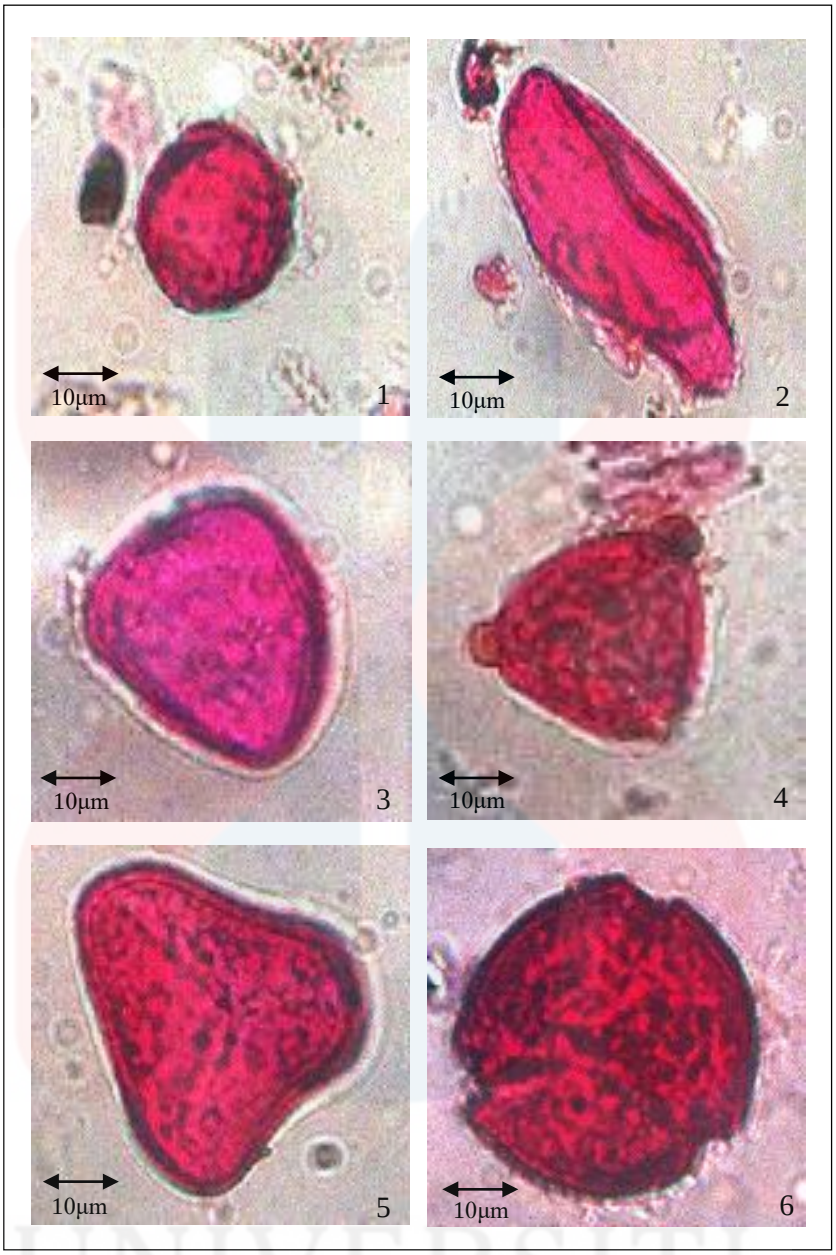


Figure 4.13 (b): Morphology of different pollen honey sample from Jitra under 40x magnification. 1- *Zea mays* (Poaceae); 2- *Veitchia merrillii* (Arecaceae); 3- *Melilotus suaveolens* (Fabaceae); 4- *Erythrina orientalis* (Fabaceae); 5- *Elaeis guineensis* (Arecaceae) and 6- *Tecoma stans* (Bignoniaceae)

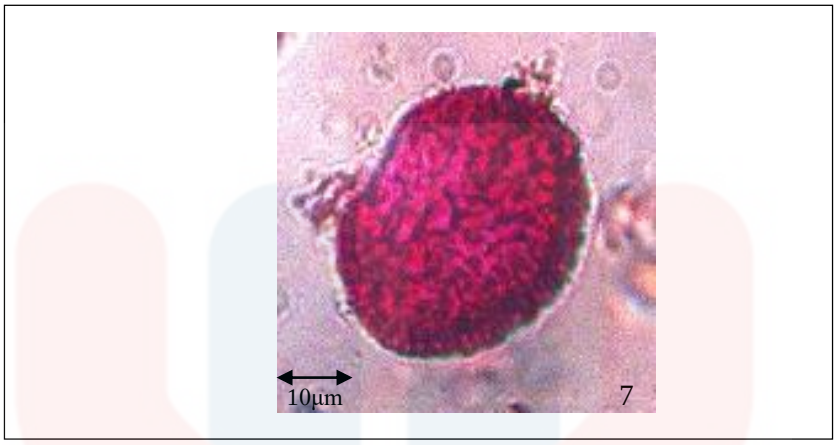


Figure 4.13 (c): Morphology of different pollen honey sample from Jitra under 40x magnification. 7- *Areca catechu* (Arecaceae)

Analysis on the honey sample from Jitra showed the occurrence of 7 different types of pollen, namely *Zea mays* (Poaceae), *Veitchia merrillii* (Arecaceae), *Melilotus suaveolens* (Fabaceae), *Erythrina orientalis* (Fabaceae), *Elaeis guineensis* (Arecaceae), *Tecoma stans* (Bignoniaceae), and *Areca catechu* (Arecaceae). The most abundance pollen species collected in Jitra was *Zea mays* or commercially known as corn plant with 78.92% of abundancy. The occurrence of predominant pollen showed this honey sample was unifloral type of honey. In this sample, 3 species were found as important minor pollen groups due to thier percentage abundance (3-15%) which were *Elaeis guineensis* (8.12%), *Tecoma stans* (4.70%), and *Veitchia merrillii* (4.13%). *Areca catechu* (1.42%), *Erythrina orientalis* (0.85%), and *Melilotus suaveolens* (1.85%) were the minor pollen type which <3% of pollen abundance. *Tecoma stans* was a scientific name for yellow bells or called 'Bunga Loceng Kuning' in Malay (Ahmad Bhat, 2019). *Areca catechu* was a scientific name for betel-nut palm or called 'Pokok Pinang' in Malay (Heatubun, 2016).

Pollen types of pollen were classified into four frequency classes, first was predominant pollen types, if >45% of total pollen counted. The second was, secondary pollen type if the pollen counted was between 16%-45%. The third was important minor pollen types, if the pollen counted was between 3%-15% and fourth was minor pollen types which referred to pollen counted <3%. If there was predominant pollen found in the sample, the honey was classified as unifloral type of honey. Otherwise, the honey was considered as multifloral honey (Jones, 2014). The honey sample from Jitra had different floral diversity in it due to the presence of different pollen species. The presence of important minor pollen and minor pollen makes this honey sample more varied.

4.14 Honey sample from Langkawi (Unifloral)

Table 4.14: The number of pollen and percentage of pollen types in the honey sample from Langkawi (LG).

Plant Species	Family	Total No. of Pollen	Percentage of Abundance (%)
Cinnamomum verum	Lauraceae	5	0.64
Cocos nucifera	Arecaceae	11	1.41
Nypa fruticans	Arecaceae	7	0.90
Psidium guajava	Myrtaceae	21	2.69
Solanum lycopersicum	Solanaceae	83	10.63
Sporobolus indicus	Poaceae	360	46.09
Veitchia merrillii	Arecaceae	16	2.05
Zea mays	Poaceae	278	35.60

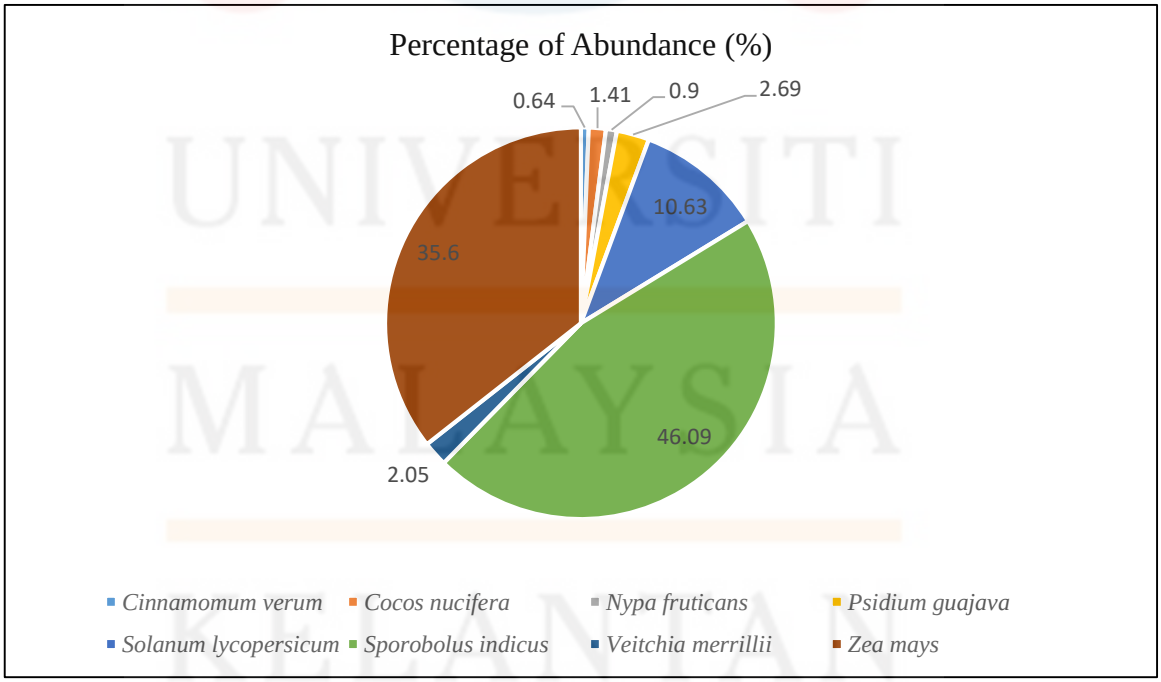


Figure 4.14: Pollen spectrum of honey sample from Langkawi

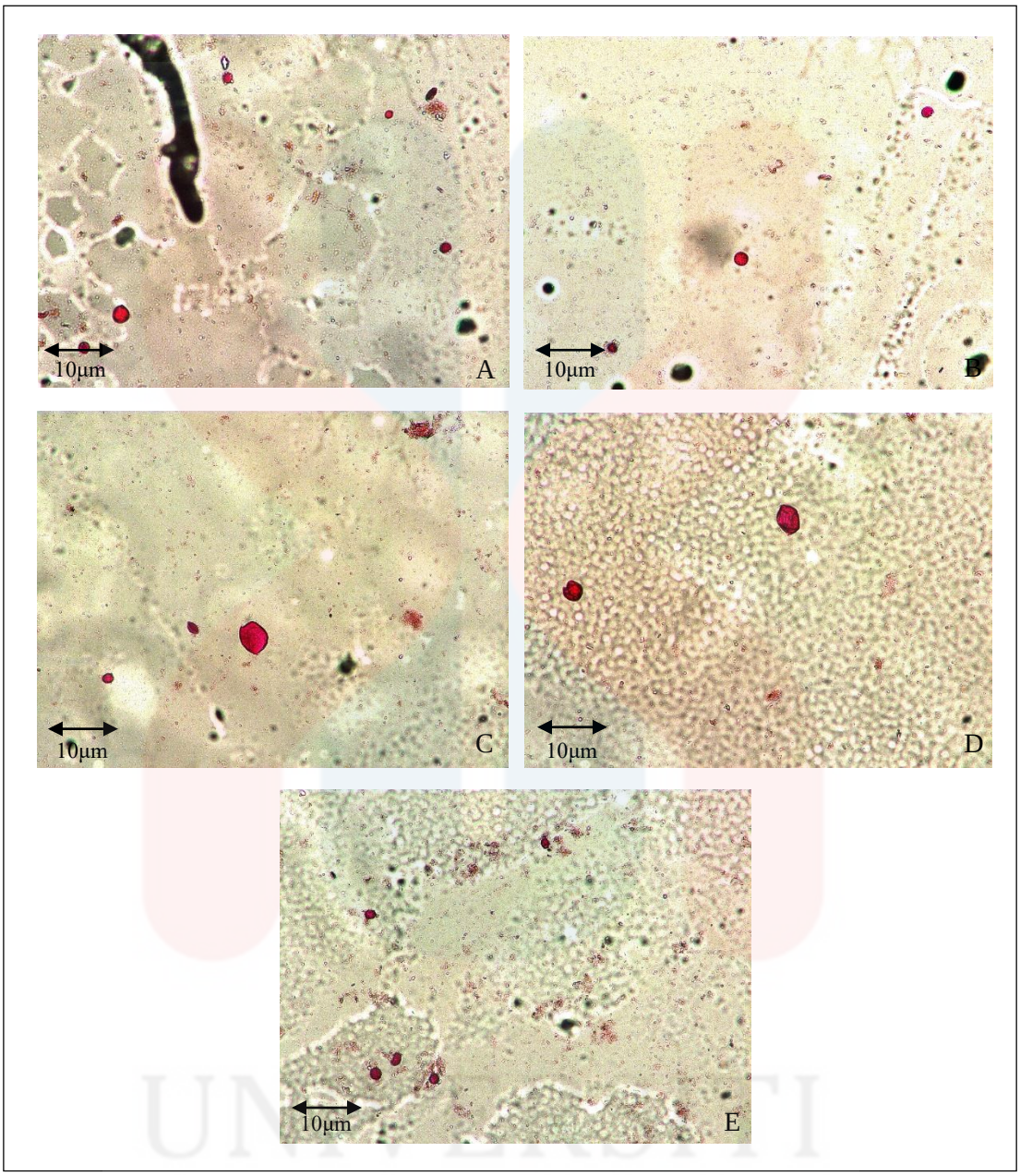


Figure 4.14 (a): Microscopic overviews of pollen density in honey sample from Langkawi under 10x magnification. Overviews of A- Right edge of cover slip (top); B- Left edge of cover slip (top); C- Right edge of cover slip (bottom); D- Left edge of cover slip (bottom) and E- centre of cover slip (middle)

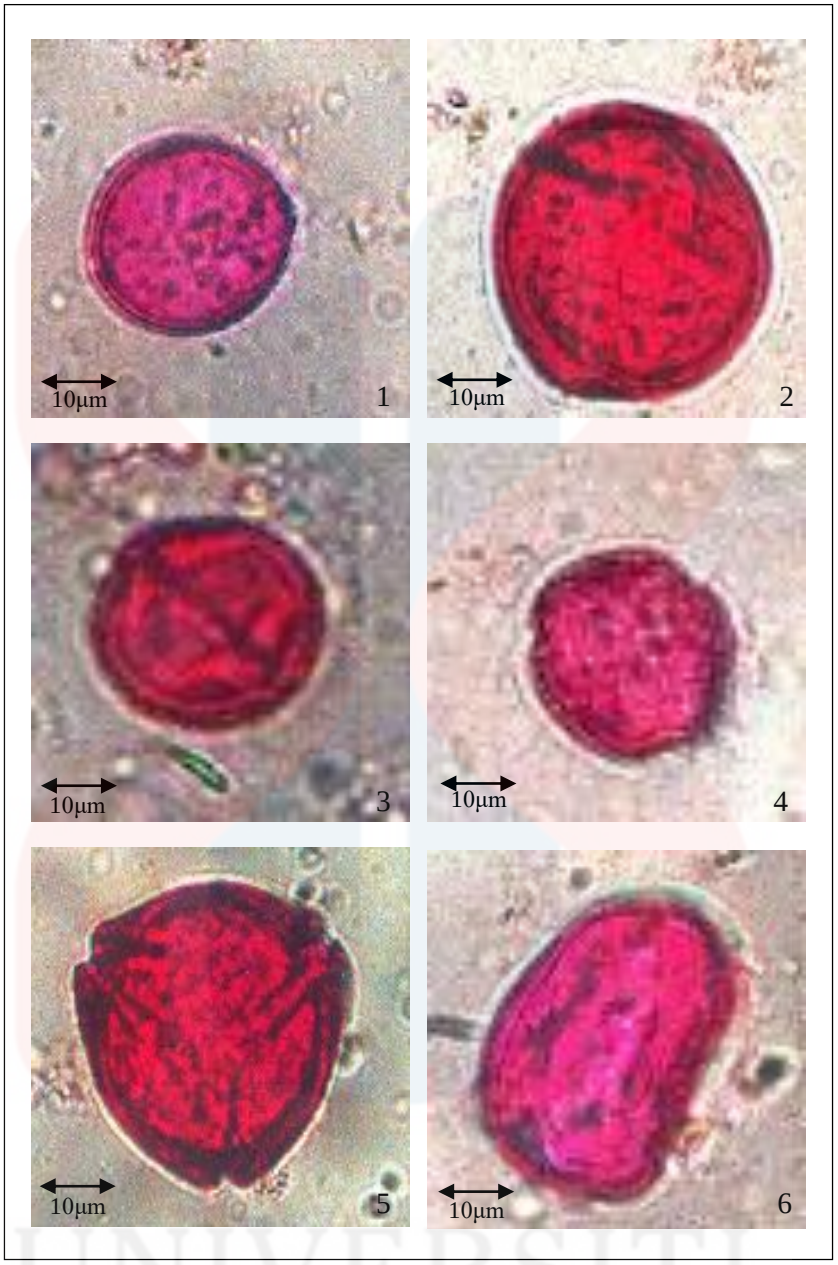


Figure 4.14 (b): Morphology of different pollen honey sample from Langkawi under 40x magnification. 1- *Sporobolus indicus* (Poaceae); 2- *Cinnamomum verum* (Lauraceae); 3- *Zea mays* (Poaceae); 4- *Solanum lycopersicum* (Solanaceae); 5- *Psidium guajava* (Myrtaceae) and 6- *Veitchia merrillii* (Arecaceae)

Figure 4.14 (c): Morphology of different pollen honey sample from Langkawi under 40x magnification. 7- *Nypa fruticans* (Arecaceae) and 8- *Cocos nucifera* (Arecaceae)

From the honey collected in Langkawi, 8 different types of pollen species had been identified, namely *Sporobolus indicus* (Poaceae), *Cinnamomum verum* (Lauraceae), *Zea mays* (Poaceae), *Solanum lycopersicum* (Solanaceae), *Psidium guajava* (Myrtaceae), *Veitchia merrillii* (Arecaceae), *Nypa fruticans* (Arecaceae), and *Cocos nucifera* (Arecaceae). The predominant pollen in this honey sample was *Sporobolus indicus* which accounted up to 46.09% of the total pollen count. Therefore, this honey sample was unifloral honey.

Zea mays species was identified as secondary pollen type with 35.60% of pollen abundance and *Solanum lycopersicum* was identified as an important minor pollen types. Other 5 species had been identified as minor pollen type which were *Cinnamomum verum* (0.64%), *Cocos nucifera* (1.41%), *Nypa fruticans* (0.90%), *Psidium guajava* (2.69%), and *Veitchia merrillii* (2.05%). *Cinnamomum verum* was a scientific name for Cinnamon tree.

In this sample, Kelulut bees preferred to forage the *Sporobolus indicus* plant which flower can be found throughout the year in the Langkawi area. Pollen analysis of honey gave reliable information on the floral components of honey and also identify the plant involved in honey production in the specific area (Ebenezer & Olugbenga, 2009). It provides knowledge about major and minor plant taxa utilized by bees (Adekanmbi & Ogundipe, 2009).

4.15 Honey sample from Taman Siswa, Jitra (Multifloral)

Table 4.15: The number of pollen and percentage of pollen types in the honey sample from Taman Siswa (TS).

Plant Species	Family	Total No. of Pollen	Percentage of Abundance (%)
Acacia auriculiformis	Fabaceae	2	1.34
Capsicum frutescens	Solanaceae	43	28.86
Cocos nucifera	Arecaceae	2	1.34
Commelina diffusa	Commelinaceae	2	1.34
Cucumis sativus	Cucurbitaceae	16	10.74
Elaeis guineensis	Arecaceae	6	4.03
Gliricidia sepium	Fabaceae	5	3.36
Melastoma malabathricum	Melastomatacea	53	35.57
Melilotus suaveolens	Fabaceae	3	2.01
Vitex pinnata	Lamiaceae	17	11.41

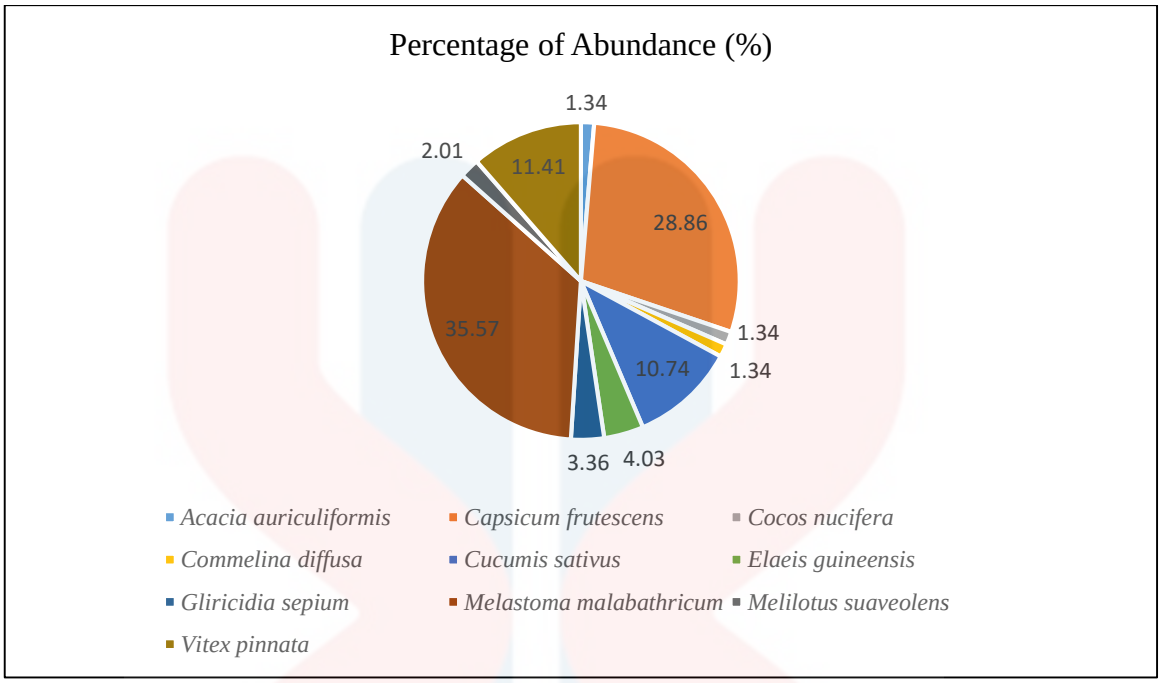


Figure 4.15: Pollen spectrum of honey sample from Taman Siswa, Jitra

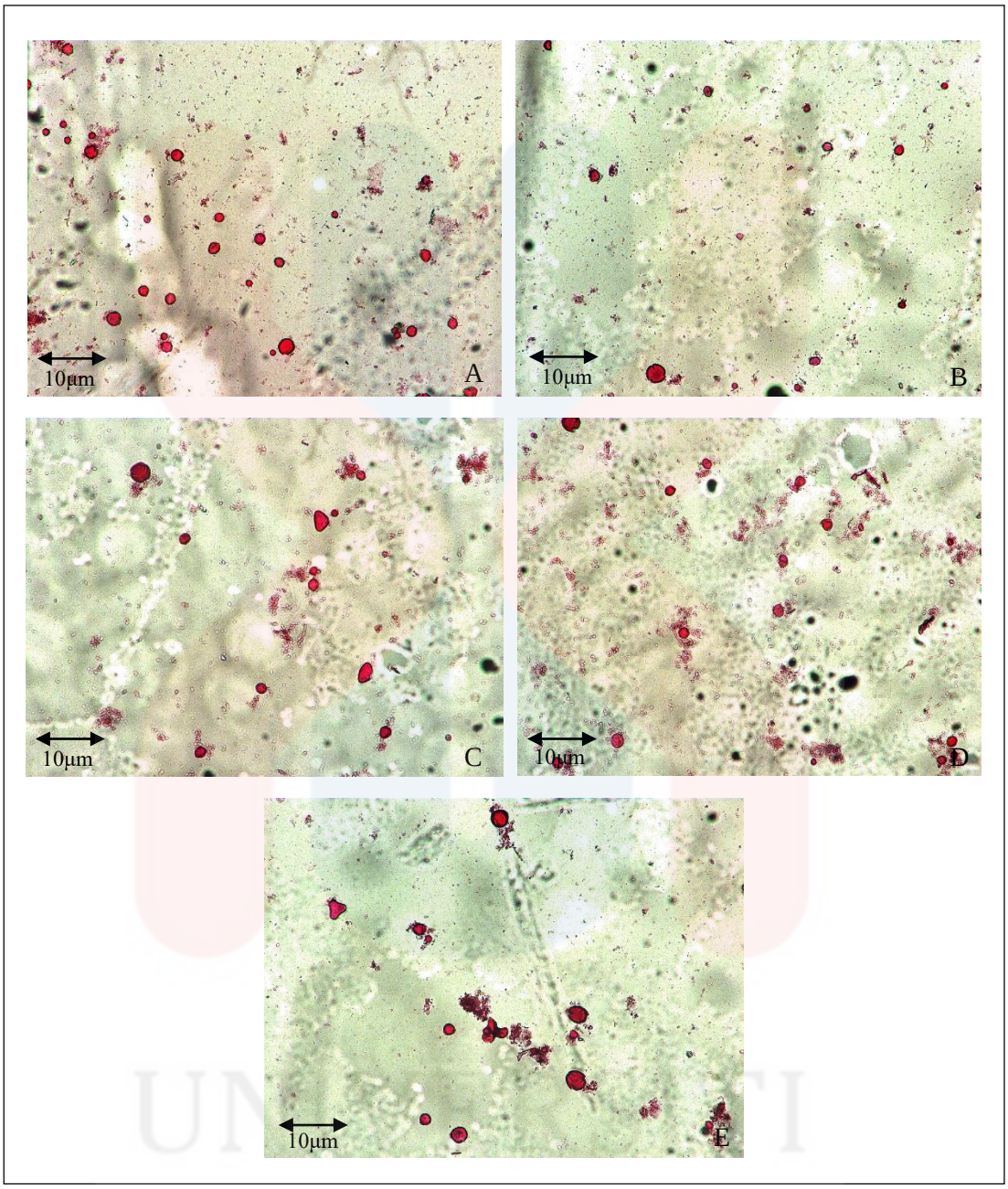


Figure 4.15 (a): Microscopic overviews of pollen density in honey sample from Taman Siswa under 10x magnification. Overviews of A- Right edge of cover slip (top); B- Left edge of cover slip (top); C- Right edge of cover slip (bottom); D- Left edge of cover slip (bottom) and E- centre of cover slip (middle)

Figure 4.15 (b): Morphology of different pollen honey sample from Taman Siswa under 40x magnification. 1- *Cocos nucifera* (Arecaceae); 2- *Vitex pinnata* (Lamiaceae); 3- *Cucumis sativus* (Cucurbitaceae); 4- *Gliricidia sepium* (Fabaceae); 5- *Elaeis guineensis* (Arecaceae) and 6- *Commelina diffusa* (Commelinaceae)

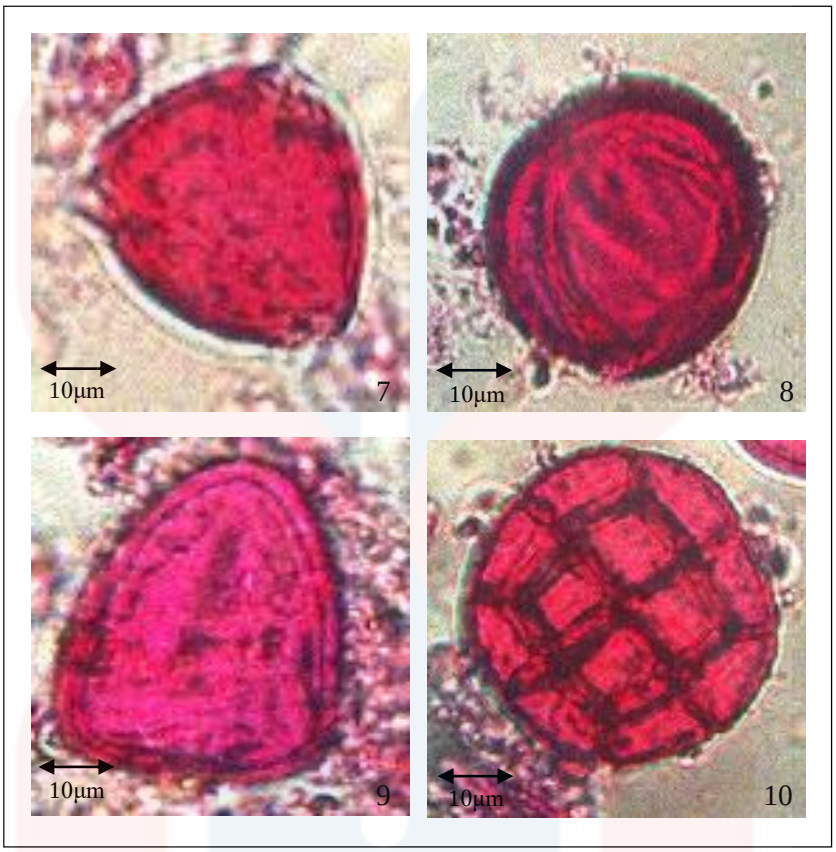


Figure 4.15 (c): Morphology of different pollen honey sample from Taman Siswa under 40x magnification. 7- *Capsicum frutescens* (Solanaceae); 8- *Melastoma malabathricum* (Melastomatacea); 9- *Melilotus suaveolens* (Fabaceae) and 10- *Acacia auriculiformis* (Fabaceae)

There were 10 different types of pollen identified from honey collected in Taman Siswa. The most abundance plant species was *Melastoma malabathricum* from the Melastomatacea family with a percentage abundance, 35.57%. In Malay term, it was called as 'Senduduk' (Nozlena et al., 2018). This result showed that Kelulut bees preferred to forage this *Melastoma malabathricum* plant in the Taman Siswa area. 'Senduduk' can be classified as secondary pollen and followed by *Capsicum frutescens* (tomato) with 35.57% of pollen abundance. *Capsicum frutescens* was from the family Solanaceae which was known as the herbaceous and woody family. Many plant species from this family was important to the economy. The pollen morphology found for the tomato plants was acolpate, bicolpate, and tricolpate. The shape was prolate-spheroidal (Song et al.,2018).

4 species were identified as important minor pollen types which were *Cucumis sativus* from family Cucurbitaceae with 10.74%, *Elaeis guineensis* from family Arecaceae with 4.03%, *Gliricidia sepium* from family Fabaceae with 3.36%, and *Vitex pinnata* from family Lamiaceae with 11.41%. Other 4 plant species were referred to as minor pollen types which were *Acacia auriculiformis* (Fabaceae) with 1.34%, *Cocos nucifera* (Arecaceae) with 1.34%, *Commelina diffusa* (Commelinaceae) with 1.34%, and *Melilotus suaveolens* (Fabaceae) with 2.01%. Based on the results, it showed that Kelulut bees in Taman Siswa more preferred to forage flowers from plant species found above. Honey contained about 80% of carbohydrates and 20% of water (Saeed et al., 2014). Knowing the origin of the flower's nectar was essential as the composition of honey depends on the nectar or pollen collected by bees and also the climatic factor (Rahman et al., 2013). Honey in Taman Siswa was multifloral honey as none of the plant species reached the requirement of being predominant pollen.

4.16 Honey sample from Kampung Padang Limau, Karangan, Kulim (Multifloral)

Table 4.16: The number of pollen and percentage of pollen types in the honey sample from Kampung Padang Limau (PL).

Plant Species	Family	Total No. of Pollen	Percentage of Abundance (%)
Cinnamomum verum	Lauraceae	16	5.05
Cleome rutidosperma	Cleomaceae	7	2.21
Cucumis melo	Cucurbitaceae	46	14.51
Cucumis sativus	Cucurbitaceae	92	29.02
Mimosa bimucronata	Fabaceae	27	8.52
Mimosa pudica	Fabaceae	9	2.84
Sporobolus indicus	Poaceae	120	37.85

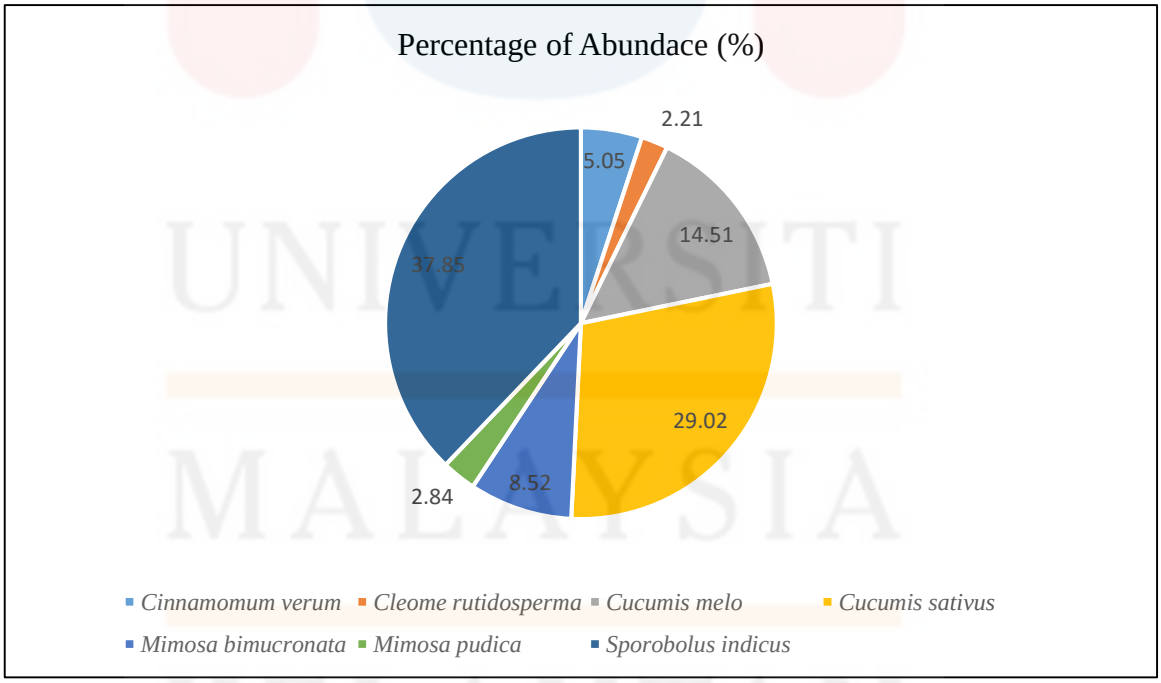


Figure 4.16: Pollen spectrum of honey sample from Kampung Padang Limau, Karangan, Kulim

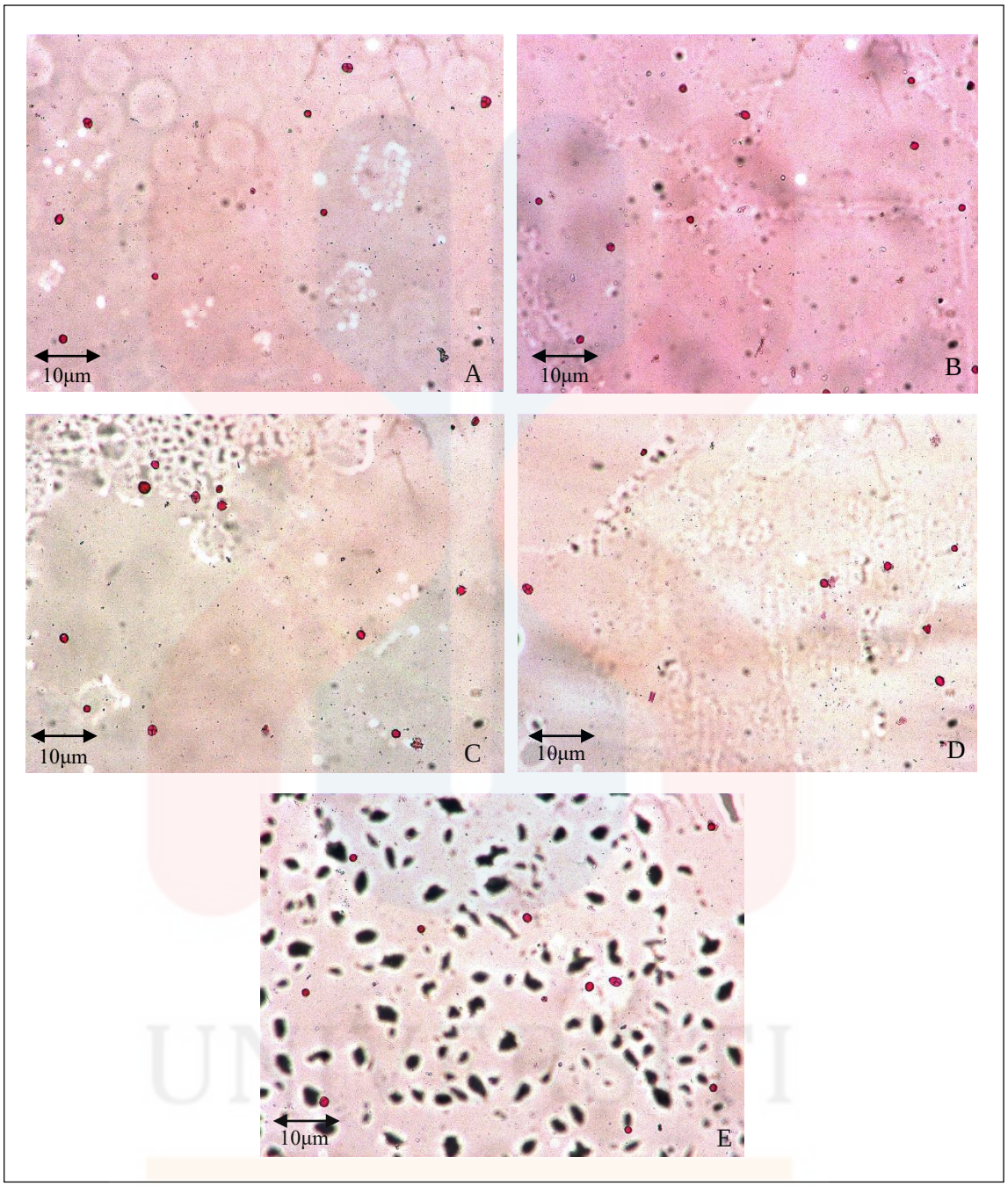


Figure 4.16 (a): Microscopic overviews of pollen density in honey sample from Kampung Padang Limau under 10x magnification. Overviews of A- Right edge of cover slip (top); B- Left edge of cover slip (top); C- Right edge of cover slip (bottom); D- Left edge of cover slip (bottom) and E- centre of cover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

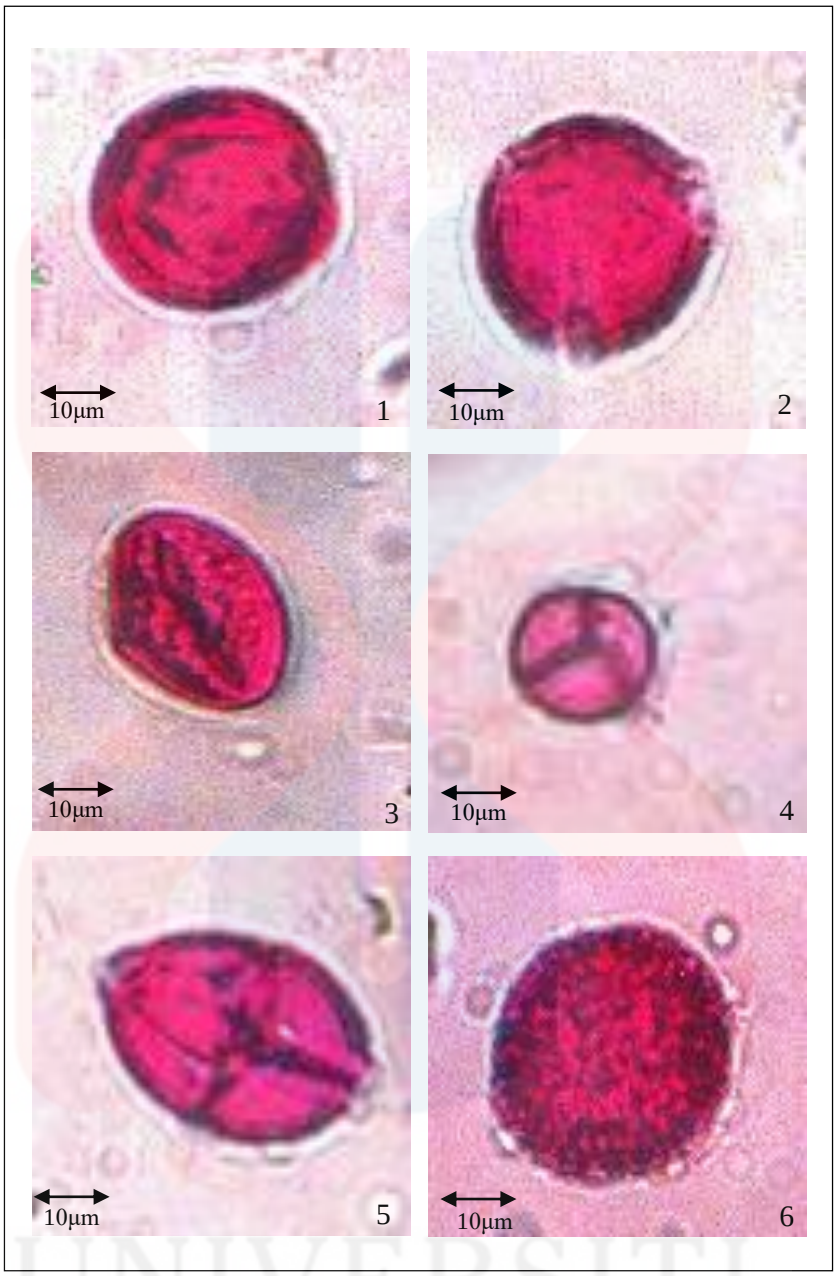


Figure 4.16 (b): Morphology of different pollen honey sample from Kampung Padang Limau under 40x magnification. 1- *Sporobolus indicus* (Poaceae); 2- *Cucumis melo* (Cucurbitaceae); 3- *Cleome rutidosperma* (Cleomaceae); 4- *Mimosa pudica* (Fabaceae); 5- *Mimosa bimucronata* (Fabaceae) and 6- *Cinnamomum verum* (Lauraceae)

Figure 4.16 (c): Morphology of different pollen honey sample from Kampung Padang Limau under 40x magnification. 7- *Cucumis sativus* (Cucurbitaceae)

From the honey sample of Kampung Padang Limau, 7 different types of pollen species were found which were *Sporobolus indicus* (Poaceae), *Cucumis melo* (Cucurbitaceae), *Cleome rutidosperma* (Cleomaceae), *Mimosa pudica* (Fabaceae), *Mimosa bimucronata* (Fabaceae), *Cinnamomum verum* (Lauraceae), and *Cucumis sativus* (Cucurbitaceae).

The highest pollen abundance came from *Sporobolus indicus* with 37.85% of pollen abundance. Therefore, the percentage does not exceed >45% and conclude that the pollen as secondary pollen type. *Cucumis sativus* was also included in secondary pollen type with 29.02% of pollen abundance. Important minor pollen also found in this sample were *Cinnamomum verum* with 5.05%, *Cucumis melo* with 14.51%, and *Mimosa bimucronata* with 8.52%. Another 2 pollen types that had been identified were *Cleome rutidosperma* (2.21%) and *Mimosa pudica* (2.84%) which were referred as minor pollen types. The honey sample from Kampung Padang Limau was identified as multilfloral

honey type because there was no predominant pollen found. Pollen analysis of honey was important to determine the floral origins of the honey and also verify apiarist claims about the purity of the honey product (Morais et al., 2011). Furthermore, comprehensive characterisation of the various honey types was necessary to minimise adulteration and to maximise honey's commercial value (Silva et al., 2009).

4.17 Honey sample from Kampung Batu Kembai, Kuala Ketil (Multifloral)

Table 4.17: The number of pollen and percentage of pollen types in the honey sample from Kampung Batu Kembai (BK).

Plant species	Family	Total No. of Pollen	Percentage of Abundance (%)
Anacardium occidentale	Anacardiaceae	14	4.09
Ananas comosus	Bromeliaceae	11	3.22
Brassica chinensis	Brassicaceae	69	20.18
Cocos nucifera	Arecaceae	34	9.94
Hevea brasiliensis	Euphorbiaceae	8	2.34
Nephelium lappaceum	Sapindaceae	17	4.97
Nypa fruticans	Arecaceae	4	1.17
Sporobolus indicus	Poaceae	96	28.07
Veitchia merrillii	Arecaceae	89	26.02

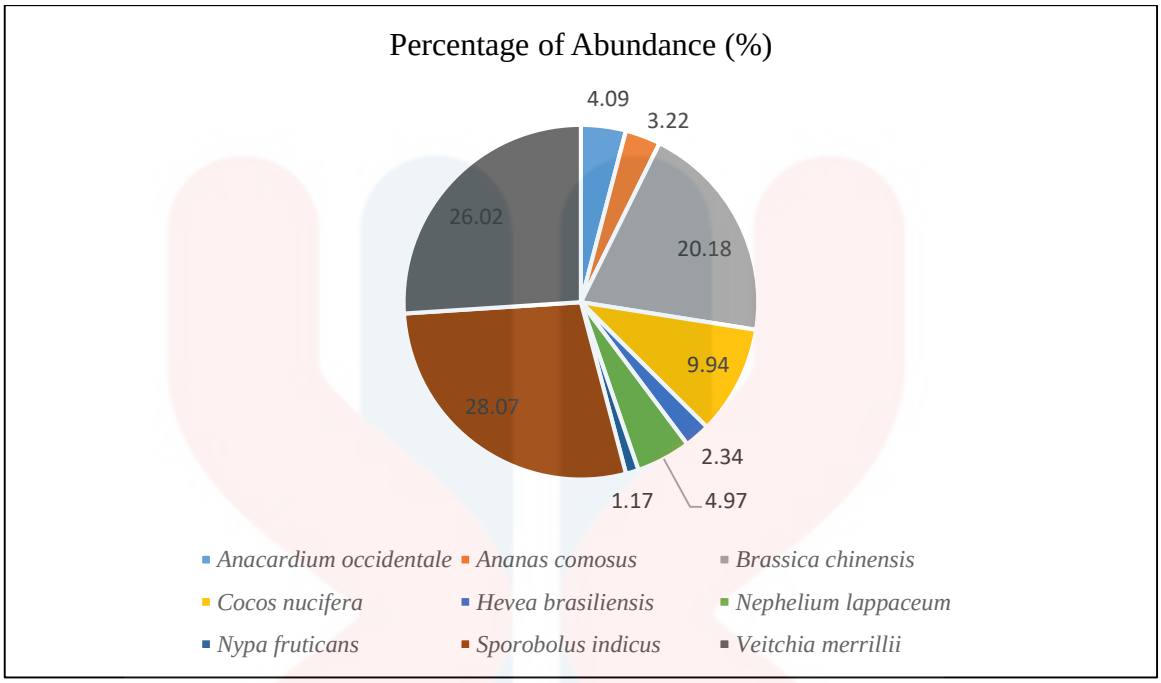


Figure 4.17: Pollen spectrum of honey sample from Kampung Batu Kembai, Kuala Ketil

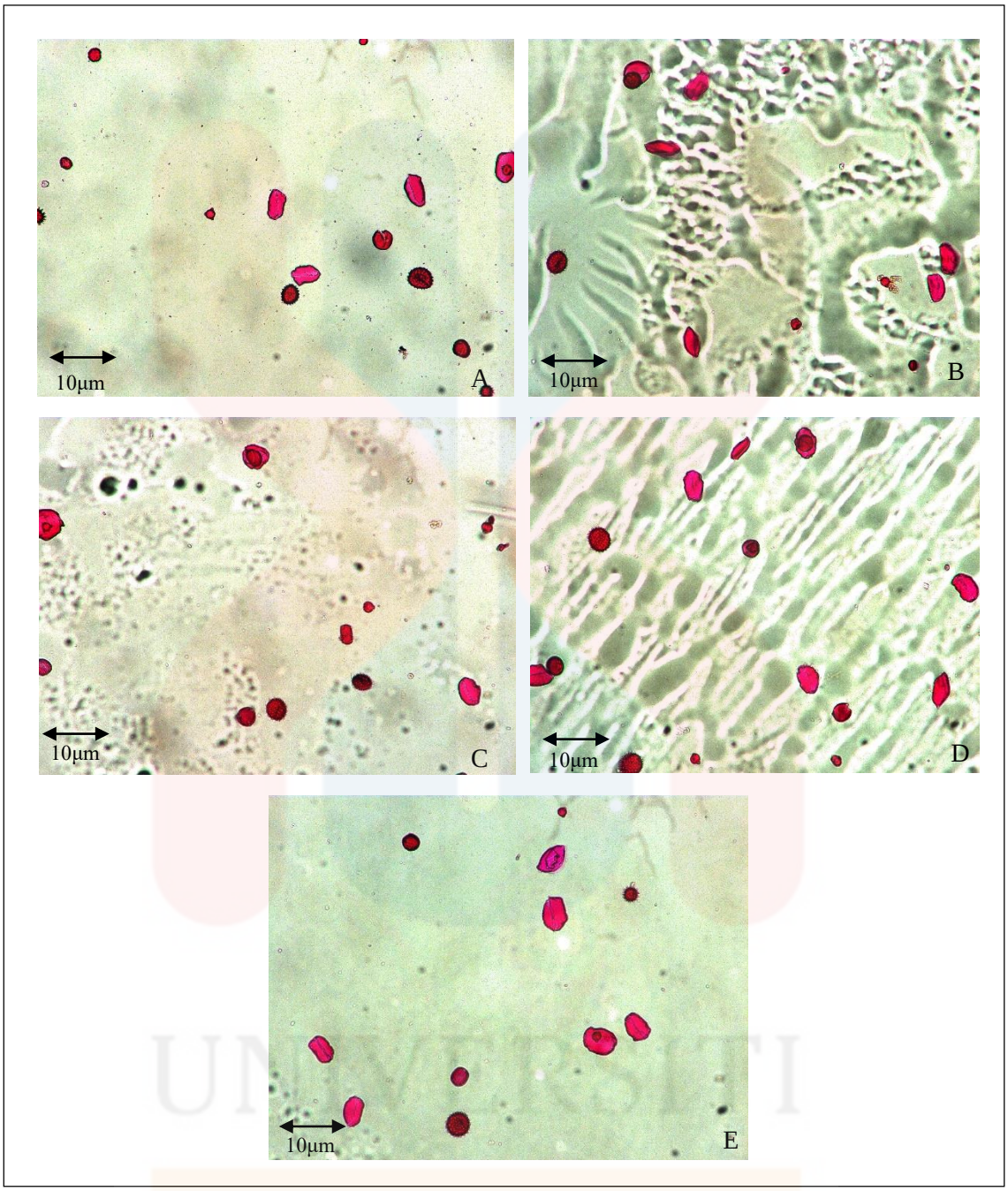


Figure 4.17 (a): Microscopic overviews of pollen density in honey sample from Kampung Batu Kembai under 10x magnification. Overviews of A- Right edge of cover slip (top); B- Left edge of cover slip (top); C- Right edge of cover slip (bottom); D- Left edge of cover slip (bottom) and E- centre of cover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

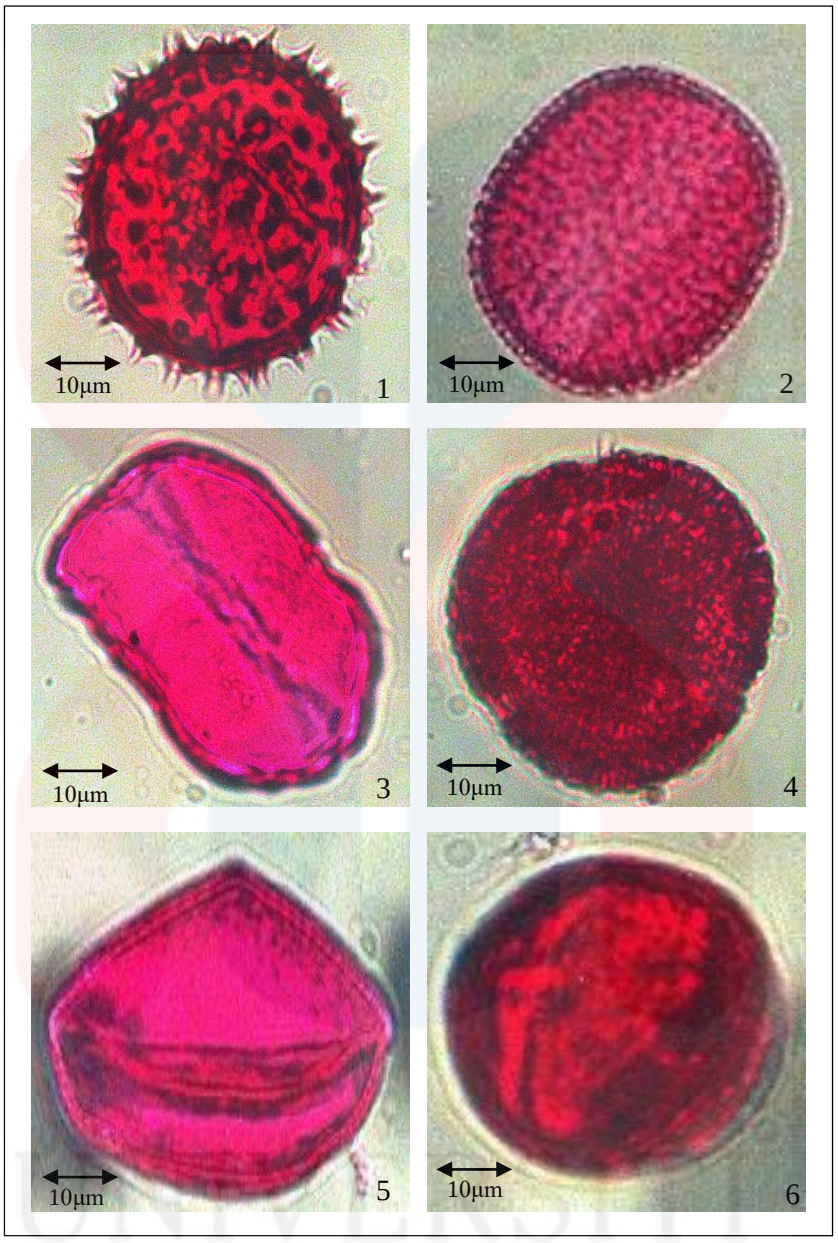


Figure 4.17 (b): Morphology of different pollen honey sample from Kampung Batu Kembai under 40x magnification. 1- *Nypa fruticans* (Arecaceae); 2- *Ananas comosus* (Bromeliaceae); 3- *Veitchia merrillii* (Arecaceae); 4- *Nephelium lappaceum* (Sapindaceae); 5- *Cocos nucifera* (Arecaceae) and 6- *Brassica chinensis* (Brassicaceae)

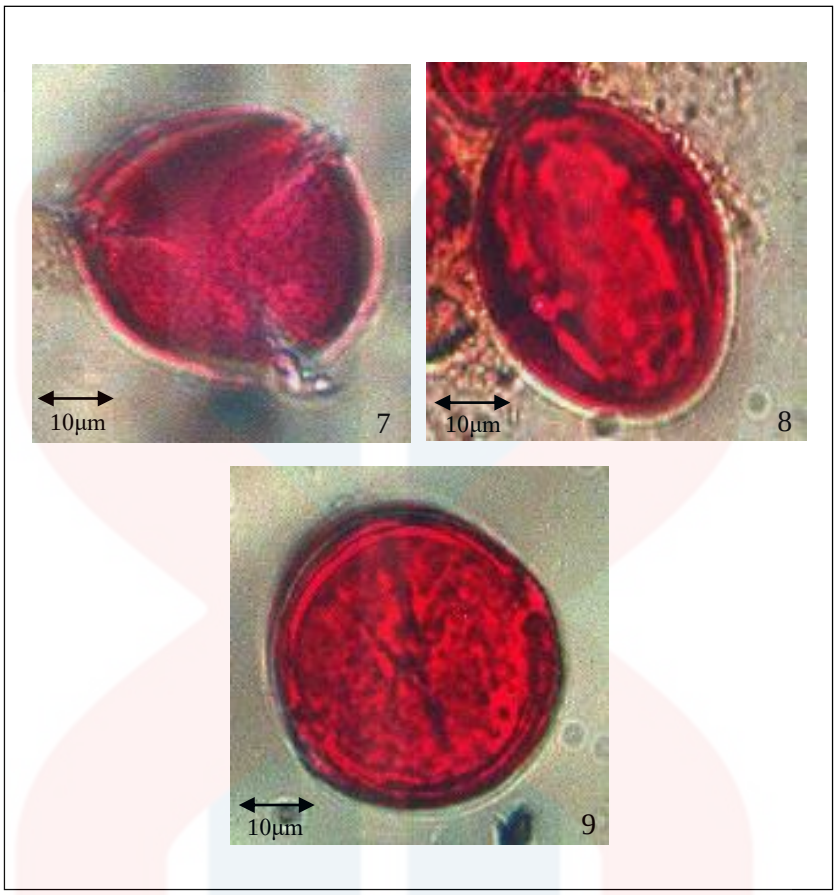


Figure 4.17 (c): Morphology of different pollen honey sample from Kampung Batu Kembai under 40x magnification. 7- *Hevea brasiliensis* (Euphorbiaceae); 8- *Anacardium occidentale* (Anacardiaceae) and 9- *Sporobolus indicus* (Poaceae)

Pollen analysis of honey from Kampung Batu Kembai showed the occurrence of 9 different types of pollen, namely *Nypa fruticans* (Arecaceae), *Ananas comosus* (Bromeliaceae), *Veitchia merrillii* (Arecaceae), *Nephelium lappaceum* (Sapindaceae), *Cocos nucifera* (Arecaceae), *Brassica chinensis* (Brassicaceae), *Hevea brasiliensis* (Euphorbiaceae), *Anacardium occidentale* (Anacardiaceae), and *Sporobolus indicus* (Poaceae). The most abundance pollen species collected in Kampung Batu Kembai area was *Sporobolus indicus* with 28.07% of pollen abundance. *Sporobolus indicus* was

categorised under secondary pollen type due to its percentage of abundance (16-45%) same as *Veitchia merrillii* with 26.02% and *Brassica chinensis* with 20.18%.

4 pollen species found were grouped into important minor pollen types which were *Anacardium occidentale* (4.09%), *Ananas comosus* (3.22%), *Cocos nucifera* (9.94%), and *Nephelium lappaceum* (4.97%). The other 2 species were identified as minor pollen types were *Hevea brasiliensis* and *Nypa fruticans* with 2.34% and 1.17% respectively. Since none of the pollen species was identified as predominant pollen, the honey sample in Kampung Batu Kembai was included in multifloral honey type. Honey characterization was important for commercial purposes. The flowering and nectar production seasons for the same species of flower in different regions might be different, honey production in an area was largely determined by the flora and climatology of that area (Herrero et al., 2002).

From all the 16 samples analysed, it can be concluded that honey in Kedah state contained pollen from coconut *(Cocos nucifera),* smut grass *(Sporobolus indicus),* and oil palm *(Elaeis guineensis)* in almost every sample. The Kelulut bees had preferred to forage this plant species which flowers intermittently throughout the year in Kedah state. Pollen from *Cocos nucifera* and *Elaeis guineensis* was one of the most essential markers for distinguishing Malaysian honey from imported honey such as Australia and China. Moreover, *Cocos nectar* contained higher sugar concentrations (Selvaraju et al., 2019). This emphasises the value of pollen analysis in identifying the botanical and geographic origins of honey.

Pollen analysis of honey or Melissopalynology was important as it gives verified information on the floral components of honey and allows beekeepers to identify the source plants utilised in honey production. This approach was not only beneficial for determining the geographical and botanical origin of a certain variety of honey, but it was also important for quality control and determining whether honey was contaminated (Ebenezer & Olugbenga, 2009). These discoveries reveal information on honey's origins, which was crucial for bees to have an appropriate and plentiful supply of nectar and pollen while maximising the honey yields (K. Hamid & A.F. Mohd, 2015).

CHAPTER 5

Conclusion

Analysis of the honey samples from 16 different locations in Kedah state in Malaysia revealed the occurrence of 61 types of pollen from 30 different plant families. 10 of the honey samples were multilfloral and 6 honey samples were unifloral. From the results, each of the locations of the samples had varieties of pollen honey. The classification of the honey spectrum was based on the existence of the predominant pollen that can be found through the percentage of pollen abundance. If the percentage of pollen was >45%, the honey was classified as uniforal honey and if no predominant found the honey was classified as multifloral honey. 3 types of pollen were continuously and repeatedly found in most of the samples which were *Cocos nucifera*, *Sporobolus indicus*, and *Elaeis guineensis.* Pollen analysis or melissopalynology was a valuable tool for the identification of the botanical and geographical origin of honey. As a recommendation, the government should prioritise the conservation and rehabilitation of plant species that Kelulut bees usually forage. Locals were encouraged to produce bee foraging plant species that were dense and abundant.

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APPENDIX

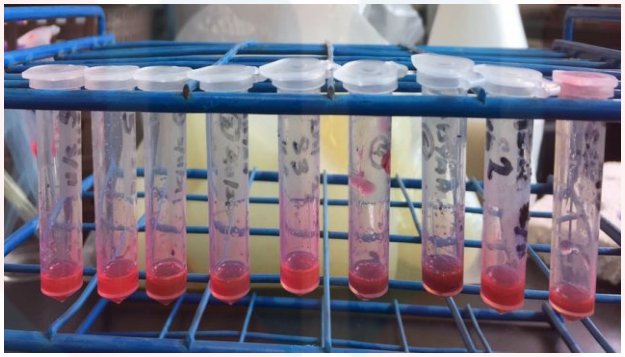

Figure a(i): Mixture of glycerine jelly with pollen material

Figure a(ii): Homogenous mixture of honey sample

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