Melissopalynology of Kelulut Honey Collected from Various Bee Forms Located in Perlis

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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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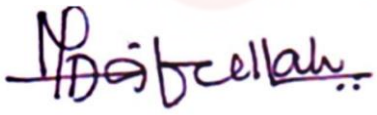

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Melissopalynologi Madu Kelulut yang Dikumpul dari Pelbagai Bentuk Madu Lebah Kelulut di Perlis

ABSTRAK

Tujuna kajian ini dilaksanakan adalah untuk mengasingkan, mengenal pasti, dan menilai jenis debunga yang terdapat di dalam sampel madu kelulut. Di samping itu, dapat mengetahui jenis spesies melalui debunga yang diperoleh daripada sampel tersebut. Terdapat dua spesies lebah kelulut yang selalu dibela iaitu spesies *Heterotrigona itama* dan spesies *Geniotrigona thoracica*. Madu yang dikumpul daripada 10 kawasan yang berbeza di Perlis diasingkan ke dalam bekas kaca. Untuk kajian yang selanjutnya Analisis Melissopalynologikal dijalankan dalam eksperimen ini. Jenis debunga yang diperoleh daripada madu dikelaskan dan pengiraan debunga juga dijalankan melalui ujian asetolisis. Selain itu, tujuan kajian ini dilaksanakan adalah kerana tiada lagi kajian analisis melissopalinologi terhadap lebah kelulut di Perlis. Oleh itu, mengambil peluang ini untuk melakukan penyelidikaan sebagai projek tahun akhir. Objektif kajian ini adalah untuk mengkaji pencirian debunga berdasarkan jenis debunga yang terdapat dalam sampel. Objektif seterusnya, adalah untuk mengira bilangan debunga yang terdapat di dalam sampel madu. Berdasarkan keputusan akhir yang diperoleh melalui kajian ini, kehadiran 64 jenis debunga daripada 39 keluarga tumbuhan yang berbeza ditemui dalam sampel madu. Tiga daripada sampel madu adalah madu uniflora manakala tujuh daripada sampel madu adalah jenis madu multiflora. Justeru itu, pengenalpastian pelbagai tumbuhan adalah penting kerana ia sepadan dengan pengeluaran madu dan membantu dalam pengesahan jenis madu.

Kata kunci: Melissopalynologikal, *Heterotrigona itama Geniotrigona thoracica*, ujian aasetolisis, kiraan debunga, uniflora, multiflora.

Melissopalynology of Kelulut Honey Collected from Various Bee Forms Located in Perlis

ABSTRACT

The purpose of this study to isolate, identify and evaluate the pollen from honey of the stingless bee honey. In addition, it is possible to know the type of species through the pollen obtained from the sample. There were 2 commonly species which are *Heterotrigona itama* and *Geniotriona thoracica.* The honey collected from 10 different region in Perlis were isolated into the glass container. For the further research, Melissopalynological analysis conducted in this experiment. The pollens obtain from the honey sample were characterized and pollen count were carried out through the acetolysis test. The purpose of this study was none had done the research yet about Melissopalynological analysis in Perlis. Therefore, took the opportunity for doing this research as the final year project. The objectives of this study are to characterize types of honey based on the pollen found in the sample. The next objectives, were to count the pollen found in the honey sample. Based on the final result obtained through the research, the presence of 64 types of pollen from 39 distinct plant families was discovered in honey samples Three of the honey samples were unifloral honey, whereas the other seven were multifloral honey. Thus, the identification of various plants is important because they correspond to the production of honey and aid in the verification of honey origins.

Keywords: Melissopalynological, *Heterotrigona itama*, *Geniotrigona thoracica*, acetolysis test, pollen count, unifloral honey, multifloral honey

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LIST OF ABBREVIATIONS AND SYMBOL

MARDI	Malaysian Agricultural Research and Development Institute
MGI	Malaysian Genome Institute
sp.	Species
H. itama	*Heterotrigona itama*
G. thoracica	*Geniotrigona thoracica*
SOP	Standard Operating Procedure
DPPH	1,1-diphenyl-2-picryhydrazy
FRAP	ferric reducing antioxidant strength
ABTS	2,2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid
ORAC	oxygen radical absorbance ability
%	Percentage
CRD	Completely Randomized Design

CHAPTER 1

INTRODUCTION

1.1 Research background

1.1.1 The definition of honey

Honey is the most common and well-known organic food made from nectar by both honeybees (*Apis* spp.) and stingless bees (*Trigona* spp.). Honey is a natural, super saturated food that is dark gold in colour that is produced in the honey sacs of different bees from flower nectar. The flowers from which the nectar is collected decide the flavour and colour (Selvaraju et. al ,2019). The local honeybee produces some of the most highly desirable honeys from wildflower (Britannica, 2021). This natural substance is also well-known antimicrobial and nutritious properties. Honey is mostly composed of simple sugars compounds and contains approximately 200 active compounds such as minerals, vitamins, hormones, chemical compounds, enzymes, and flavonoids (Britannica, 2021)

1.1.2 The Source of Honey

Honey is a delicious food produced by bees from flower nectar. Honey bees use regurgitation and evaporation to convert nectar into honey. They keep it in wax honeycombs within the beehive as a primary food supply. Honey's flavour is derived from monosaccharides, carbohydrates, and glucose, and it has about the same relative sweetness as white sugar. Honey has a long tradition of human use and is used as a sweetener and flavouring in a variety of foods and drinks, Khan (2014). In Malaysia, honey is obtained from two forms of beekeeping: commercial Apiculture using honey bees (Apini) and Meliponiculture using stingless bees (Meliponini). Both types of bees are strongly eusocial corbiculate bees belonging to the same bee family. Honey bees are made up of 11 species in the genus Apis, with Apis mellifera being the most common. There are over 500 species (60 genera) of stingless bees, most of which are found in tropics and subtropics such as United states and south America, Australia, and South Asia (Siok,et. al, 2018). Based on 145 specimens from the Malaysian Agricultural Research and Development Institute (MARDI), the Malaysian Genome Institute (MGI), and sampling conducted in multiple stingless bee farms in Peninsular Malaysia, 35 species of stingless bee (Apidae: Meliponini) from eight genera were investigated and revised in Malaysia (Samsudin, et al.., 2018).

1.1.3 Introduction of Stingless Bee

The Malaysian Agricultural Research and Development Institute (MARDI) introduced stingless beekeeping in 2004 with the expectation of providing new alternative species that could complement existing Apis spp. horticulture projects in terms of honey development and pollination operations. (Mahani, 2020). The aim of this analysis is to classify pollen honey extracted from the stingless bee *Heterotrigona itama*. Bee pollen is flower pollen that accumulates on the feet and body of worker bees. It can also contain honey and bee saliva. Since honey pollens come from a wide variety of plants, the composition of bee pollen can vary greatly. Honey pollen may be used to increase slowly, increase resilience and efficiency, and has a strong antioxidant content that protects against oxidants and related illnesses (Samsudin, et al.., 2018).

Malaysia is considered one of the world's mega biodiversity hubs. Pollinator species are one of the indications of Malaysia's high biodiversity. A total of 29 stingless bee species have been discovered in Peninsula Malaysia, with 17 species known to live in virgin forest, according to earlier reports. Beekeepers have been flocking to stingless bees for their honey and as a productive pollinator species in agroecosystems since 2012. *Heterotrigona itama* and *Geniotrigona thoracica*, two kinds of stingless bees, were used as pollinators for several key crops in Malaysia, as well as for meliponiculture (Hafizudin & Rosliza , 2016).

1.2 Problem statement

The purpose of this study was none had done the research yet about Melissopalynological analysis in Perlis. Therefore, took the opportunity for doing this research as the final year project. The process for this study to isolate, identify and evaluate the pollen from honey of the stingless bee *Heterotrigona itama*, (H. itama) and *Geniotrigona thoracica* (G. thoracica) The pollen honey was collected from different region in Perlis. In this experiment, Melissopalynological analysis performed in this study, and the isolates be identified and pollen counted using the acetolysis procedure (Zulkhairi, 2020). Taking all of these factors into account, the current research was conducted to determine the pollen identifications, types of honey in the sample, the botanical and the geographical origin of plants of honey produced by stingless bees (*H. itama*) and (*G. thoracica*) in Perlis, Malaysia.

1.3 Hypothesis

The pollen contents of stingless bee honey vary by Perlis region. The stingless bee honey contains pollen from various species of floral regions in Perlis.

1.4 Research questions

1. What are the similarities and differences in the honey pollen collected from stingless bee in Perlis?
2. Does honey pollen collected in all area of study have the same properties of pollen?
3. Where stingless bee will collect the pollen from the unifloral flower or multifloral flower? What are the types and species of pollen found in the sample of honey?
4. How to count the pollen based on the acetolysis analysis?

1.5 Scope of the study

10 honey samples collected from stingless bee *Heterotrigona itama* and *Geniotrigona thoracica* investigated their plant origins, types of pollen identified, and pollen counted. Then honey samples will be acetolyses and microscopically classification, and the acetolyses pollens were placed in glycerine jelly for pollen identification. The observations highlighted the impact of plant origins on the pollen analysis activities of stingless bee honey. These results may have a huge impact on the survival of the stingless bee community as well as the global promotion of Malaysian stingless bee honey.

1.6 Significance of the study

Melissopalynological analysis research can aid in the detection and identification species sources. The identification of various plant species is essential because they correspond to the composition of honey and aid in the verification of honey authenticity. The identification of numerous plants is important since they relate to honey production and help in the verifying of honey origins. As an outcome, the expected result was to evaluate the chemical composition and antioxidant effects of stingless bee honey (Mahani, 2020). The aim was to assess the chemical characteristics and antioxidant effects of stingless bee honey. In this analysis, the pollen needed to be counted according to the five parallel uniform equidistant lines to establish pollen types and abundances (Selvaraju, 2019). As a result, acetolysis tests were often used to determine the geographical and floral sources of honey. As a consequence, pollen analysis of honey can assist in deciding the origin of plant species. The identification of numerous plants is important since they contribute to honey production and help in the authentication of honey origins (Mahani, 2020).

1.7 Limitation of the study

The following observations were made when doing this analysis. It was thought that the purposive sampling method reduces the results' generalizability. This research would not be applicable to all areas of Perlis. Next, owing to the spread of Covid-19, movement is restricted to find samples of honey comb of kelulut honey in the state of Perlis due to increasingly tight of SOP by the government. Lastly, the sample collection only 10 sample due to the many beekeepers have been closed.

1.8 Research objectives

1. The goals of this research are to identify at pollen that has been characterised based on the pollen present in the sample.
2. This distinguishes the features of pollen obtained from unifloral flowers or multifloral flowers.
3. Aside from that, the goals are to determine how many pollens are present in the pollen honey sample.

CHAPTER 2

LITERATURE REVIEW

2.1 The structure of honeycomb

Stingless bees that nest above ground make their nests in hollow tree trunks, other cavities, under the roofs of houses, and in empty log hives. The primary nest walls orb tumen, which can be several centimetres thick, often delimit the cylindrical nesting cavity in a tree hollow. Concentric sheets of involucrum, which may encircle brood but rarely the food storage pots, are even more complex. Brood cells form a cluster comb of spherical to oval brood cells and naked pupalcells, which are always ovoid, when they are not packed into combs, whose cells are then pushed on all sides and become hexagonal (David, 2020). Honeycomb structural materials are usually created by sandwiching a honeycomb material between two thin layers to have tension strength. This results in a sheet assembly (Siok, 2018).

Honeycomb structures are commonly used in applications requiring smooth or gently curved surfaces, and their high specific strength is highly valued. While stingless bees yield fewer honey, their honey is more valuable than honey from *Apis* honey bee. The price of bee pollen can be up to ten times that of *Apis mellifera* honey in Africa, Asia, Colombia, and Bolivia (Siok, 2018). Honey is a delicious food produced by bees from flower nectar. Honey bees use regurgitation and water loss to convert nectar into honey. They keep it in wax honeycombs within the beehive as a primary food supply. Honey's flavour is derived from glucose, and fructose, and it has about the same relative sweetness as white sugar (Khan, 2014).

2.2 The characteristics of different floral type of honey

Both two stingless bee species, *Heterotrigona itama* and *Geniotrigona. Thoracica* have been widely reared for the purchase of agricultural honey production in Malaysia in the southern part of Malaysia. These two species was the species that is easily obtained in the forests and is widely sought after by beekeepers. Honey is divided into categories based on the floral sources from which bees obtain nectar. Honey is classified into two groups: multifloral honey and unifloral honey (Fao, 2011). Wildflower honey is another name for this honey. This classification is based on the location of the bee's nectar collection. Multifloral or polyfloral honey is made up of a blend of unifloral honeys from various species and is collected by bees from a wide variety of flowers (Agashe & Rangaswamy, 1997). "Unifloral honey is made up of nectar from a single species of flower." Depending on the type of flower from which the nectar is collected, they have a particular flavour and colour."

Despite their small body size, *Heterotrigona itama* and *Geniotrigona thoracica* will collect a wide variety of nectar and pollen while foraging (Samsudin et.., 2018). The difference in floral sources as well as importance of environmental which result in different varieties of honey each with their own distinct flavour, which can be used to distinguish them from each other. Current interest about the sources of bee products, as well as economic advantages in evaluating their quality, stimulated research activities that used pollen grain morphology as an investigative method. However, a few Melissopalynological studies, especially on stingless bee species, have been documented in Malaysia (Mahani, 2020)

According to Agashe and Caulton (2009), the morphological features of pollen can be researched more thoroughly if a correct technique is used. There are a variety of approaches to employ, and must choose intelligently in order to achieve study goals. Pollen analysis is divided into two types: quantitative (which refers to pollen count when observed under a microscope) and qualitative (refer to the types of pollen present in honey samples). Pollen count can be different categorized of classes which are predominant pollen (> 40%), secondary pollen (16% - 40%), important minor pollen (3% - 16%) and minor pollen (<3%) (Paulino et.al, 2010).

If a honey sample contains only one main pollen type, it is classified as unifloral, and it often contains pollen from a single species or genus taxonomy. By dividing the number of samples by the total number of honey samples, the value may be calculated. Pollen study demonstrated the botanical content across the pollen spectrum diversity in terms of multifloral honeys, while unifloral honey can be classified by its own physico-chemical properties, according to Selvaraju et al. (2019).

2.3 Identification of pollen

Classifying pollen returning to the hive offers a clear indicator of pollen harvesting, while pollen contained in honey provides a longer-term analysis of plants used for both nectar and pollen (Philip, 2019). These techniques are often used to determine the horticultural composition of honey in order to verify its geographic origin for food consistency and traceability. They have been used even less often to examine foraging preferences. Before honey can be commercialised, the major honey-producing countries need correct labelling, including floral recognition, which has historically been accomplished by Melissopalynological.

The presence of pollen grains in honey can be attributed to either the production of pollen grains in flower nectar or to environmental factors (Philip, 2019). Honey pollen profiles represent forest vegetation, floral diversity, and plant species distribution harvested by honey bees. The relative pollen abundance is used for labelling and ensuring regional origin, both of which have a significant impact on honey's market importance. It is also used as a record keeping tool by food safety organizations. When honey is unifloral, it comes from an entirely or partly botanical origin, including pollen, physicochemical, and sensory features that are unique to that source. As a result, the three fundamental systems are complementary honey characterisation tests (Phillip, 2019).

Pollen in honey tells us which flowers the bees visited for nectar, while pollen in corbiculae tells us which flowers pollen was collected from. As a result, pollen can be used as a signpost to the bee's food sources. It is frequently possible to determine which plants are of big relevance and which are of little importance based on the relative abundance of each pollen type. Management measures can be implemented to preserve the colony's strength and maximise honey yields based on a thorough understanding of which plants give nectar, pollen, or both, and their relative value (Ruth & Mohammaed ,1991). Pollen, like fingerprints, can be used to identify plants because it is often unique to a group of plants. By combining the size, shape, and surface pattern of pollen, most plants can be recognised to genus, and some can be identified to species. However, the distinctions between species and genera are so minor that only a family level identification can be made with assurance. According to the pollen atlas, pollen types are classified by size, shape (round, ovoid, or triangular), and surface pattern (smooth, spiny, net-like, or with holes and other features) (Ruth & Mohammaed ,1991).

2.4 The melissopalynology analysis

Melissopalynogical experiments are the recognition and quantitative analysis of pollen in honey. Apart from physicochemical analysis and polyphenol or sensory analysis, it is one of the most effective methods of identifying the Botanical sources of honey. Melissopalynological is another word for pollen analysis (Phillip, 2019). These experiments are quantitative and qualitative microscopic determinations of pollen existing in honey, which aids in identifying floral or botanical sources, which is required for honey standardisation, geological origin of honey, and honeybee foraging biodiversity. Pollen analysis will also help determine whether honey has been polluted with toxic pollen or whether it is mislabelled (Phillip, 2019). These studies support in distinguishing multifloral honeys from unifloral honeys or particular types of honey samples of high commercial value. Furthermore, the pollen samples discovered will include details about the flowering plants used by bees in the study field. Beekeepers must be knowledgeable about the botanical sources of honey in order to increase pollination (Izzah, 2019).

According to researchers, floral honey always contains a large amount of pollen grains, primarily from the plant species foraged by honey bees. Melissopalynological, or honey pollen analysis, is very useful for determining and controlling both botanical and geographical origin. The entire pollen spectrum must be consistent with the flora of a certain place, as well as any reference spectra or descriptions in the literature, to determine geographical origin (Aronne et al., 2010).

2.5 The pollen count and determination

Pollen grains are technically the tiny male reproductive parts of plants that fertilise the female portions. Insects, water, wind, and gravity transport the microscopic particles from plant to plant. The number of pollen grains that fall on a certain region over a given time period (Charles, 2019). A rotating rod that flies through the air at regular intervals takes the count. The pollen grains that adhere are dyed and analysed under a microscope. To calculate the frequency of each pollen type, the number of pollen grains of each type that may be detected in a microscopic field in each honey collection sample will be counted. This means that if a person is especially vulnerable to pollen, even a low count of 15 to 20 grammes per cubic metre can be depressing. Pollen counts of 50 or fewer, on the other. hand, are generally considered poor. A pollen count of 1,000 or more is expected to result in increased (Charles, 2019).

2.6 Statistical analysis of pollen honey

Statistical analyses of honey samples determining the types of honey in the sample collected. By using the completely randomized design (CRD) method for analysing data in identifying the pollen types in honey sample. Then, the percentage of abundance of every species calculated in the sample using the equation.

The simplest design for comparison trials is a completely randomised design (CRD), which employs only two basic principles of experimental design: randomization and replication. Its potency is best understood in the context of agricultural trials (for which it was originally conceived), and it will be explained from that standpoint, but real experimental designs are valuable in the social sciences and in medical experiments, where possible. Any difference between experimental units receiving the same treatment is regarded as experimental error by the CRD. As a result, CRD is only suitable for investigations involving homogenous experimental units, such as laboratory experiments, where environmental effects are easily controlled. The CRD is rarely employed in field experiments since there is usually a lot of variances in environmental parameters between experimental plots (Sofiev, & Bergmann, 2012).

2.7 Antioxidant properties of honey

The difficulty of stingless bee honey composition is the significant obstacle in honey science, so profiling and identifying honey produced in different regions is critical. As a result, honey from common sources sparked a lot of interest due to their unique phenolic profile similar to botany roots (Mahani, 2020). Honey's antioxidants capacity is determined not only by the presence of total phenolic compounds, but also by the presence of flavonoids, which play a vital role in minimising oxidative damage. Honey's physiological and medicinal effects are enhanced by the presence of phenolics and flavonoids. Previous research has found a strong connection between botanical roots and phenolic compounds like phenolics and flavonoids (Mahani, 2020).

Despite the fact that stingless bee honey has a stronger composition than Apis spp. honey, there have been few experiments on the biological function of stingless bee honey. Thus, research into the therapeutic benefits of honey samples bee is needed to combat a variety of diseases, as there have been few studies in Southeast Asia, including Malaysia (Mahani, 2020).

2.8 Physiochemical properties of honey

Honey's physicochemical characteristics are critical parameters to understand. The colour of honey, for example, influences its price, its moisture aids in structural analysis, and its surface tension is needed for the design of processing equipment. These characteristics are often used to assess the consistency of pollen (Khan, 2014). Honey contains carbohydrates like monosaccharides, as well as minerals like magnesium, potassium, calcium, sodium chlorine, sulphur, copper, and phosphate. Honey contains nutrients B1, B2, C, B6, B5, and B3 focusing on the consistency of the nectar and pollen. Honey's pH is usually among 3.2 and 4.5. This comparatively acidic pH level inhibits bacterial growth. Honey is largely composed of two glucose and fructose. The water activity of this mixture is minimal.

The majority of the water molecules are connected with the sugars, and only a few continue to be available for microorganisms to develop in, making it an unfavourable environment for their development. When water is combined with honey, the honey loses its low water content and hence loses its antibacterial activity (Khan, 2014). The physicochemical properties of honey are used as criteria in verification tests, such as determining its botanical and geographic sources and detecting unapproved compounds. Scientists discovered that honey has potent anti-bacterial effects on at least sixty different types of bacteria, unlike antibodies, which are often ineffective against some kinds of microorganisms (Khan, 2014).

2.9 Applications of honey

Honey is a healthy source of natural antioxidants, which help to lower the risk of heart disease, cancer, immune system decline, cataracts, and other chronic inflammation. The natural antioxidants of stingless bee honey piqued the attention of several researchers, who discovered that stingless bee honey has strong antioxidant ability. (Siok, 2018). Honey is used for more than just sweetening and flavouring. It is also eaten as food and beverage, and it is used as an ingredient in food, cosmetics, and wellness items. A wide range of antioxidant properties has been identified based on measurements of phenolic compounds, flavonoids, 1,1-diphenyl-2-picryhydrazyl (DPPH) scavenging action, ferric reducing antioxidant strength (FRAP), 2,2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical activity, -carotene bleaching activity, and oxygen radical absorbance ability (ORAC) (Siok, 2018).

The natural antioxidants of stingless bee honey piqued the attention of several researchers, who discovered that stingless bee honey has high antioxidant ability. Because of the high market demand of honey for its medicinal properties, it has become a target for honey fraud, such as replacement of low-valued honey and other sugars as food additives or mislabelling of its source and origin to achieve a higher retail value (Siok, 2018).

CHAPTER 3

MATERIALS AND METHODS

3.1 Collection of Honey Samples

Honey samples collected from the different regions in Perlis. 10 honey samples with minimum 50 grams each sample collected in different mukim of Perlis. The samples collected were bought from seller and some were collected from beehives. The locations of honey samples were from Titi Tinggi, Beseri, Chuping, Padang Pauh, Guar Sanji, Sanglang, Tambun Tulang, Sena, Kayang, and Bintong.

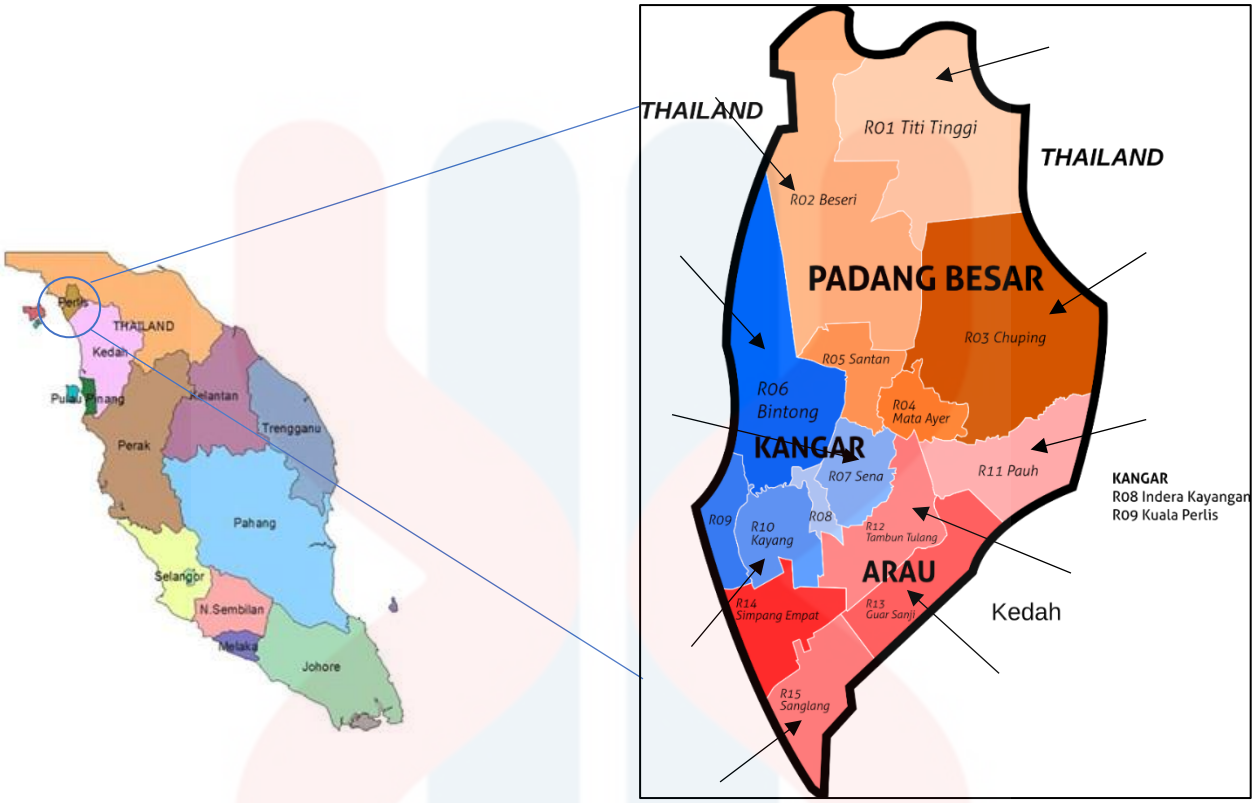

Figure 3.1 shown the map of Malaysia and map of Perlis for the locations of honey sample.collected

(Source: Google Map)

3.2 Materials

3.2.1 Honey samples

Honey samples were collected from different regions of Perlis in Titi Tinggi, Beseri, Chuping, Pauh, Guar Sanji, Sanglang, Tambun Tulang, Sena, Kayang, Bintong. These samples were brought to the chemistry and biology laboratory, Faculty of Agrobased Industry, UMK Jeli and examined for the presence of different types of pollen.

Figure 3.2: shown honey sample 1

Location: Titi Tinggi

Collection date: 20/9/2021

Colour of honey: Dark brown

Figure 3.3: shown honey sample 2

Location: Beseri

Collection date: 20/9/2021

Colour of honey: Dark brown

Figure 3.4: shown honey sample 3

Location: Chuping

Collection date: 4/10/2021

Colour of honey: Brown

Figure3.5: shown honey sample 4

Location: Pauh

Collection date: 12/10/2021

Colour of honey: Brown

Figure 3.6: shown honey sample 5

Location: Guar Sanji

Collection date: 4/9/2021

Colour of honey: Light brown

Figure 3.7: shown honey sample 6

Location: Sanglang

Collection date:23/9/2021

Colour of honey: Light brown

Figure 3.8: shown honey sample 7

Location: Tambun Tulang

Collection date: 10/9/2021

Bee species:unknown

Colour of honey: Brown

Figure 3.9: shown honey sample 8

Location: Sena

Collection date: 13/9/2021

Bee species: *Heterotrigona Itama*

Colour of honey: Light brown

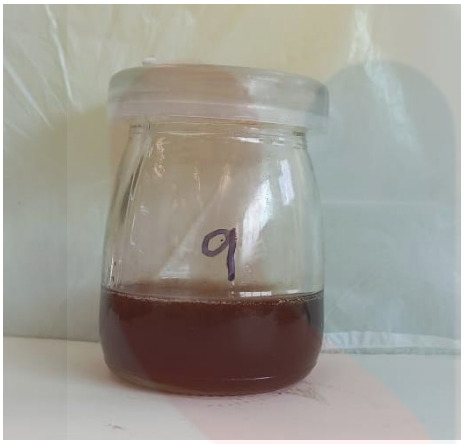

Figure 3.10: shown honey sample 9

Location: Kayang

Collection date: 6/9/2021

Bee species: unknown

Colour of honey: Brown

Figure 3.11: shown honey sample 10

Location: Bintong

Collection date: 6/9/2021

Bee species: *Heterotrigona Itama*

Colour of honey: Light brown

3.2.2 Chemicals and apparatus

Table 3.1. List of chemicals for Acetolysis process

Chemicals	Amount used
Sulphuric acid	5 mL
Acetic anhydride	45 mL
Acetic acid	10 mL
Alcohol 70%	100 mL
Acetolysis mixture	5 mL

Table 3.2 List of chemicals for preparation of Glycerine Jelly

Chemicals	Amount used
Gelatine powder	25g
Distilled water	87.5 mL
Glycerine	75 mL
Safranin powder	0.1g
Phenol crystals	3.5g

Table 3.3 List of apparatus and quantity

Apparatus

1. Compound microscope with camera Leica DM 2500	1 set
2. Hot plate	1 set
3. Water bath	1 set
4. Micropipette 100µL-100µL	1 set
5. Laboratory Centrifuge 5810R	1 set
6. Laboratory Centrifuge 5415R	1 set
7. Microscopic slides	40 pieces
8. Cover slips	40 pieces
9. Polystyrene foam board	1 piece
10. Microcentrifuge tube 2mL	10 pieces
11. Petri dish	10 pieces
12. Falcon tube 15mL	10 pieces
13. Centrifuge tube adapter 15mL	6 pieces
14. Falcon tube stand	1 piece
15. Micro spatula	1 piece
16. Glass rod	1 piece

17. Aluminium foil	1 piece
18. Clear Glass bottle 100mL	1 bottle
19. Clear Reagent bottle 50mL	2 bottles
20. Dark Reagent bottle 50mL	2 bottles
21. Beaker 50mL	2 containers
22. Beaker 100mL	2 containers
23. Beaker 250mL	1 container
24. Measuring cylinder 10mL	1 container
25. Measuring cylinder 100mL	1 container
26. Muslin cloth	1 roll
27. 100µL-1000µL Blue tip	1 box

3.3 Methods

3.3.1 Preparation of Glycerine Jelly

25 g of gelatine powder was dissolved in 87.5 mL of distilled water. Heating and continuous stirring was done till the mixture became homogeneous and boiled. 75 mL of glycerine was added together with the boiling mixture and was stirred using a glass rod continuously. Then, 0.1 g of safranin powder was added to the mixture. The addition of safranin was to stain the jelly into pinkish colour so that it facilitated the process of observing pollen under the microscope. The mixture was allowed to boil and stirring had been continued. 3.5 g of phenol crystals were added with continuous stirring. Addition of phenol crystals was done in a fume hood as phenol crystals are hazardous when they come into contact with the skin, eyes or even when inhaled. The mixture was stirred continuously. When the jelly started turning into transparent mixture, boiling was stopped and it had been removed from the hot plate. The hot glycerine jelly was filtered using a muslin cloth and poured into petri dish where it was left to solidify. Solidification of the jelly was further enhanced by storing the petri dish into refrigerator. The glycerine jelly prepared was to be used in order to mount the pollen material for observation of pollen morphology under compound microscope. Normally, Kisser's method of glycerine jelly preparation was followed by palynologist in the studies of pollen (Agashe & Caulton, 2009)

3.3.2 Preparation of acetolysis mixture

The preparation of the acetolysis mixture were done in the fume board. The Acetolysis mixture was prepared by dissolving 5 mL of sulphuric acid in 45 mL of acetic anhydride. 45mL of acetic anhydride were poured in the dark reagent bottle. Then, 5mL of sulphuric acid were added into the same bottle slowly. The bottle of the mixture was close and open while adding the sulphuric acid. The bottle was gently swirled to mix the acid. This mixture was the first-class acid therefore, following the precaution steps were applied. Lastly, the bottle was labelled with the warning sign to avoid any danger (Selvaraju et al., 2019).

3.3.3 Pollen acetolysis

Acetolysis refers to the acidic hydrolysis / acetylation of pollen grain for removing the protoplasmic contents in it leaving only pollen with its exine layer. In the studies of palynology, the secondary effect of Acetolysis involved is the darkening of the pollen grain so that it can be viewed with good transparency under the microscope (Agashe & Caulton, 2009). In this experiment, Acetolysis method as suggested by Erdtman (1960) is applied.

In this experiment, 5mL of honey samples was measured cautiously and mixed with 10mL of 70% ethanol in 15mL of falcon tube. The mixture was agitated manually by using vortex to form the homogenous mixture of the honey sample and the ethanol. The falcon tube the placed into the centrifuge adapter and then placed in the centrifuge machine. The mixture was centrifuged at 5000 r.p.m, at room temperature 24 C for 10 minutes. After 10 minutes, a clear of separation of the supernatant and pellet were obtained. The supernatant was discarder while the pellet was retained. In the meantime, water bath was switched on to preheat until 80 C. 0.5mL of glacial acetic acid were added by using micropipette to the pellet for acidic hydrolysis process occurred so that the protoplasmic content of the pollen grains was removed. The residue was transferred into the 2mL of microcentrifuge tube by using micropipette. The residue was mix agitatedly by using vortex. Next, centrifugation process was repeat for the mixture at 5000 r.p.m at 24 C for 10 minutes and after the centrifuge the supernatant were removed and pellet retained in the tube. Following that, 0.5mL of acetolysis mixture were added by using micropipette into the residue and the pellet were slowly tap to mix with the acetolysis mixture. Next, the mixture was attached to the polystyrene as floating bath and heated into the water bath for 20 minutes to enable the protoplasmic digestion. After 20 minutes in water bath, the mixture was centrifuged again at 5000 r.p.m at 24 C for 10 minutes. Then, the supernatant was removed the residue retained. The residue which contains the pollen material was washed with 1mL of glacial acetic acid by using micropipette and centrifuged again. After centrifuge process, the supernatant was removed and the pollen material remained.

Lastly, small amount of glycerine jelly was put in the pollen material by using micro spatula and was preheat for a few minutes to melt the glycerine jelly in the microcentrifuge tube. The pollen material with glycerine jelly was transfer onto the slides for further observation. If the jelly solidifies, it is better to gently heat it on the hot plate so that it will spread evenly throughout the glass slides. The pollen pellet which had been mounted into glycerine jelly placed onto the glass slide was covered with a cover slip. In this method, the identification of pollen can be observed and the pollen count can be done under microscope with camera. (Selvaraju et al., 2019).

3.12 Flowchart for the pollen acetolysis method

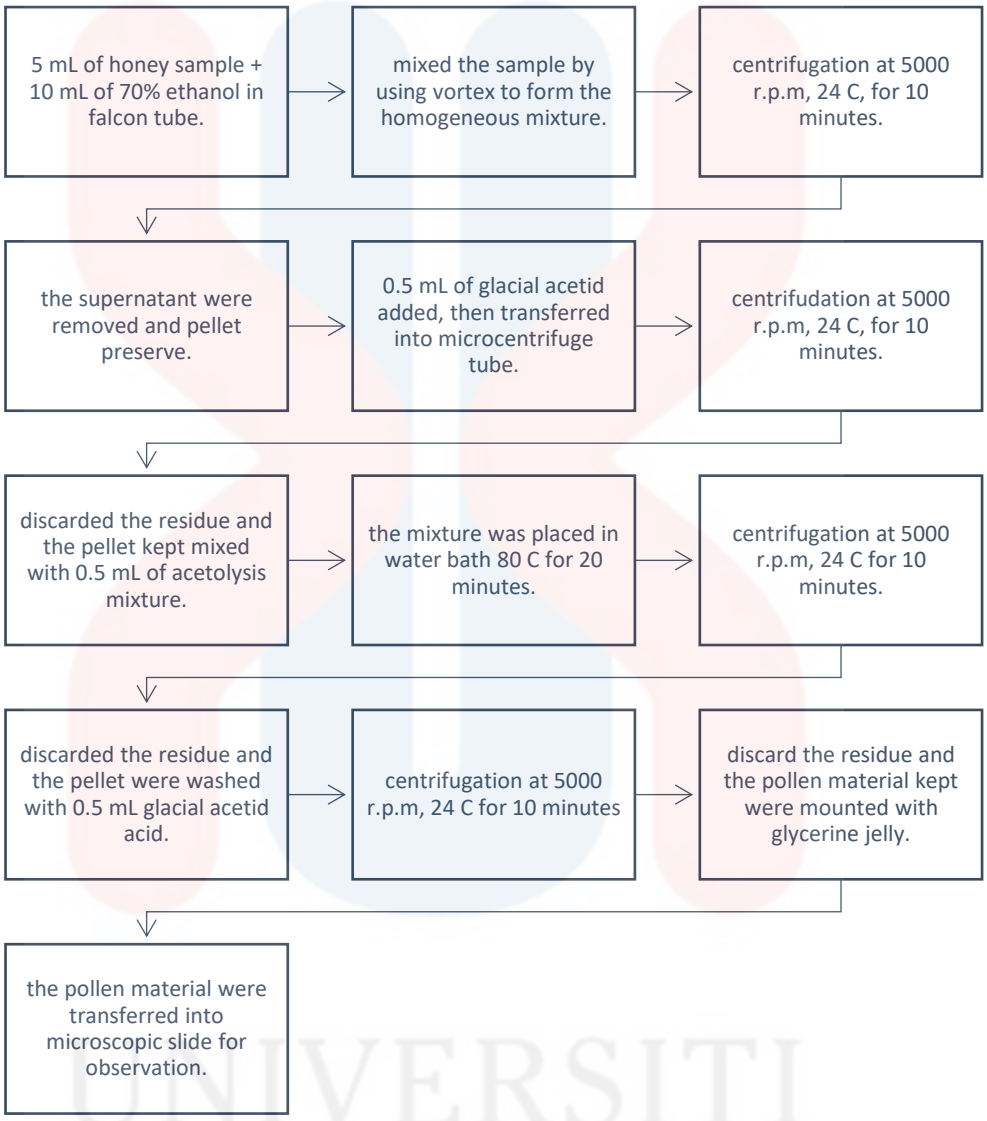

Figure 3.12 the flowchart of pollen acetolysis

3.3.4 Pollen Count

Pollen counts will be performed using microscopic examination in order to establish pollen forms and densities (Ohe et al., 2014). This approach detects and counts pollens in groups of 100 using five parallel uniform equidistant lines drawn from one side of the cover slip to the other. If the relative frequencies are not stabilized, or if 500 pollen grains are insufficient for interpretation, (complex spectrum, over-represented pollen, abundant pollen of nectarless plants or other conditions that can mask the actual nectar source of the honey), then the pollen count should be continued to 1 000 according to another 5 parallel lines located between the first 5 (Selvaraju et al., 2019). To obtain the overall pollen count per slide and proceed with the experiment, the following equation (Eq. 1). In this experiment, the recommendation for a square coverslip = 50 views per slide.

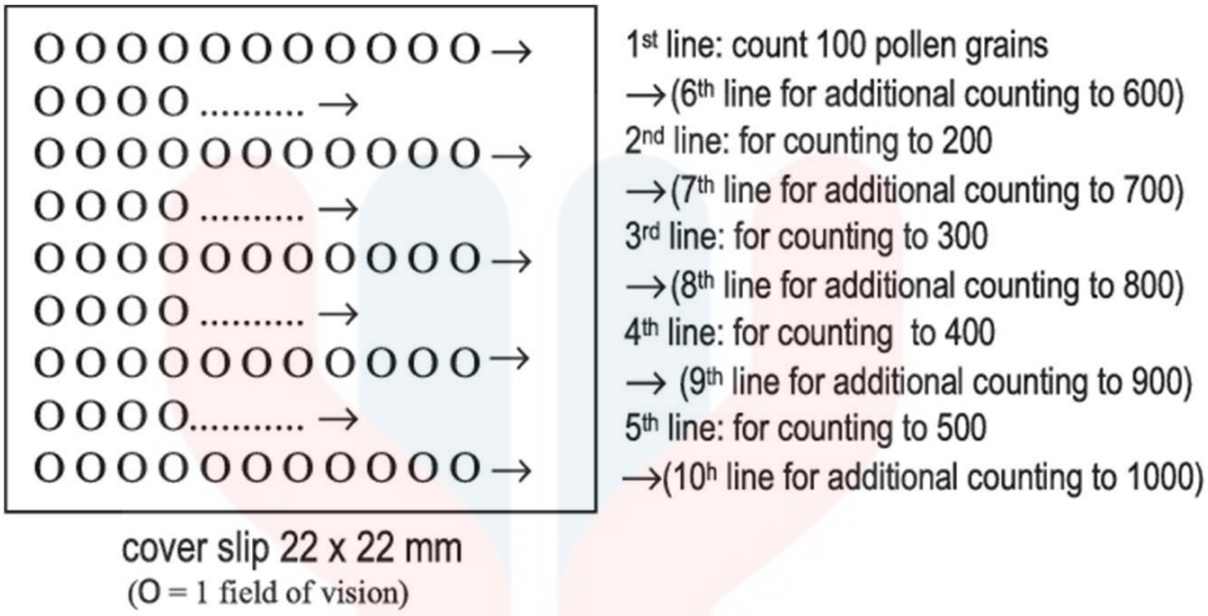

Figure 3.13 Matrix for counting the pollen grains (O = a whole microscopic field of view), Source: (Ohe et al., 2019)

Equation 1:

Percentage of abundance, %

$$= \frac{\text{Total number of pollens of a particular species}}{\text{Total number of observed pollens}} \times 100$$

3.3.5 Experimental Design

In the experiment, completely randomized design (CRD) was used to analyse data on pollen after calculating the percentage of abundance of pollen besides identifying pollen types. The tables and charts in result were accomplished using the Microsoft Excel Data Sheet.

3.4 Sample analysis

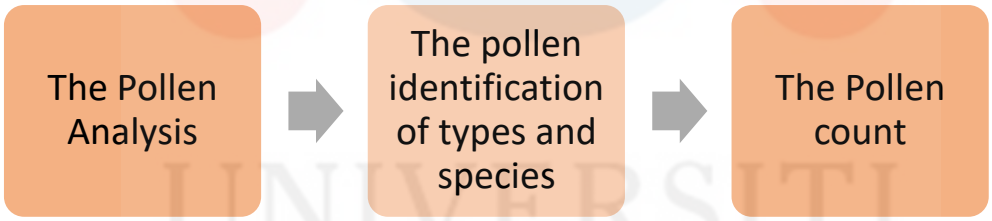

Figure 3.14 Flow chart of Sample analysis

CHAPTER 4

RESULT AND DISCUSSION

4.1 Pollen Analysis of Overall Honey Samples

Pollen analysis and pollen identification had been done for 10 honey samples collected from 10 different locations in Perlis such as Titi Tinggi, Beseri, Chuping, Padang Pauh, Guar Sanji, Sanglang, Tambun Tulang, Sena, Kayang and Bintong. Pollen analysis was done in the laboratory for honey samples by using the glacial acetic acid for centrifugation and proceed to acetolysis mixture which immersed in water bath for 20 minutes at 80-degree Celsius. After that, the pollens were added the glycerine jelly to stain the pollen and easy to clearly seen the pollens under the microscope. Then, pollen counting and identification was done by using Leica DM 2500 microscope under magnification of 10x for counting and magnification 40x for identification respectively for every honey sample.

Lastly, the method for counting the pollen in every slide were following 5 parallel equidistant lines uniformly distributed from one edge of the cover slip to the other, until 500 grains are counted and continue the count to 1000 following another 5 parallel lines situated between the first 5 (Ohe et al., 2004). Lastly, the pollen was counted for every species of pollen found in each of honey samples.

Table 4.1 shown that 3 of honey samples were unifloral while the other 7 were multifloral. The unifloral honey from Guar Sanji, Sanglang and Bintong. The multifloral honey from Titi Tinggi, Beseri, Chuping, Padang Pauh, Tambun Tulang, Sena, and Kayang. According to Figure 4.1, the honey samples collected from Tambun Tulang has highest number of pollens which was 1860. Padang Pauh had the least number of pollens, which was 132 compared to other honey samples collected. To distinguish either types of honey multifloral or unifloral, the species in each sample must exceed 40% of percentage abundance to clarify as unifloral honey. If not, the sample labelled as multifloral honey.

Table 4.1: Total number of pollens within their areas of origin and types of honey

Location	**Total number of pollens**	**Types of honey**
Titi Tinggi	628	multifloral
Beseri	359	multifloral
Chuping	152	multifloral
Padang Pauh	132	multifloral
Guar Sanji	778	unifloral
Sanglang	963	unifloral
Tambun Tulang	1860	multifloral
Sena	1474	multifloral
Kayang	271	multifloral
Bintong	179	unifloral

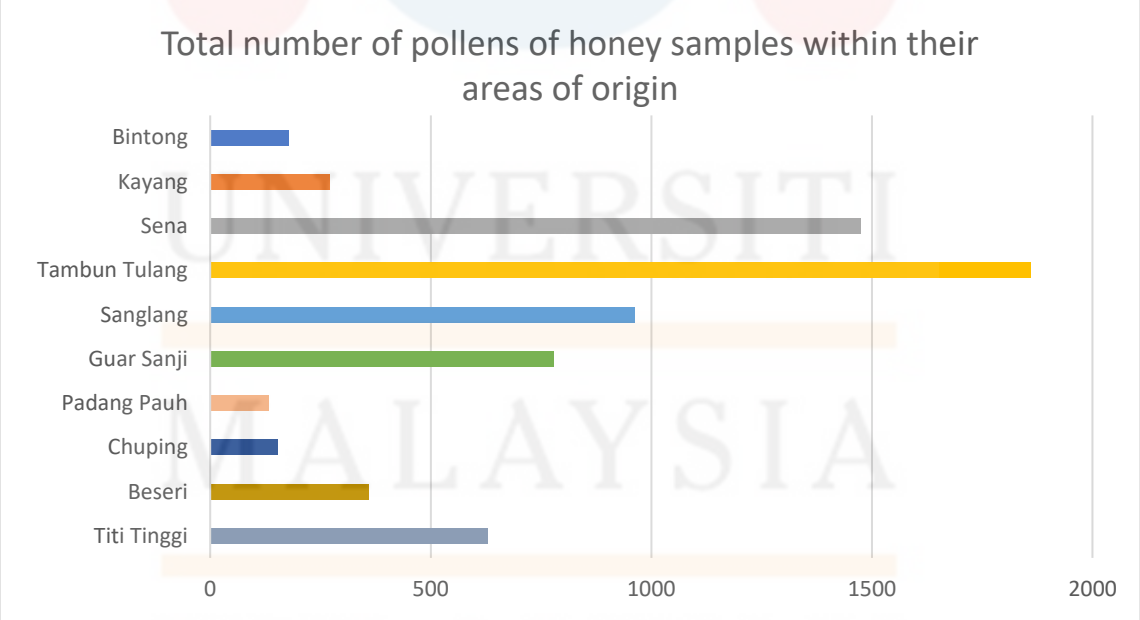

Figure 4.1: Total number of pollen honey samples within their areas of origin

Table 4.2 showed that the both *Gliricidia sepium* and *Capsicum fructescenes* shared the highest abundance of pollen which were 11.46% in the honey sample from Titi Tinggi. The least abundance of pollen was *Ptychosperma macarthurii* from Asteraceae family which got only 0.48% of pollen abundance. Types of honey present in sample from Titi Tinggi was multifloral due to none of them getting more than 40% pollen abundance. Apparently, different in Table 4.3, species *Gliricidia sepium* and *Capsicum fructescenes* did not present in sample from Beseri. The highest pollen was *Sesbania grandiflora* from Fabacae family with percentage abundance of 18.66%. the least number was belonged to *Bruiguiera gymnorrhiza* with 0.84% of pollen abundance. Types of honey in the sample from Beseri was multifloral due to none of the pollen exceed 40% of the percentage pollen abundance. As for Table 4.4, shown that the honey sample from Chuping most abundant with species *Artocarpus heterophyllus* with 23.03% and *Benincasa hispida* shown rare occurrence with 1.32% of pollen abundance. Types of honey in sample from Chuping was multifloral honey because the percentage of species did not exceed 40%.

Table 4.5 stated that the sample from Padang Pauh has species *Veptorium odoratum* from asteracea family as the highest percentage of pollen abundance of 25.76%. *Benincasa hispida* has the lowest percentage of 2.27% in the pollen abundance. Types of honey found in Padang Pauh sample was multifloral. The similarity between sample 3 and sample 4 that both have the least abundance of pollen were species *Benincasa hispida* from Cucurbitaceae family.

Based on the observation in all sample, *Combretum indica* and *Antigonan leptopus* were both the favourite pollens found in the many of the collected sample. It can be said that these species usually planted in the location of the beehives. These species were the favourite for bees to collect their honey from these flowers. Table 4.6 depicted that the sample from Guar Sanji has *Mutingia calabura* was the plant species that predominant with percentage abundance of 43.70%. The lowest abundance percentage was *Mimusops elangi* as the percentage was 2.27%. Types of honey in this sample was unifloral honey because there was present of species that more than 40% as clarified. In table 4.7, Combretum indica was the most abundance present in the sample from Sanglang with 15.48% while Bougainvillea sp. was the least abundance pollen present in the sample with 0.21%. The highest and the lowest percentage of pollen abundance in all sample was from sample in Sanglang. Types of honey from Sanglang sample was unifloral due to there was a species exceed 40% of percentage abundance.

Table 4.8 revealed there was none predominant species in sample from Tambun Tulang and the most abundance species was *Antigonan leptopus* with 15.48%. The least abundance of pollen was *Bougainvillea sp.* with percentage of 1.61%. Table of 4.9 shown that there is no predominant for Sena and therefore the sample selected as multifloral honey. The highest percentage of abundance species was *Antigonan leptopus* with percentage of 14.25%. Meanwhile, the lowest percentage abundance was *Amaranthus lividus* with 2.17%.

Based on the Table 4.10, the highest abundance of pollen found in the sample from Kayang was *Acacia auriculiformis* with percentage of 16.61% and the lowest abundance of pollen found was *Ananas cosmosus* with percentage of 1.48%. for the last sample in table 4.11 which was from Bintong, the highest percentage of pollen was species from *Antigonan leptopus* with abundance percentage 41.90%. the least abundance pollen in this sample was *Bruiguiera cylindra* with abundance of 1.12%. Thus, there was a species from this sample that more than 40% of percentage abundance and the types of honey in this sample was unifloral.

4.2 Honey samples from Titi Tinggi, Perlis

Table 4.2: The number of pollen and percentage of abundance of pollen types in the honey samples from Titi Tinggi.

Plant species	Family	No. of pollen	Percentage of abundance (%)
Gliricidia sepium	Fabaceae	72	11.46%
Nerium indicum	Apocynaceae	24	3.82%
Hevea brasiliensis	Euphorbiaceae	17	2.71%
Muntingia calabura	Muntingiaceae	16	2.55%
Amischotolype griffithii	Commelinaceae	20	3.18%
Ischaemum muticum	Poaceae	4	0.64%
Citrus aurantifolium	Rutaceae	8	1.27%
Moringa pterygosperma	Moringaceae	16	2.55%
Ipomea pes-caprae	Convolvulaceae	4	0.64%
Cyperus bevifolius	Cyperaceae	8	1.27%
Asystasia gangetica	Acanthaceae	7	1.11%
Veitchia merrillii	Arecaceae	60	9.55%
Manihot esculenta	Euphorbiaceae	12	1.91%
Melastoma malabathricum	Melastomataceae	28	4.46%
Ptychosperma macarthurii	Arecaceae	3	0.48%

Lycopersicon esculentum	Solanaceae	16	2.55%
Solanum torvum	Solanaceae	44	7.01%
Capsicum fructescenes	Solanaceae	72	11.46%
Cascabela thevetia	Apocynaceae	4	0.64%
Asystasia intrusa	Acanthaceae	16	2.55%
Jacaranda 0078obtusifolia	Bignoniaceae	20	3.18%
Anacardium occidentale	Anacardiaceae	24	3.82%
Neomarica longifolia	Iridaceae	31	4.94%
Zea mays	Poaceae	17	2.71%
Artocarpus heterophyllus	Moraceae	35	5.57%
Tecomaria capensis	Bignoniaceae	50	7.96%
TOTAL NUMBER OF POLLEN		628	

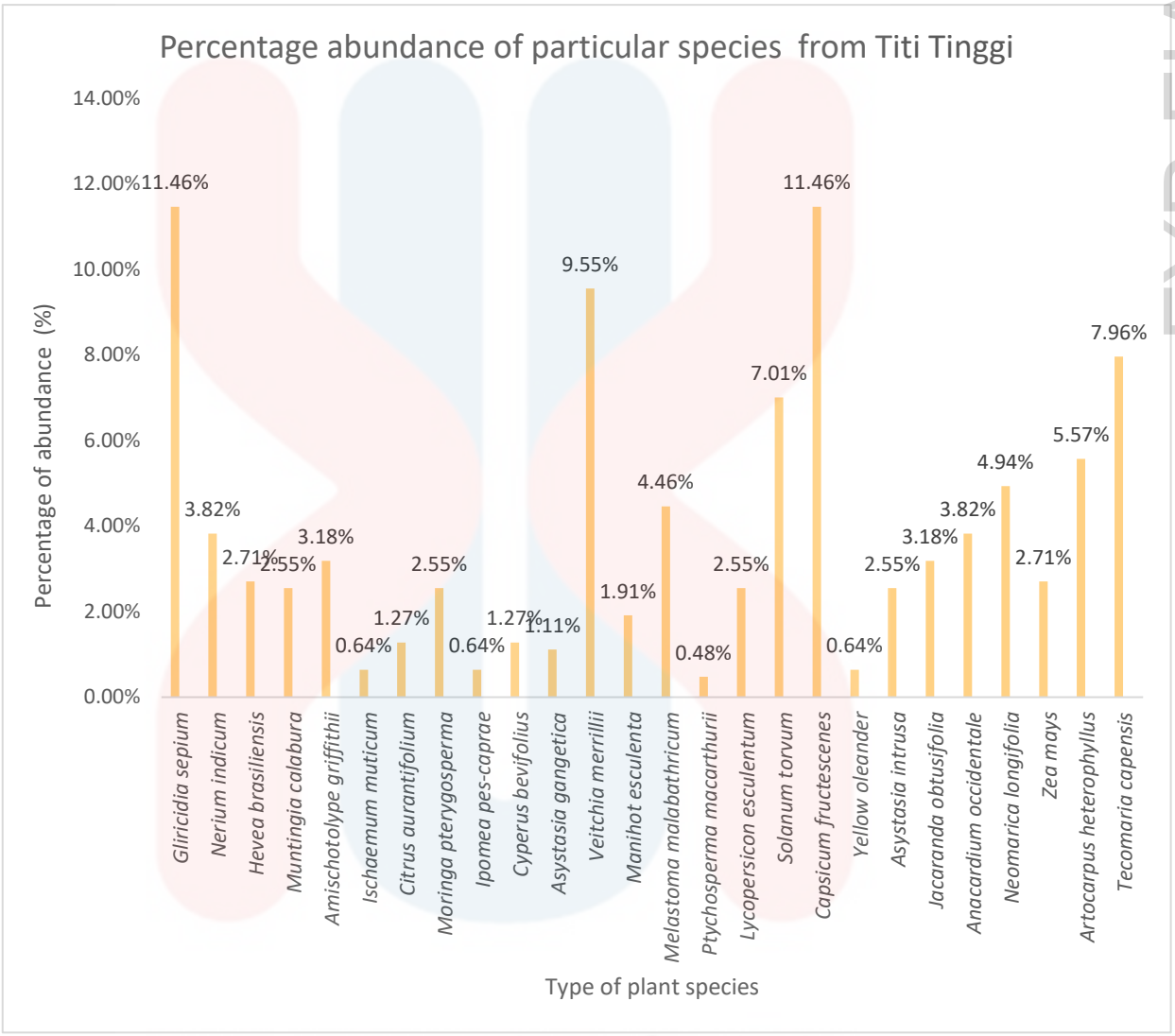

Figure 4.2: Percentage abundance of particular species from Titi Tinggi

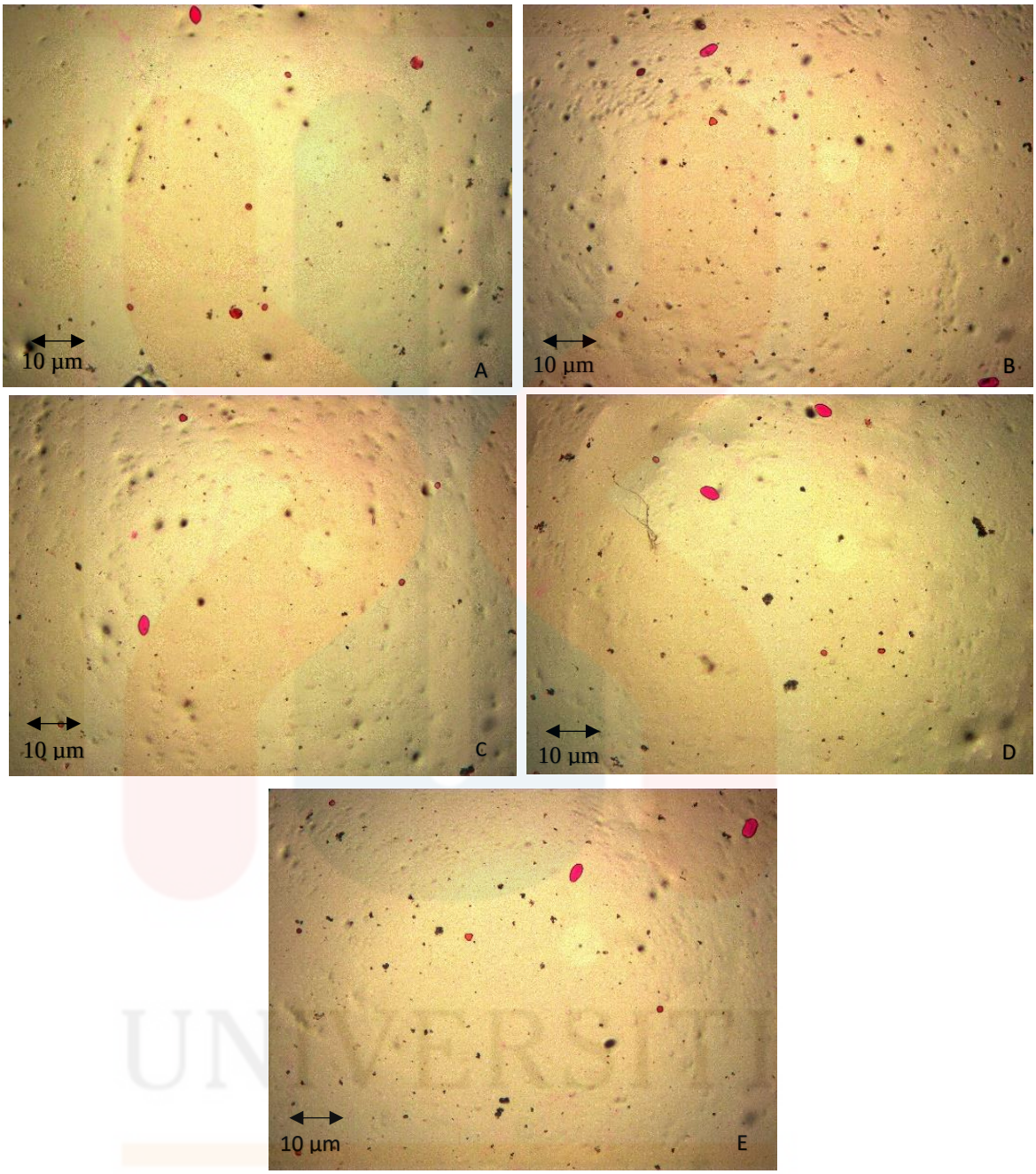

Figure 4.2 (a): Microscopic overviews of pollen density of honey sample from Titi Tinggi under 10x magnification. Overviews of A- left edge of cover slip (top); B- right edge of cover slip (top); C- left edge of the cover slip (bottom); D- right edge of the cover slip (bottom); E-middle of the cover slip

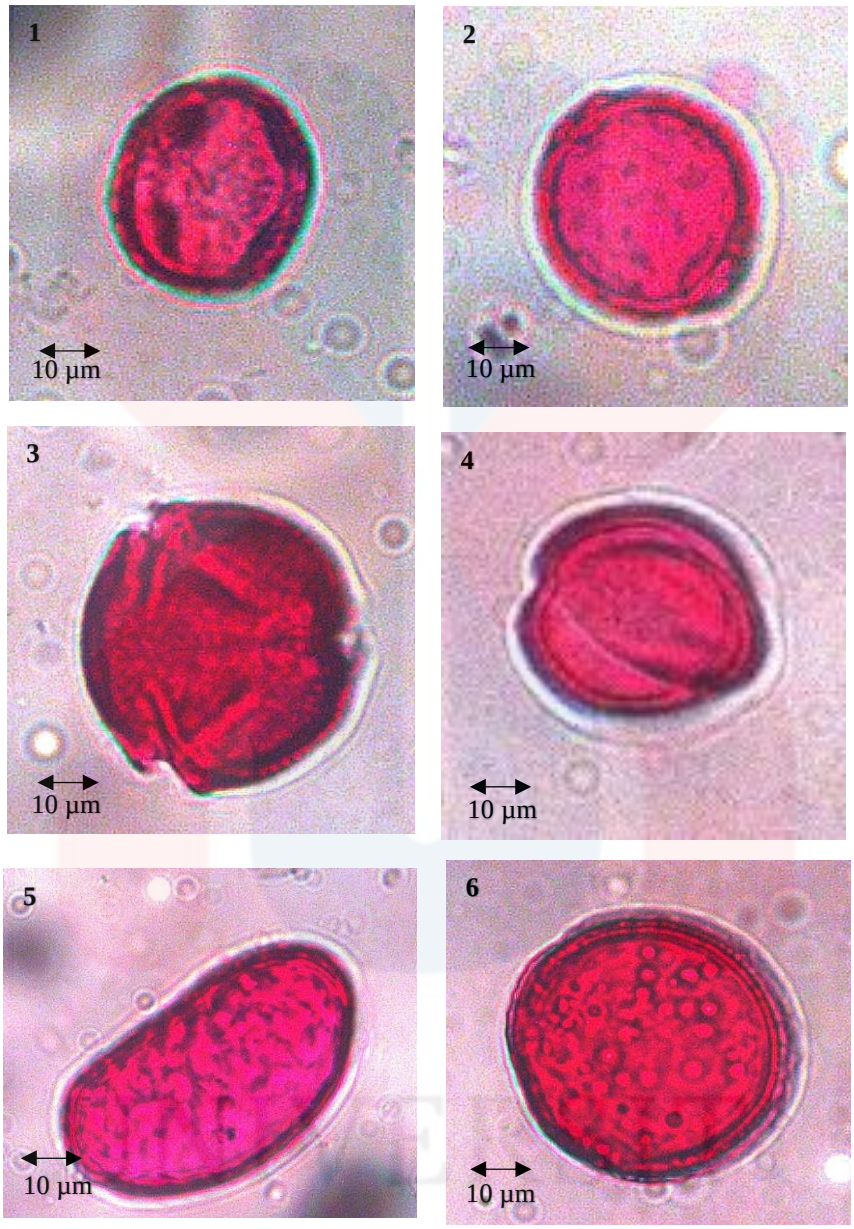

Figure 4.2 (b): The morphology of different pollen honey sample from Titi Tinggi under 40x magnification. 1- *Gliricidia sepium* (Fabaceae); 2- *Nerium indicum* (Apocynaceae); 3- *Hevea brasiliensis* (Euphorbiaceae); 4- *Mutingia calabura* (Mutingiaceae); 5- *Amischolotype griffithii* (Commelinaceae); 6- *Iscaemum muticum* (Poaceae)

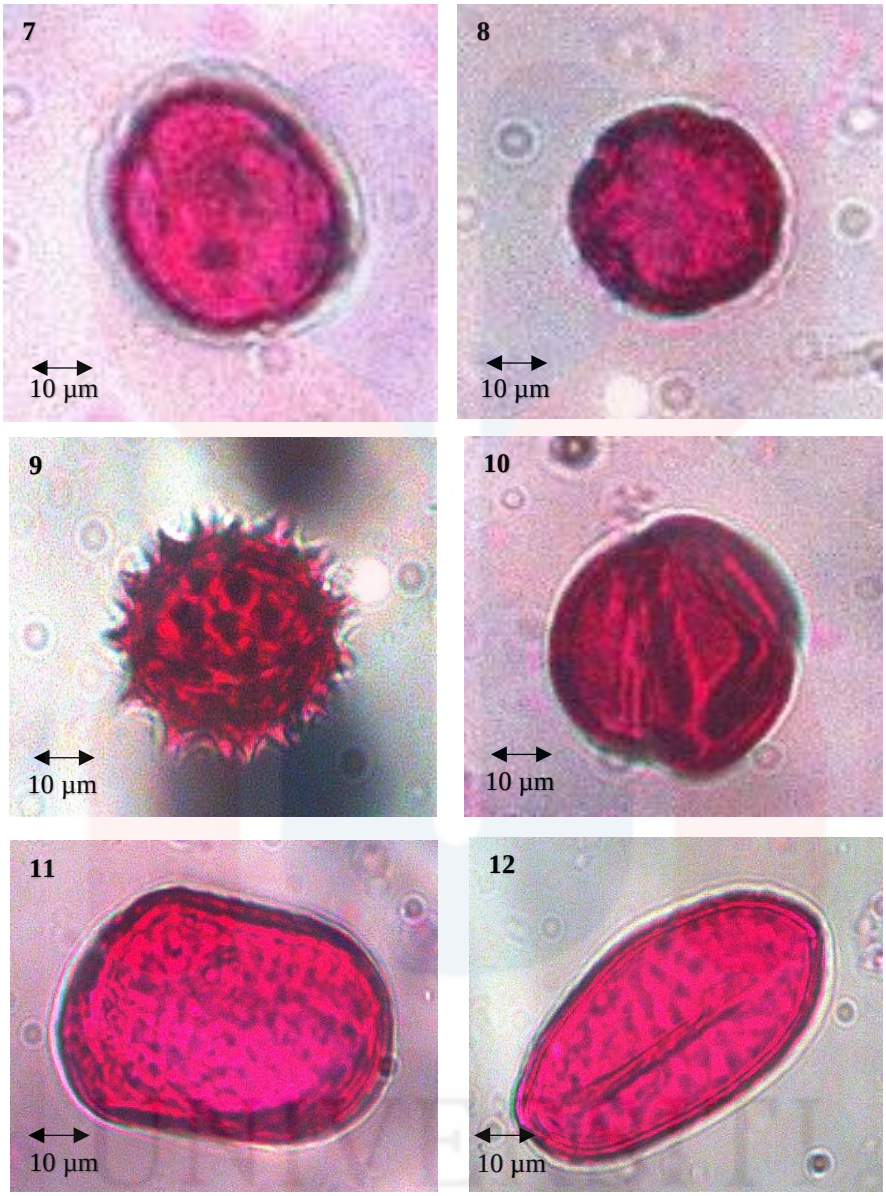

Figure 4.2 (c): The morphology of different pollen honey sample from Titi Tinggi under 40x magnification. 7- *Citrus aurantifolium* (Rutaceae); 8- *Moringa pterygosperma* (Moringaceae); 9- *Ipomea pes-caprae* (Convolvulaceae); 10- *Cyperus brevifolius* (Cyperaceae); 11- *Asystasia gangetica* (Acanthaceae); 12- *Veitchia merrillii* (Arecaceae)

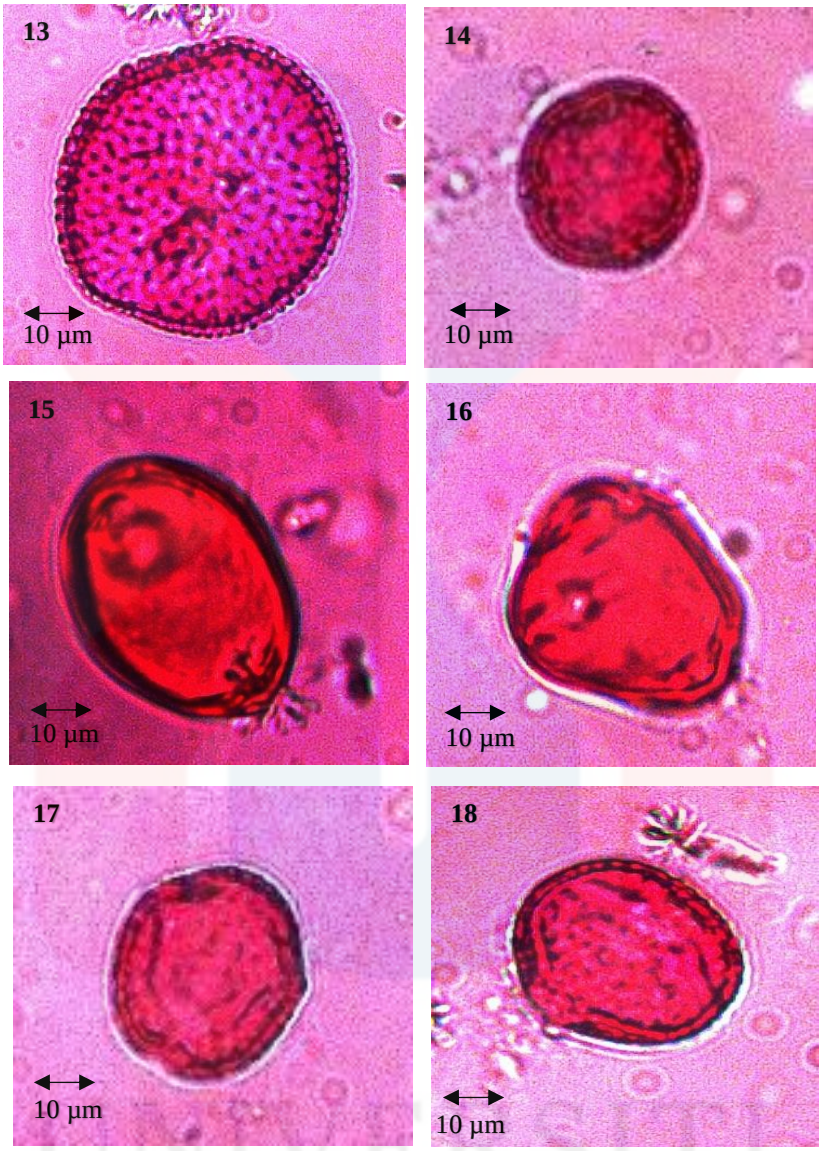

Figure 4.2 (d): The morphology of different pollen honey sample from Titi Tinggi under 40x magnification. 13- *Manihot esculenta* (Euphorbiaceae); 14- *Melastoma malabathricum* (Melastomataceae); 15- *Ptychosperma macarthurii* (Arecaceae); 16- *Lycopersicon esculentum* (Solanaceae); 17- *Solanum torvum* (Solanaceae); 18- *Capsicum fructescenes* (Solanaceae)

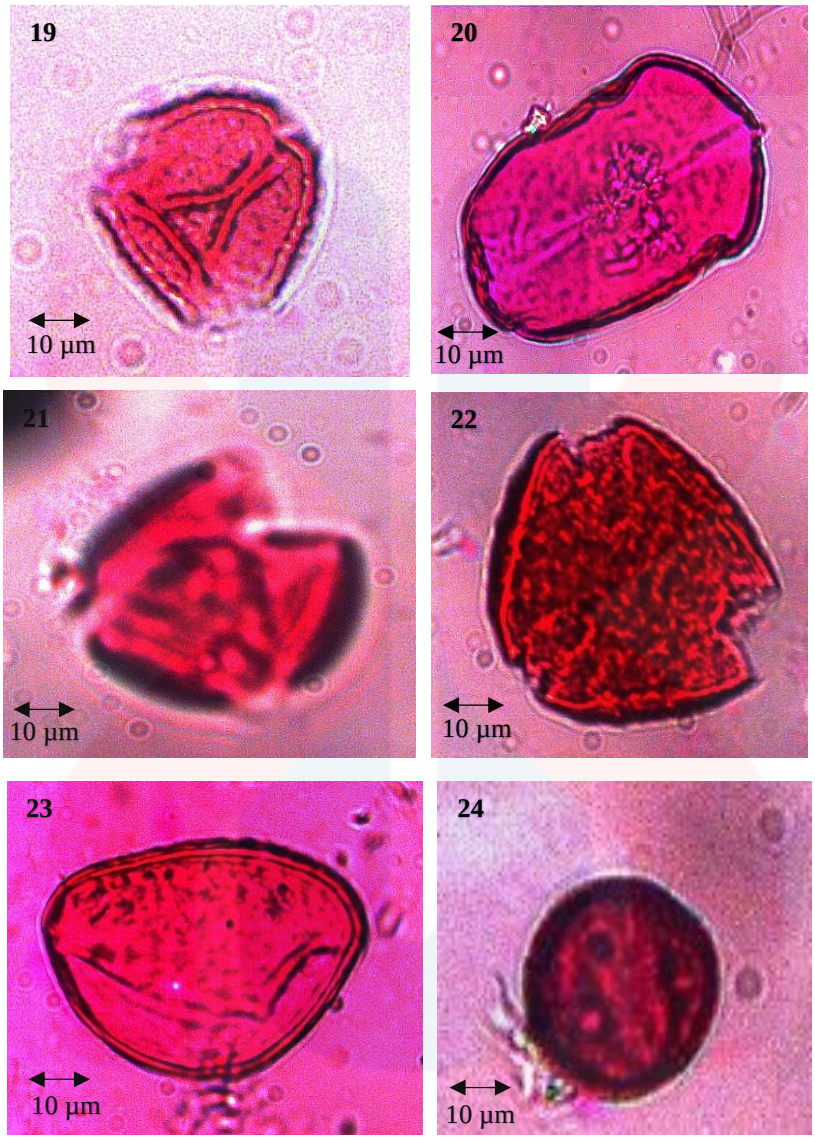

Figure 4.2 (e): The morphology of different pollen honey sample from Titi Tinggi under 40x magnification. 19- *Cascabela thevetia* (Apocynaceae); 20- *Asytasia intrusa* (Acanthaceae); 21- *Jacaranda obtusifolia* (Bignoniaceae); 22- *Anacardium occidentale* (Anacardiaceae); 23- *Neomarica longifolia* (iridaceae); 24- *Zea mays* (Poaceae)

Figure 4.2 (f): The morphology of different pollen honey sample from Titi Tinggi under 40x magnification. 25- *Artocarpus heterophyllus* (Moraceae); 26- *Tecomaria capensis* (Bignoniaceae)

There were 26 different types of pollen identified from the honey collected in Titi Tinggi. From all the honey sample collected, honey sample from Titi Tinggi had the highest types of plant species. This sample consist of diversity species of plant due to the location of beehives near the forest and hilly areas. the types of honey in this sample were multifloral honey because none of the species of plant more than 40% percentage of abundance. The most abundance plant species were *Gliricidia sepium* from fabaceae family and *Capsicum fructescenes* from Solanaceae family with percentage of 11.46%. In common name, *Gliricidia sepium* was called as 'Mexican lilac'. The plants sheds leaves before the start of flowering. All the trees start flowering from late January and show full flowering in mid-February. This species was favourite to bees collected their pollen (Simons, Stewart, 2007) The least abundance of plant species was *Ptychosperma macarthurii* with percentage of 0.48%.

In this sample *Gliricidia sepium, Nerium indicum, Amischotolype griffithii, Veitchia merrillii,Melastoma malabathricum, Solanum torvum, Capsicum fructescenes, Jacaranda obtusifolia, Neomarica longifolia, Artocarpus heterophyllus* and *Tecomaria capensis* were categorized under the important minor pollen because their pollen counted were range from 3%-16%. *Hevea brasiliensis, Muntingia calabura, Ischaemum muticum, Citrus aurantifolium. Moringa pterygosperma, Ipomea pes-caprae, Cyperus bevifolius, Asytasia gangetica, Manihot esculenta, Ptychosperma macarthurii, Lycopersicon esculentum, yellow oleander, Asystasia intrusa*, and *Zea mays* were categorized as minor pollen due to percentage of abundance less than 3%.

There are some clear pictures of the pollen due to the absence of their cytoplasm leaving behind a hollow pollen together with their respective exine surfaces. Proper analysis method is vital to ensure the removal of pollen's cytoplasm to produce clear pictures under compound microscope views. Only then the pollen morphology would be clear. The digestion of the pollen was done by using Acetolysis mixture whereas it had been left in water bath for 20 minutes. Pollen wall is generally made up of 2 layers, inner layer (intine) and outer layer (exine). Intine layer is less resistant to acids and it is composed of cellulose and pectin. This layer can be destroyed during Acetolysis process leaving behind only the exine layer which is resistant, hard and sclerodermatous. In this case, Acetolysis function to dissolve the cellulose layer of the pollen facilitating the examination of its morphological aspects. Indirectly, the effectiveness of Acetolysis was increased when the temperature and time of digestion increased (Agashe & Caulton, 2009).

4.3 Honey samples from Beseri, Perlis

Table 4.3: The number of pollen and percentage of abundance of pollen types in the honey samples from Beseri.

Plant species	Family	No. of pollen	Percentage of abundance (%)
Nerium indicum	Apocynaceae	27	7.52%
Sporobolus indicum	Poaceae	24	6.69%
Sporobolus diander	Poaceae	11	3.06%
Anacardium accidentale	Anacardiaceae	12	3.34%
Solanum torvum	Solanaceae	21	5.85%
Neomarica longifolia	Iridiceae	25	6.96%
Areca catechu	Aracaceae	18	5.01%
Averrhoa carambola	Oxalidaceae	22	6.13%
Sesbania grandiflora	Fabaceae	67	18.66%
Amischotolype griffithii	Commelinaceae	16	4.46%
Citrus grandis	Rutaceae	20	5.57%
Ananas cosmosus	Bromeliaceae	12	3.34%
Zea mays	Poaceae	20	5.57%
Passiflora edulis	Passifloraceae	10	2.79%
Jacaranda obtusifolia	Bignoniaceae	26	7.24%
Elaeis guineensis	Aracaceae	4	1.11%
Bruiguiera gymnorrhiza	Rhizophoraceae	3	0.84%
Ptychosperma macathurii	Aracaceae	21	5.85%
TOTAL NUMBER OF POLLEN		359	

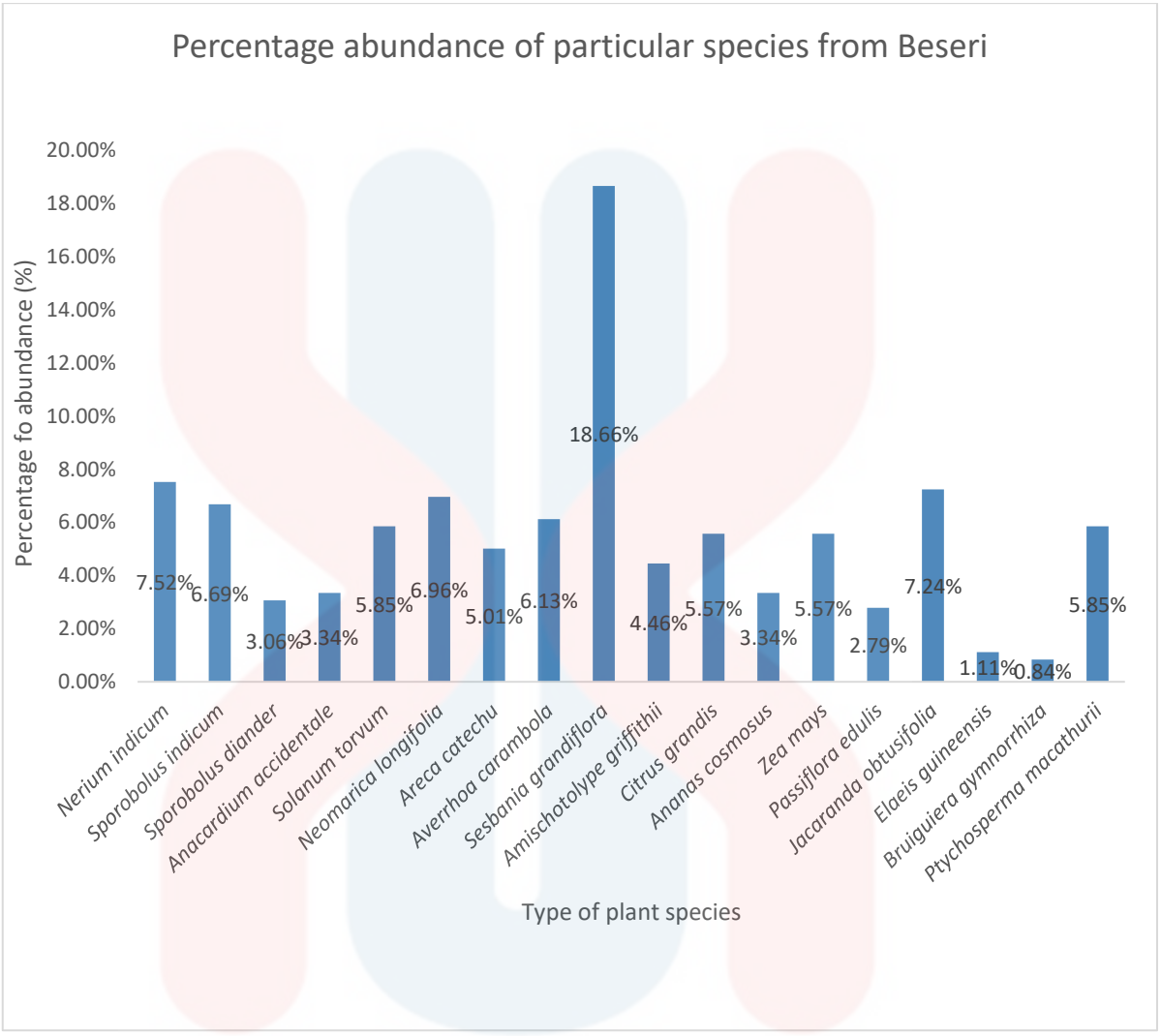

Figure 4.3: Percentage abundance of particular species from Beseri

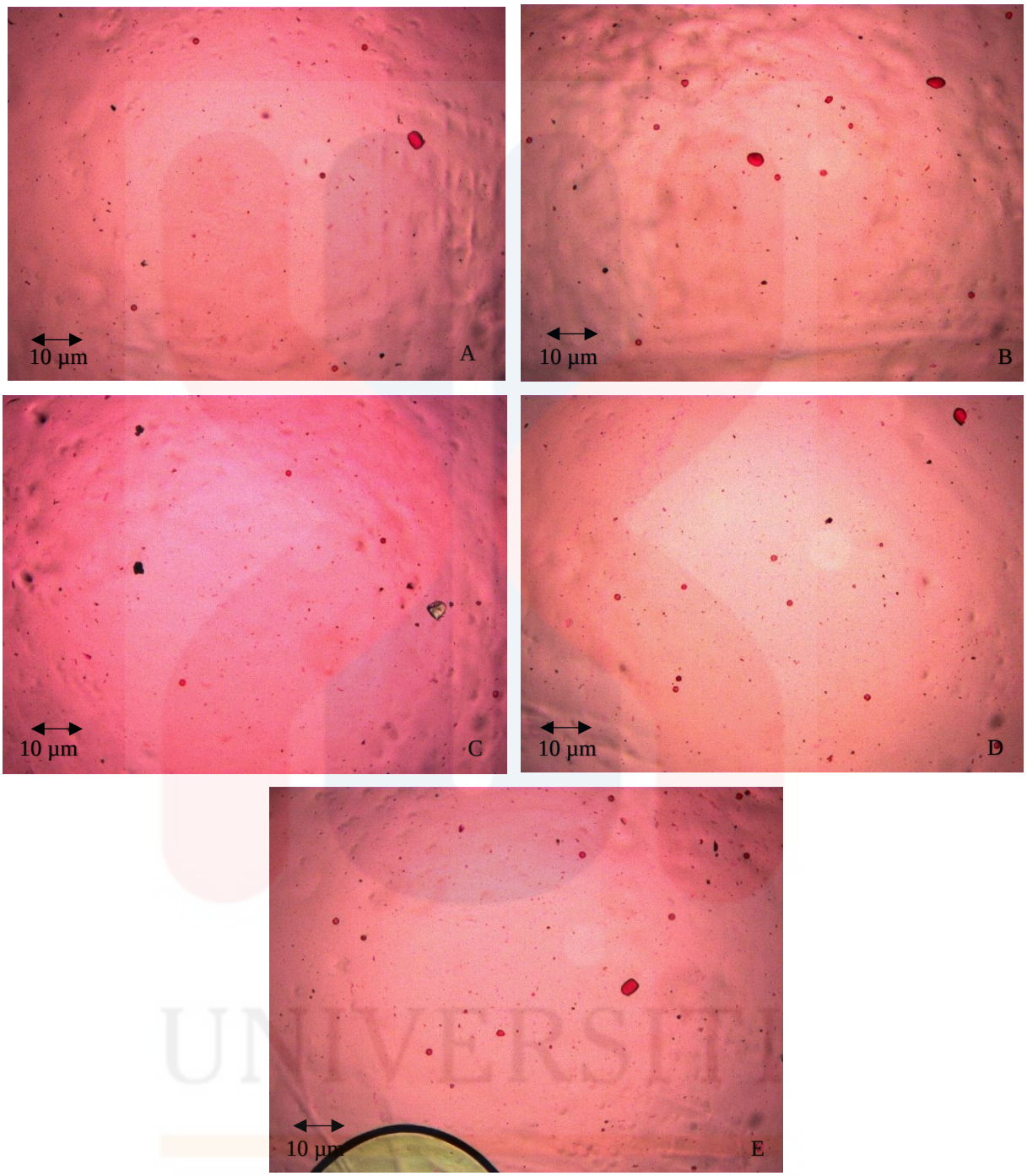

Figure 4.3 (a): Microscopic overviews of pollen density of honey sample from Beseri under 10x magnification. Overviews of A- left edge of cover slip (top); B- right edge of cover slip (top); C- left edge of the cover slip (bottom); D- right edge of the cover slip (bottom); E-middle of the cover slip

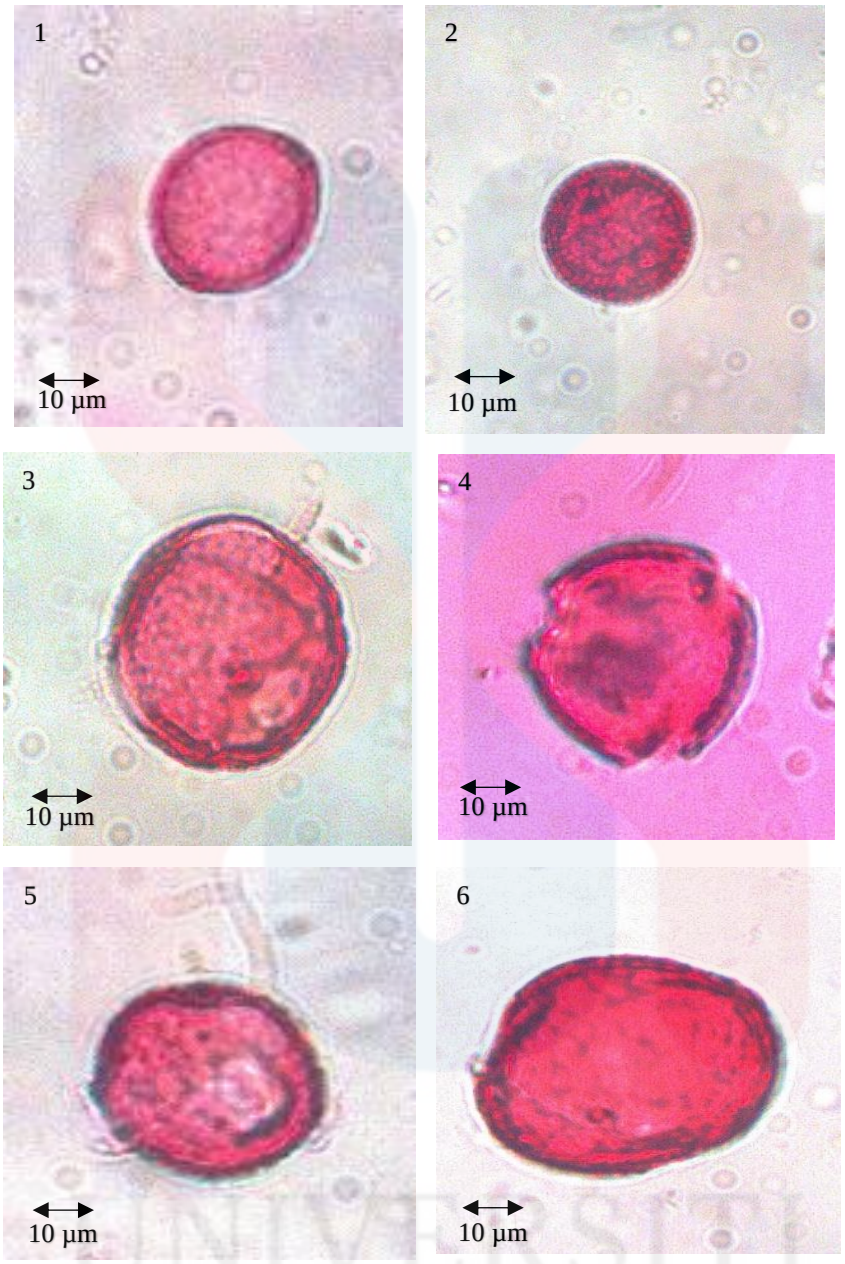

Figure 4.3 (b): The morphology of different pollen under 40x magnification.1- *Nerium indicum* (Apocynaceae); 2-*Sporobolus indicum* (Poaceae); 3-*Sporobulus diander* (Poaceae); 4-*Anacardium accidentale* (Anacardiaceae); 5-*Solanum torvum* (Solanaceae); 6-*Neomarica longifolia* (Iridiceae).

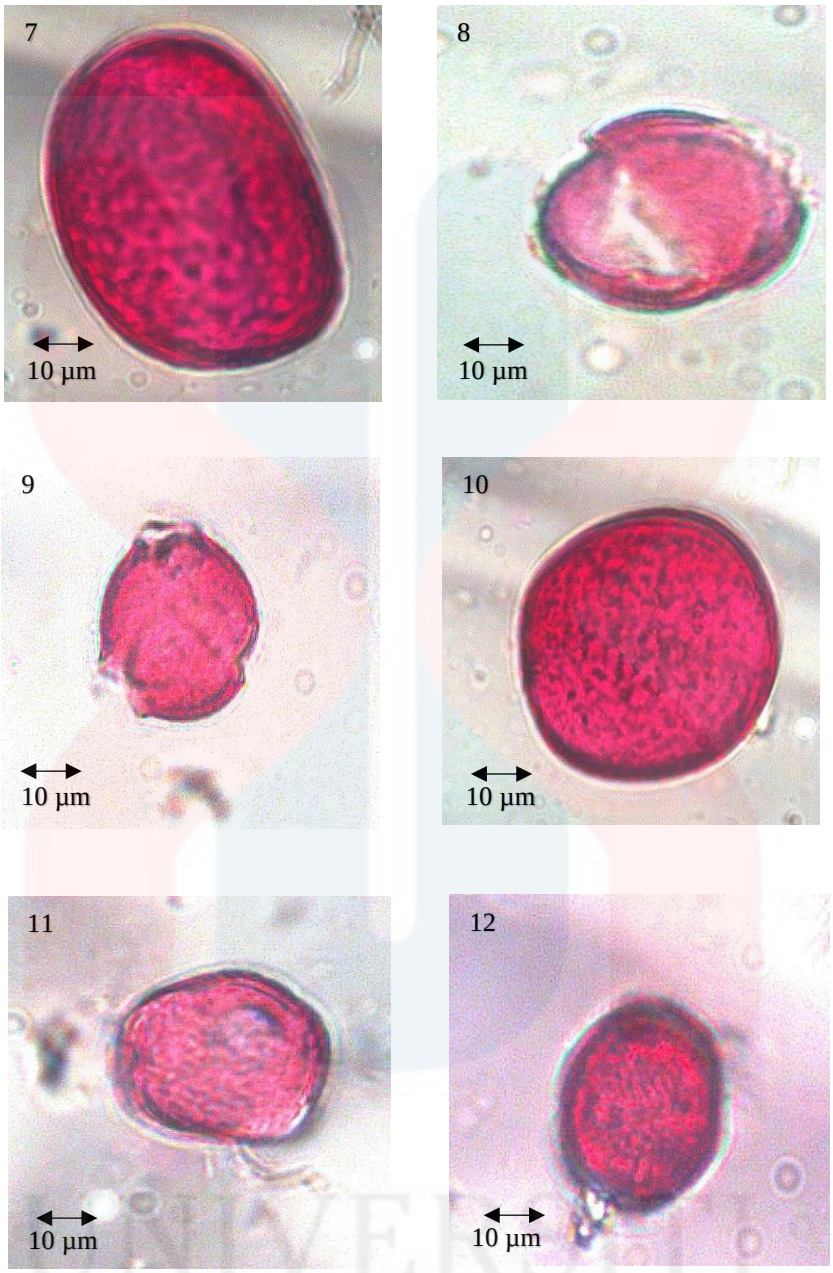

Figure 4.3 (c): The morphology of different pollen under 40x magnification.7-*Areca catechu* (Arecaceae); 8- *Averrhoa carambola* (Oxalidaceae); 9- *Sesbania grandiflora* (Fabaceae); 10- *Amischotolype griffithii* (Commelinaceae); 11 *Citrus grandis*-(Rutaceae); 12- *Passiflora edulis* (Passifloraceae).

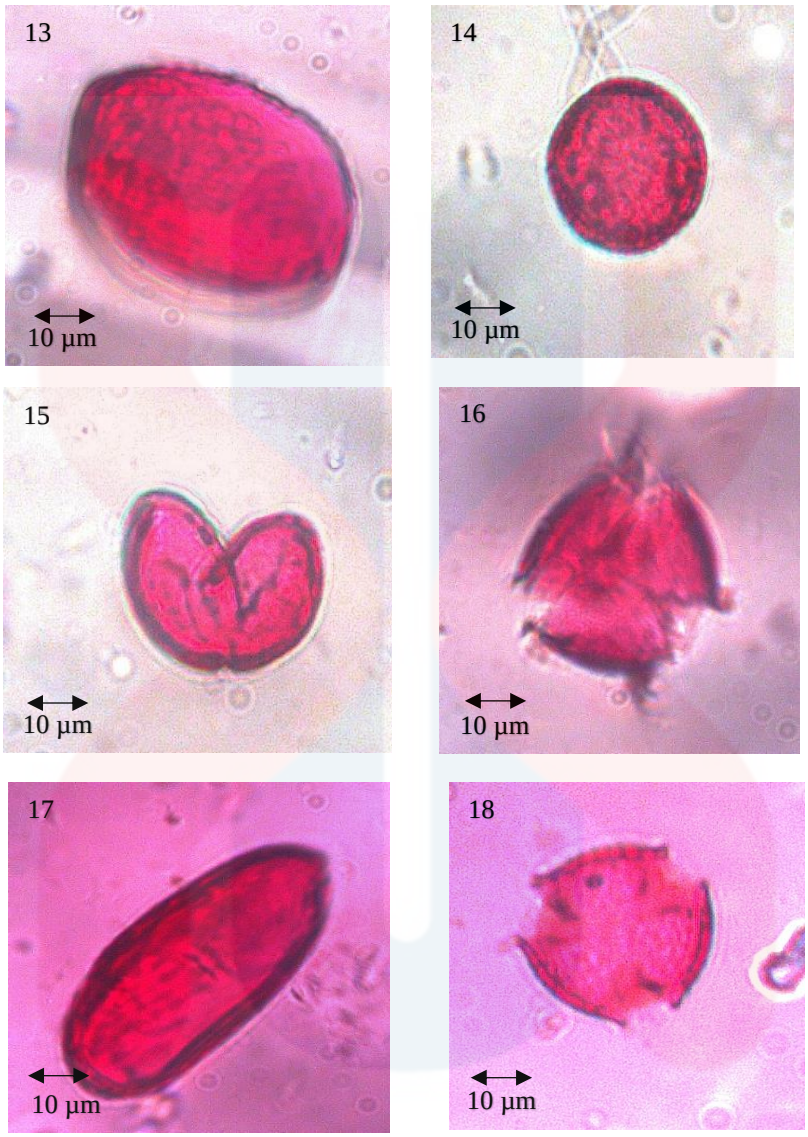

Figure 4.3 (d): The morphology of different pollen under 40x magnification 13-*Ananas cosmosus*(Bromeliaceae)*;*14-*Zea mays* (Poaceae)*;* 15-*Elaeis guineensis*(Arecaceae)*;* 16-*Jacaranda obtusifolia;* 17-*Ptchosperma macarthurii* (Arecaceae); 18-*Bruiguiera gymnorrhiza* (Rhizophoraceae)

17 varieties of pollen species were discovered from the sample in Beseri. There were about 359 total of pollen in the sample honey. Honey samples from Beseri had a different floral diversity in it due to the presence of different pollen species. *Sesbania grandiflora* was the only species that classified as secondary pollen with percentage of abundance of 18.66% of pollen found in the sample. Thus, the sample from Beseri was multifloral honey because none the species become predominant pollen.

Sesbania grandiflora commonly known as vegetable hummingbird was from Fabaceae family. This species was perennial crop and it was a tree in form of large shrub that can grow up to 15m tall and 30cm in trunk diameter. Besides that, *Sesbania grandiflora* is a flowering plant that is native to tropical Asia Its twice-pinnately compound leaves are alternately arranged and up to 30cm long, with 20 to 50 leaflets. Each leaflet is 1.2-4.4cm length and 0.5-1.5cm wide, with an oblong to elliptical shape. The huge, butterfly-shaped blooms bloom on the leaf axils and are white, yellow, pink, or red in colour. The fruit is a pod that is linear to slightly bent. Because it produces a lot of flowers, it can attract stingless bee honey (Noviany et.al, 2018).

Important minor pollen and minor pollen were also present making this honey samples more varieties. Important minor pollen has 14 species in the sample such as *Nerium indicum* (7.52%), *Sporobolus indicum* (6.69%), *Sporobolus diander* (3.06%), *Anacardium accidentale* (3.34%), *Solanum torvum* (5.85%), *Neomarica longifolia* (6.96%), *Areca catechu* (5.01%), *Averrhoa carambola* (6.13%), *Amischolotype griffithii* (4.46%), *Citrus grandis* (5.57%), *Ananas cosmosus* (3.34%), *Zea mays* (5.57%), *Jacaranda obtusifolia* (7.24%), *Ptychosperma macarthurii* (5.85%).

Among 17 species found in the sample, there were 3 species such as *Passiflora edulis* (2.79%), *Elaeis guineensis* (1.11%) and *Bruiguiera gymnorrhiza* (0.84%) categorized as minor pollen. The lowest percentage abundance was *Bruguiera gymnorrhiza* (0.84%). The three Arecaceae species *Areca catechu, Elaeis guineensis*, and *Ptychosperma macarthurii* were present in Beseri honey sample as important pollen and minor pollen, respectively. *Areca catechu* is a culturally significant perennial plant that provides nectar and pollen to bees. Despite the fact that *Eleais guineensis* does not generate nectar, bees prefer to visit it for pollen. Pollen is one of the most important characteristics that distinguishes Malaysian honey from imported honey. The research study's melissopalynological research determined a diversity of pollen grains gathered by stinglees bees. However, the bees' trophic niche can fluctuate during the year due to a variety of reasons, including flower richness within a vegetative zone where colonies are located and climatic conditions. As a result, the variety of floral sources visited by bees may result in different varieties of honey (Mahani, et al.., 2020).

4.4 Honey samples from Chuping, Perlis

Table 4.4: The number of pollen and percentage of abundance of pollen types in the honey samples from Chuping.

Plant species	Family	No. of pollen	Percentage of abundance (%)
Melastoma malabathricum	Melastomataceae	28	18.42%
Mimosa pudina	Fabaceae	14	9.21%
Cucumis sativus	Cucurbitaceae	5	3.29%
Sporobolus indicum	Poaceae	30	19.74%
Nerium indicum	Apocynaceae	8	5.26%
Amaranthus lividus	Amaranthaceae	3	1.97%
Capsicum fructescenes	Solanaceae	5	3.29%
Solanum torvum	Solanaceae	7	4.61%
Artocarpus heterophyllus	Moraceae	35	23.03%
Benincasa hispida	Cucurbitaceae	2	1.32%
Clerodendrum inerme	Lamiaceae	15	9.87%
TOTAL NUMBER OF POLLEN		152	

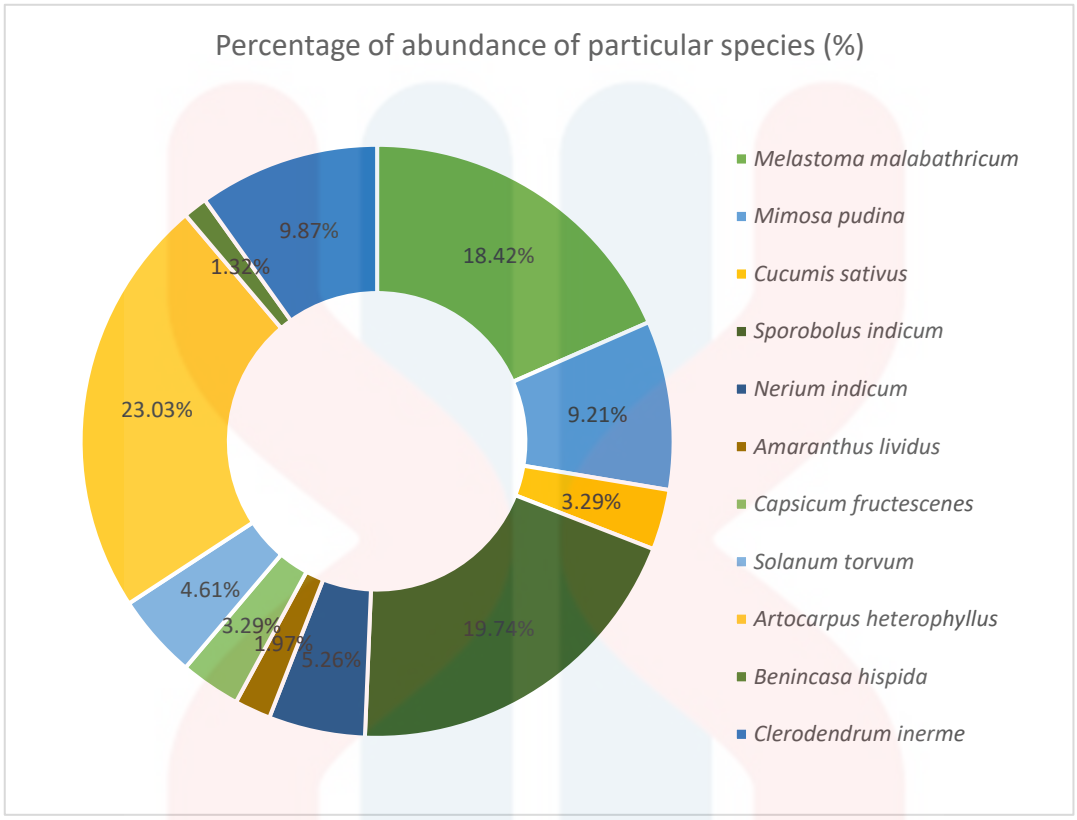

Figure 4.4: Percentage abundance of honey sample from Chuping

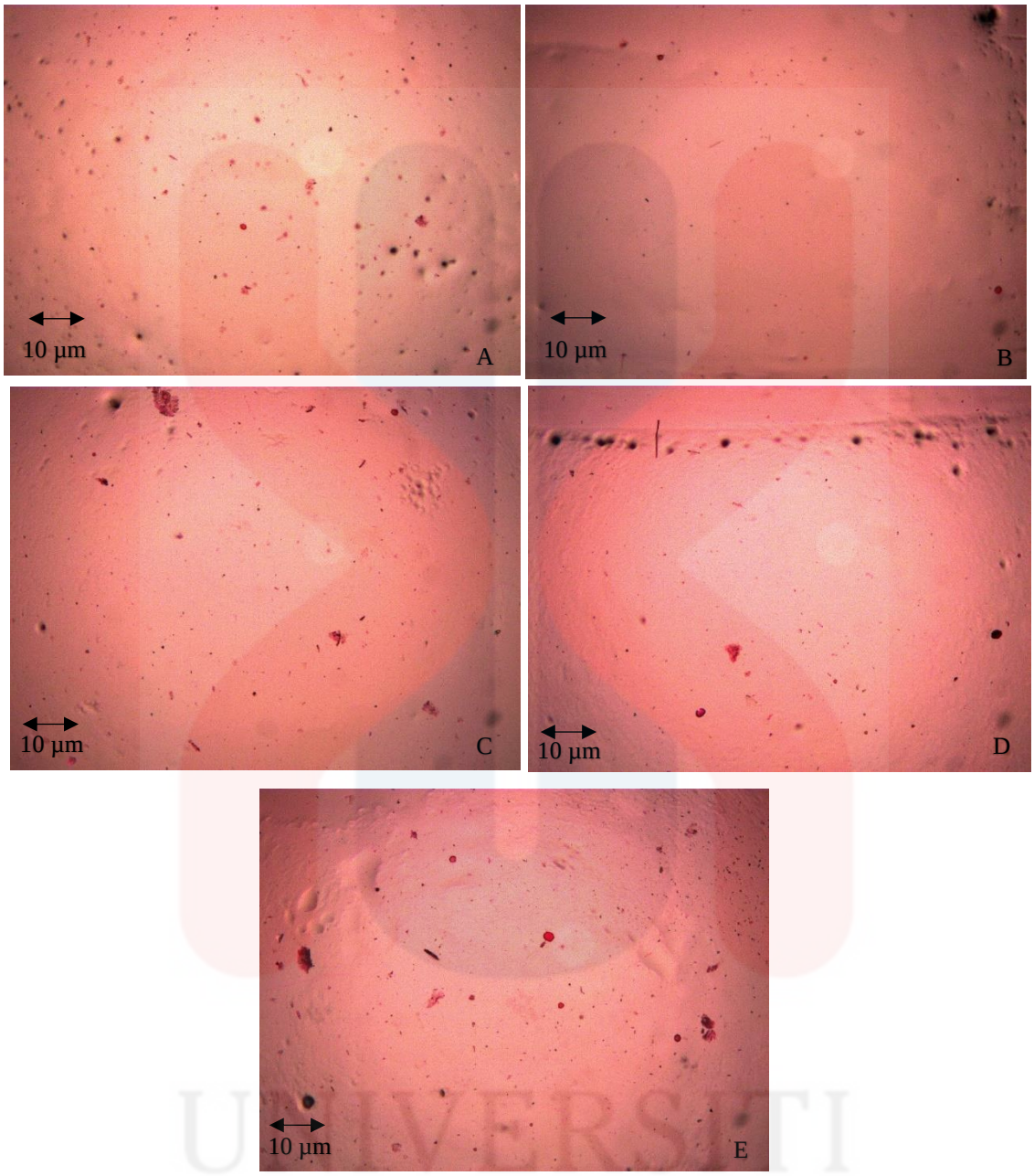

Figure 4.4 (a): Microscopic overviews of pollen density of honey sample from Chuping under 10x magnification. Overviews of A- left edge of cover slip (top); B- right edge of cover slip (top); C- left edge of the cover slip (bottom); D- right edge of the cover slip (bottom); E-middle of the cover slip

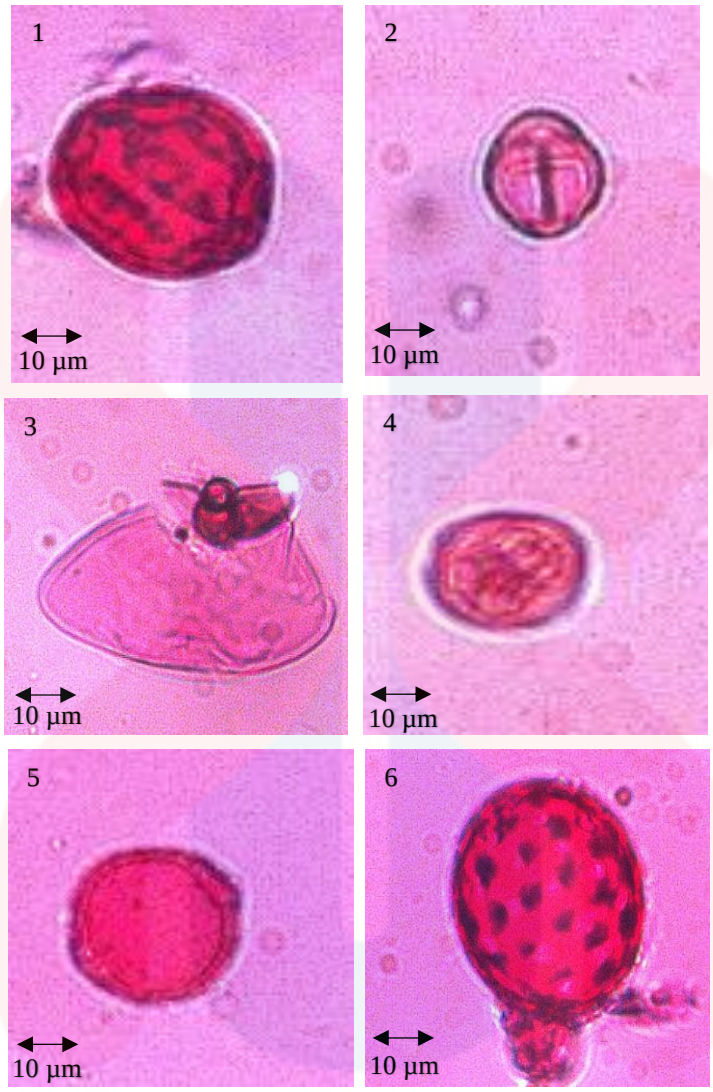

Figure 4.4 (b): The morphology of different pollen under 40x magnification. 1-*Melastoma malabathricum* (Melastomataceae)*;* 2-*Mimosa pudina* (Fabaceae)*;* 3-*Cucumis sativus* (Cucurbitaceae)*;* 4-*Sporobolus indicum* (Poaceae)*;* 5-*Nerium indicum (*Apocynaceae*);* 6-*Amaranthus lividus* (Solanaceae)

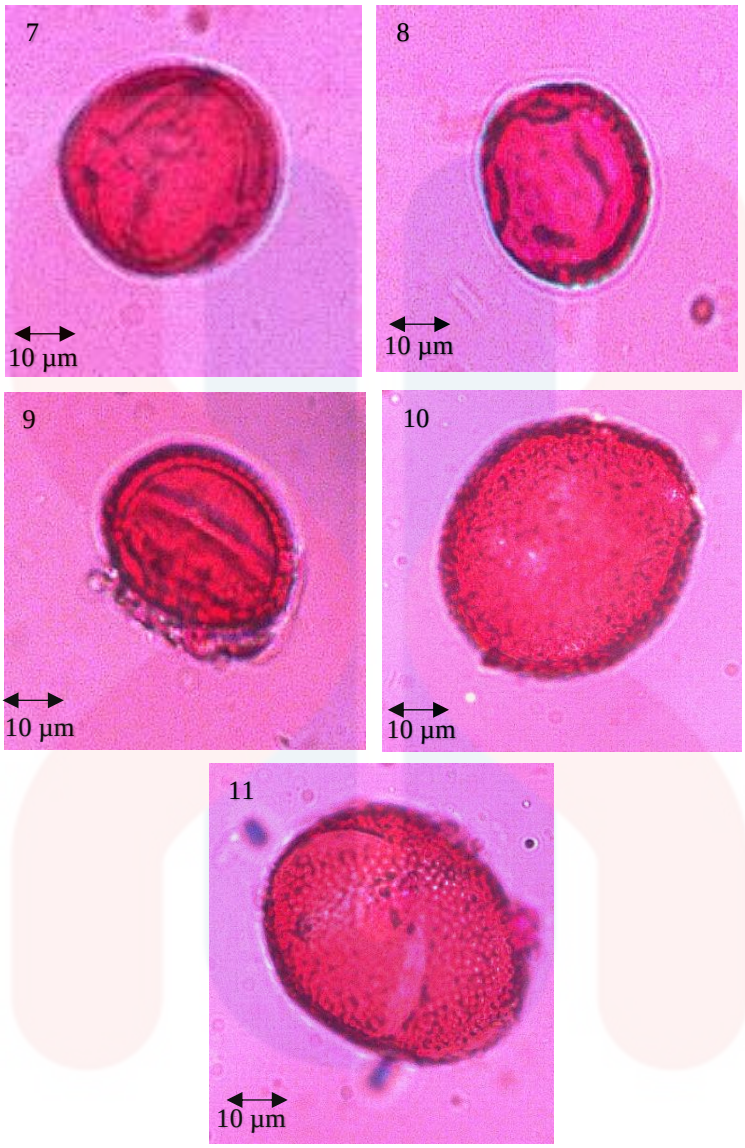

Figure 4.4 (c): The morphology of different pollen under 40x magnification. 7-*Capsicum fructescenes* (Solanaceae); 8-*Solanum torvum* (Solanaceae); 9-*Artocarpus heterophyllus* (Moraceae); 10-*Benincasa hispida* (Cucurbitaceae); 11-*Clerodendrum inerme* (Lamiaceae);

Because of their surroundings, the sample collected from Chuping contained relatively contain small amount of pollen counted under microscope (152). Whereas Chuping once was historically a sugarcane farming area that is now devoid of sugarcane production. The region is presently used for industrial purposes. The sample contained 11 different species. The species *Artocarpus heterophyllus* of the Moraceae family has the highest percentage pollen abundance of 23.03%. Meanwhile, the species *Benincasa hispida* of the Cucurbitaceae family had the lowest percentage abundance of 1.32%. The sample contained significant minor pollen with percentage abundances ranging from 3% to 16%, such as *Melastoma malabathricum, Mimosa pudina, Cucumis sativus, Sporobolus indicum, Nerium indicum, Capsicum fructescenes, Solanum torvum,* and *Clerodendrum inerme.* Only two species were recognized as minor pollen in this sample, with a percentage abundance of less than 3%, such as *Amaranthus lividus* from Amaranthaceae family and *Benincasa hispida*.

Based on the research, the fragrance produced of the tree, *Artocarpus heterophyllus* can attract bees to collect nectar from the tree. The popular name jackfruit tree was one of the most important trees in Malaysian backyards, and it was also the most ubiquitous and useful species in the Artocarpus genus. The size was medium-sized evergreen trees that may grow up to 25 metres tall and are easily identified by their fruits, which are the largest among cultivated species. The trees can adapt to a wide range of soil conditions, from acidic to neutral. the aromatic and flavourful fruit can be eaten fresh or preserved. the trees can adapt to freely draining, acid to neutral soil (Craig & Harley, 2006)

Benincasa hispida commonly name as winter melon was the least pollen found in the sample. The winter melon was the herbaceous vine that grew once a year. It has a twining growth form and uses tendrils to climb. The broad, plain, coarsely textured leaves are 5 lobed and can reach a width of 10 to 15cm. The leaves are arranged in a zigzag pattern along the stem. The stems have coarse hairs on them. The yellow flowers appear in the axils of the leaves. The dark green fruit has white flesh and is cylindrical to oblong in shape. The ripe fruit can reach a length of 30 to 45cm and has a waxy coating that allows it to be stored for extended periods of time. The flowers produce low from *Benincasa hispida* only once per year and cause less attraction to stinglees bee honey to collect nectar and pollen from this species (Mohammad et, al,2019).

Thus, because the pollen taxon distribution in these samples varied, and none of the samples satisfied the requirements for having predominant species, the sample collected from Chuping was multifloral. Each local flora has its own distinct qualities, which will be represented in the form of pollen spectrum from a variety of honey samples, as well as assisting in the geographical identification of the honey gathered (Agashe & Caulton, 2009).

4.5 Honey samples from Padang Pauh, Perlis

Table 4.5: The number of pollen and percentage of abundance of pollen types in the honey samples from Padang Pauh.

Plant species	**Family**	**No. of pollen**	**Percentage of abundance (%)**
Mimosa pudina	Fabaceae	8	6.06%
Capsicum sp.	Solanaceae	15	11.36%
Citrullus lanatus	Cucurbitaceae	22	16.67%
Antigonan leptopus	Polygonaceae	16	12.12%
Dillenia suffructicosa	Dilleniaceae	13	9.85%
Bougainvillea sp.	Nyctaginoceae	5	3.79%
Mimusops elengi	Sapotaceae	3	2.27%
Zea mays	Poaceae	6	4.55%
Brassica chinensis	Brassicaceae	10	7.58%
Veptorium odoratum	Asteracea	34	25.76%
TOTAL NUMBER OF POLLEN		132	

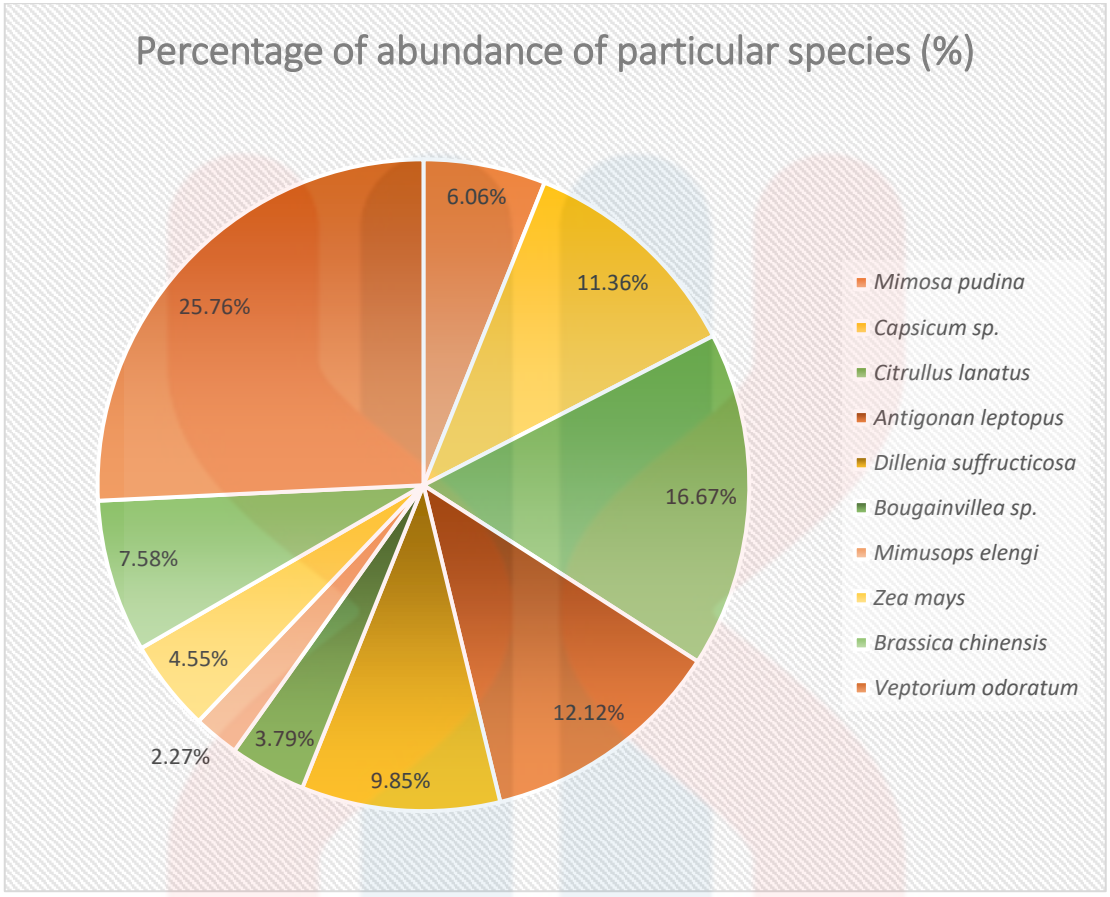

Figure 4.5: Percentage abundance of honey sample from Padang Pauh.

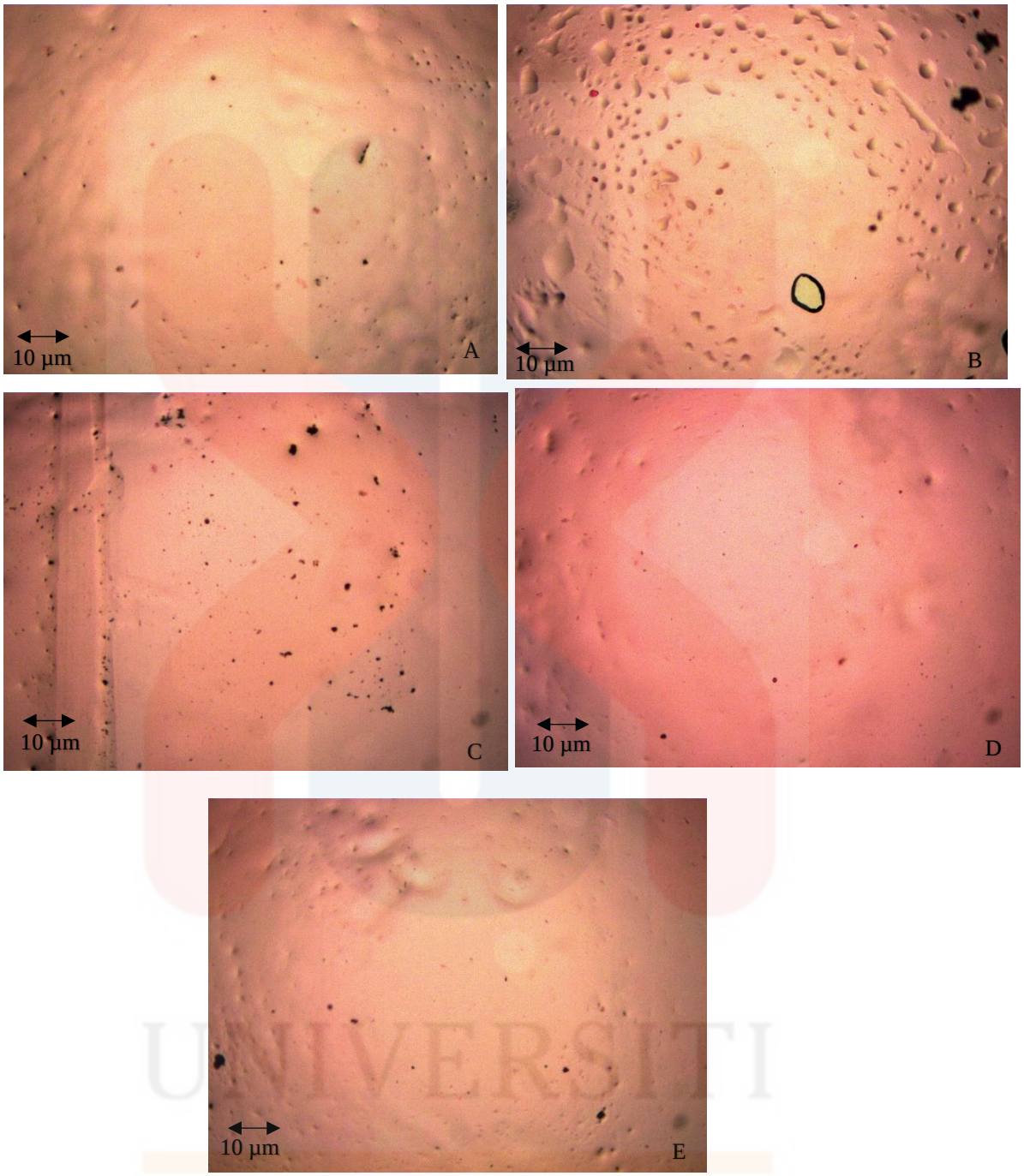

Figure 4.5 (a): Microscopic overviews of pollen density of honey sample from Padang Pauh under 10x magnification. Overviews of A- left edge of cover slip (top); B- right edge of cover slip (top); C- left edge of the cover slip (bottom); D- right edge of the cover slip (bottom); E-middle of the cover slip

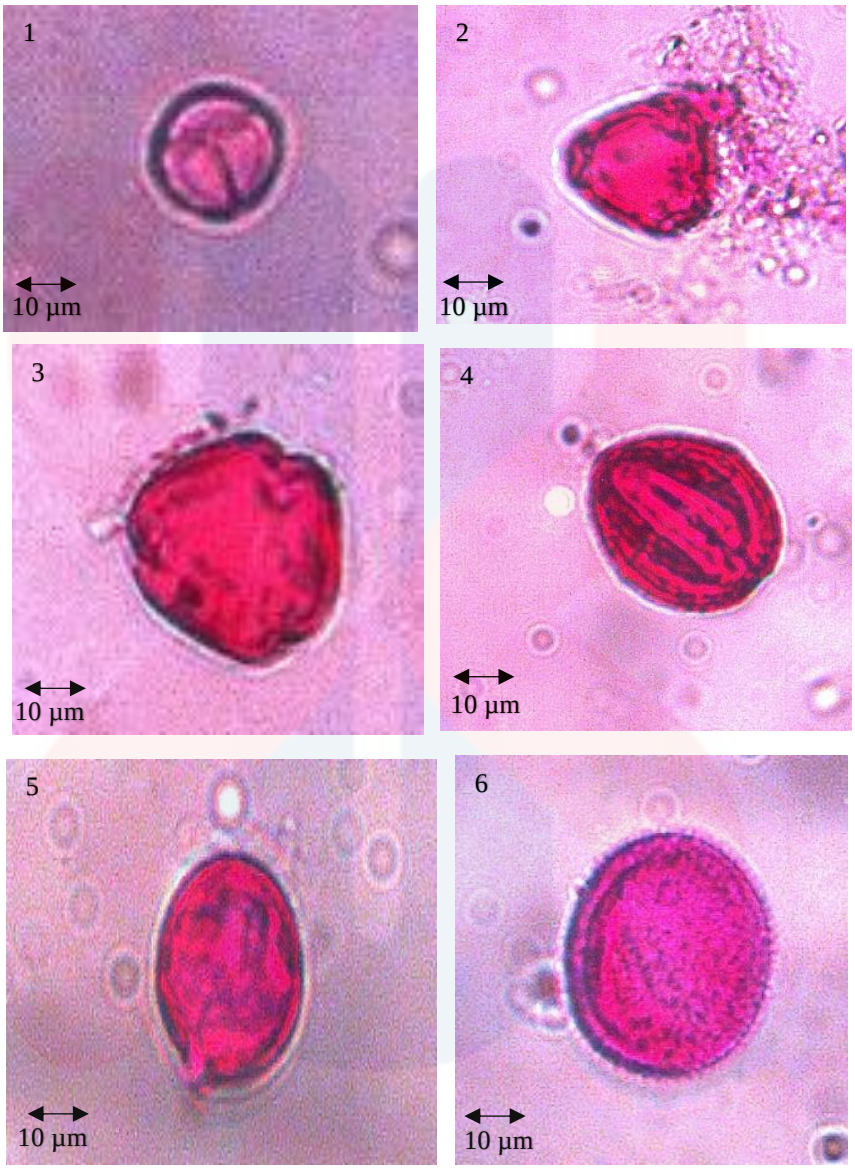

Figure 4.5 (b): The morphology of different pollen under 40x magnification. 1-*Mimosa pudina* (Fabaceae); 2-*Capsicum sp.* (Solanaceae); 3-*Citrullus lanatus* (Cucurbitaceae); 4-*Antigonan leptopus* (Polygonaceae); *Dillenia suffructicosa* (Dilleniaceae) 6-*Bougainvillea sp.* (Nyctaginoceae)

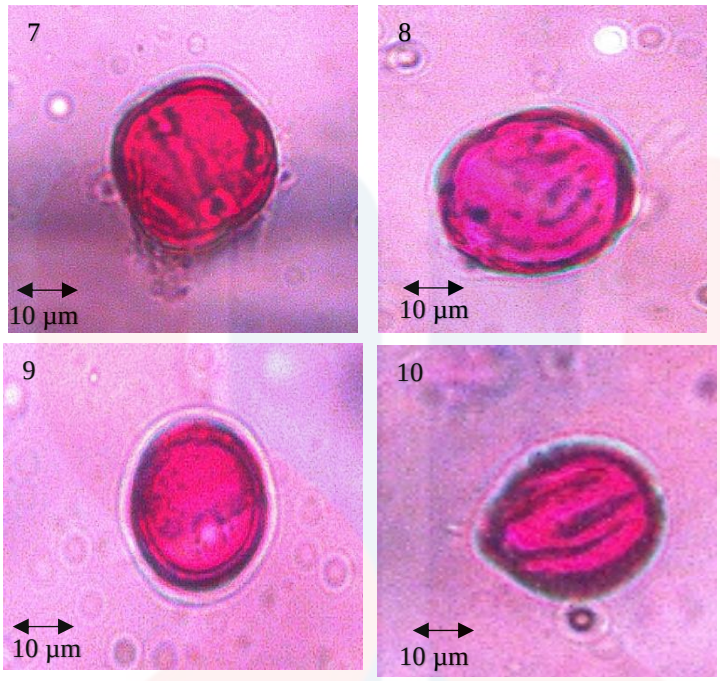

Figure 4.5 (c): The morphology of different pollen under 40x magnification. 7-*Mimusop elengi* (Sapotaceae); 8-*Zea mays* (Poaceae); 9-*Brassica chinensis* (Brassicaceae); 10-*Veptorium odoratum* (Asteraceae).

Sample from Padang Pauh was limited in total of pollen compared to other sample with total number of pollens only 132 pollens. In addition, this sample and previous sample had similarity because there was less pollen in this sample than in the previous one, Chuping (152). From the results obtained in the pollen analysis of honey samples from Padang Pauh, the sample also features the shortest number of species detected, with just 10 different types of pollen had been successful detected such as *Mimosa pudina* (6.06%), *Capsicum sp.* (11.36%), *Citrullus lanatus* (16.67%), *Antigonan leptopus* (12.12%), *Dillenia suffructicosa* (9.85%), *Bougainvillea sp.* (3.79%), *Mimusops elengi (*2.27%*)*, *Zea mays* (4.55%), *Brassica chinensis* (7.58%), *Veptorium odoratum* (25.76%).

Furthermore, because there was no dominant species, the sample was classified as multifloral honey based on the observation. In the secondary pollen with percentage abundance of more than 16% included 2 species such as *Citrullus lanatus* from Cucurbitaceae family and *Veptorium Odoratum* from Asteraceae family. The important minor pollen with percentage abundant between 3% to 16% included 7 species from Mimosa pudina from Fabaceae family, Capsicum sp. from Solanaceae family, *Antigonan leptopus* from Polynaceae family, *Dillenia suffructicosa* from Dilleniaceae family, *Bougainvillea sp.* from Nyctaginoceae family, *Zea mays* from Poaceae family, and *Brassica chinensis* from Brassicaceae family. The category of minor pollen with percentage below than 3% included 1 species from *Mimusops elengi* from Sapotaceae family.

Common name for *Citrullus lanatus* was watermelon. Watermelon A monoecious annual vine. It has a twining growth habit and can reach a length of 1.5 to 5 metres. The root system is shallow and widespread. The deeply lobed leaves are oblong-ovate in shape with a cordate leaf base and alternately placed along the stem. The angular, grooved herbaceous stem is coated in long, white hairs. The solitary pale-yellow flowers are borne on the leaf axils. The male flowers contain three stamens, while the female blooms have a 3-lobed stigma and an inferior ovary. Both blooms have a 5-parted corolla and a 5-lobed calyx. The fruit is a pepo, and it can be globular, oblong, or elliptical in shape (Thapa & Sirwat, 1997).

Watermelon crops in Malaysia are grown using cultivars imported totally from other countries, such as Taiwan, China, and Japan, where the environment is different from Malaysia and the compatibility element of the variety must be considered. Malaysian watermelon agriculture (agroclimatic zone) (Anim Agro Tech,2010).

Veptorium odoratum was the perennial weed of plantation crops and cleared land from family Asteraceae. The plant species is formerly known as Eupatorium odoratum or locally known as Pokok Kapal Terbang or Pokok Malaysia. It is a multi-stemmed shrub that can reach a height of 2.5 metres (100 inches) in open settings. The stems are delicate, but the shrub's base is woody. It becomes etiolated and behaves like a creeper in shaded regions, growing on other vegetation. It can then grow to a height of up to 10 metres (33 ft). The plant is hairy and glandular, and when crushed, the leaves emit a pungent, aromatic odour. The leaves are triangular to elliptical in shape and have serrated edges (Matawali et,al, 2019).

4.6 Honey samples from Guar Sanji, Perlis

Table 4.6: The number of pollen and percentage of abundance of pollen types in the honey samples from Guar Sanji.

Plant species	Family	No. of pollen	Percentage of abundance (%)
Cyperus aromaticus	Cyperaceae	13	1.67%
Mutingia calabura	Mutingiaceae	340	43.70%
Gliricidia sepium	Fabaceae	24	3.08%
Combretum indicum	Combretaceae	47	6.04%
Tecomaria capensis	Bignoniaceae	38	4.88%
Veitchia merrillii	Arecaceae	7	0.90%
Ipomea pes-caprae	Convolvulaceae	3	0.39%
Neomarica longifolia	Iridaceae	11	1.41%
Ischaemum muticum	Poaceae	15	1.93%
Artocarpus heterophyllus	Moraceae	22	2.83%
Sporobolum indicum	Poaceae	7	0.90%
Anacardium accidentale	Anacardiaceae	5	0.64%
Melastoma malabathricum	Melastomataceae	9	1.16%
Cyperus brevifolius	Cyperaceae	180	23.14%
Antigonan leptopus	Polygonaceae	45	5.78%
Pterocarpus indicus	Fabaceae	4	0.51%
Manihot esculenta	Euphorbiaceae	8	1.03%
TOTAL NUMBER OF POLLEN		778	

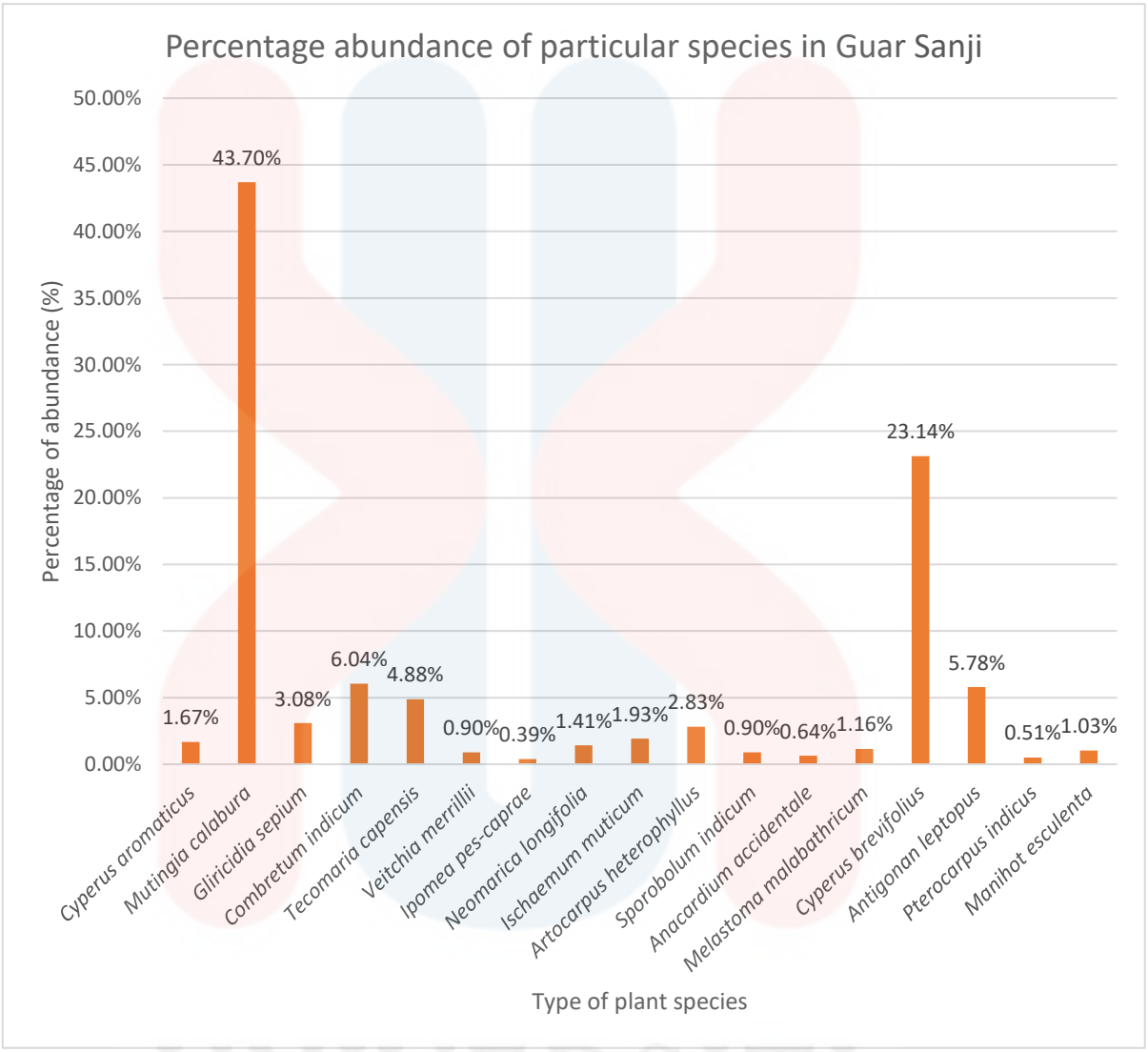

Figure 4.6: Percentage abundance of honey sample from Guar Sanji

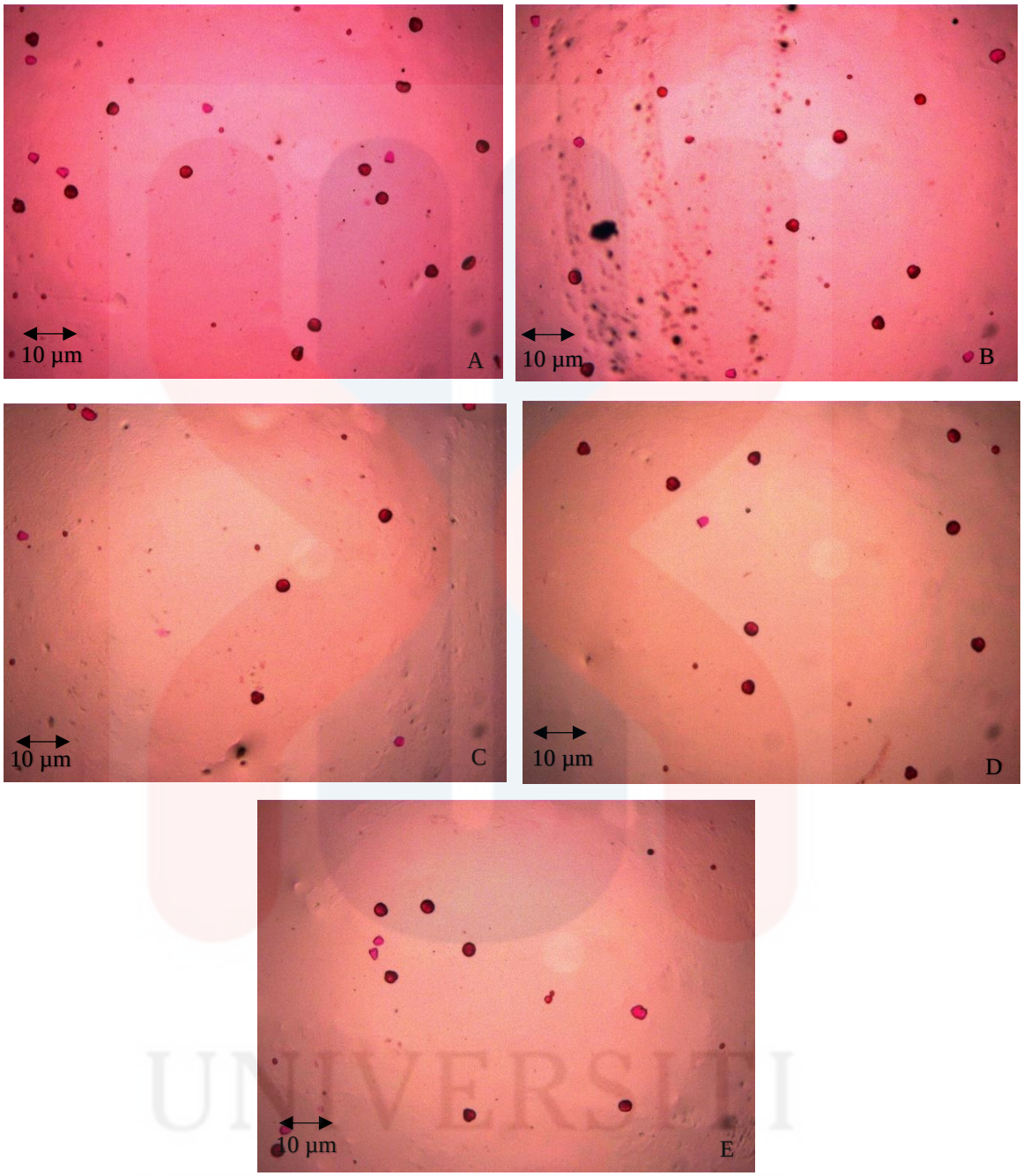

Figure 4.6 (a): Microscopic overviews of pollen density of honey sample from Guar Sanji under 10x magnification. Overviews of A- left edge of cover slip (top); B- right edge of cover slip (top); C- left edge of the cover slip (bottom); D- right edge of the cover slip (bottom); E-middle of the cover slip

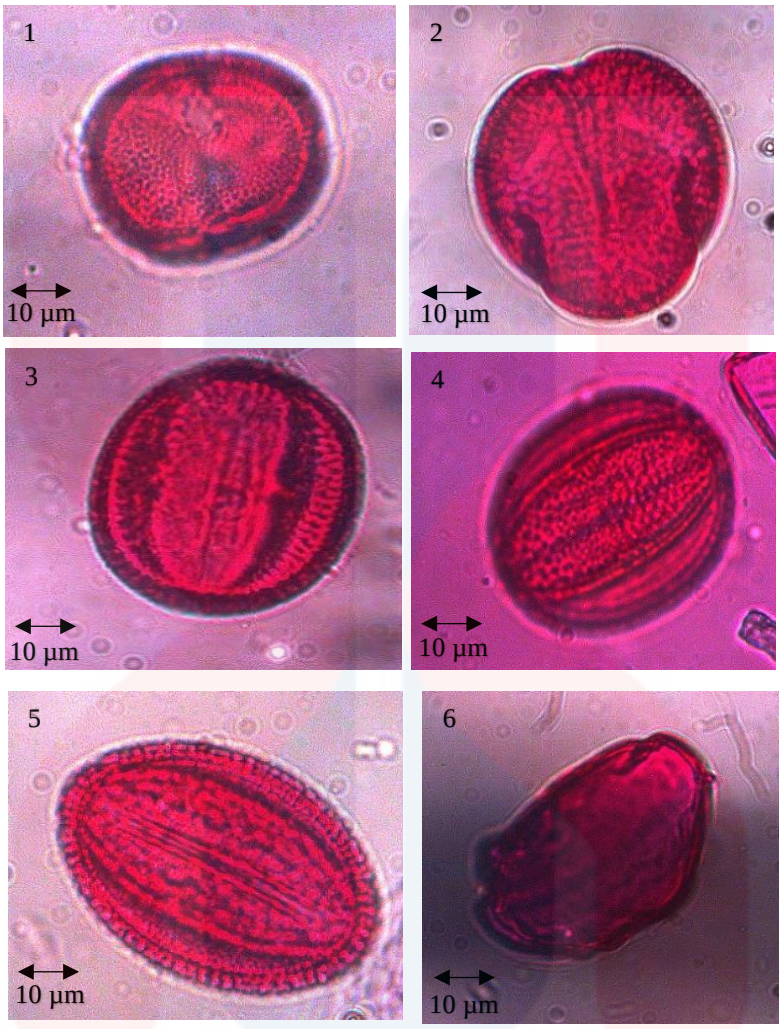

Figure 4.6 (b): The morphology of different pollen under 40x magnification. 1- *Cyperus aromaticus* (Cyperaceae); 2-*Mutingia calabura* (Mutingiaceae) 3- *Gliricidia sepium* (Fabaceae); 4- *Combretum indicum* (Combretaceae); 5-*Tecomaria capensis* (Bignoniaceae); 6-*Veitchia merrillii* (Arecacea).

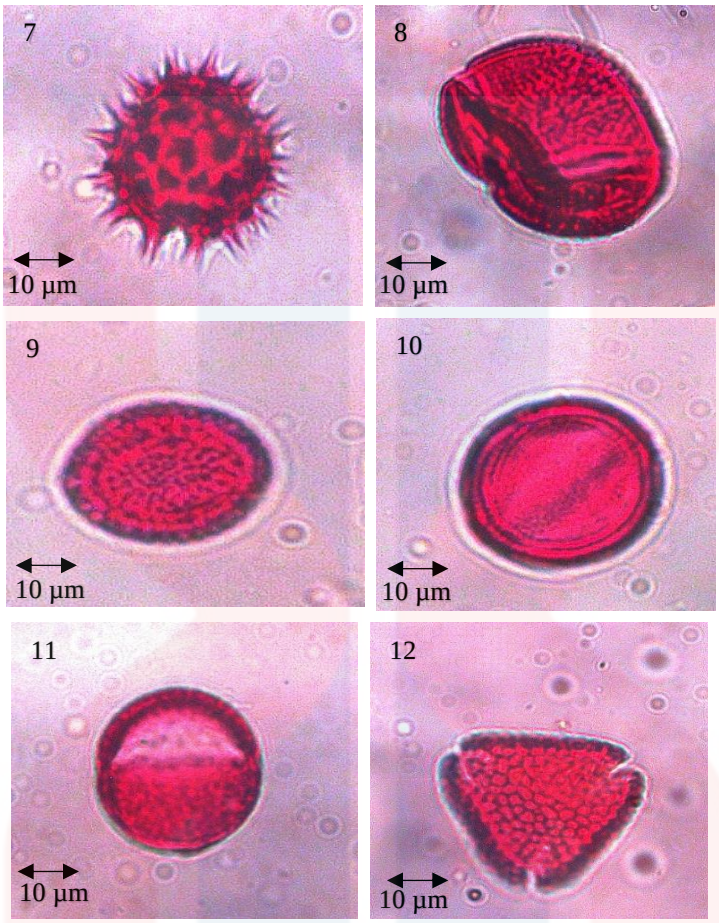

Figure 4.6 (c): The morphology of different pollen under 40x magnification.7-*Ipomea pes-caprae* (Convolvulaceae); 8-*Neomarica longifolia* (Iridaceae); 9-*Ishaemum muticum* (Poaceae); 10-*Artocarpus heterophyllus* (Moraceae); 11-*Sporobolus indicum* (Poaceae); 12-*Anacardium accidentale* (Anacardiaceae).

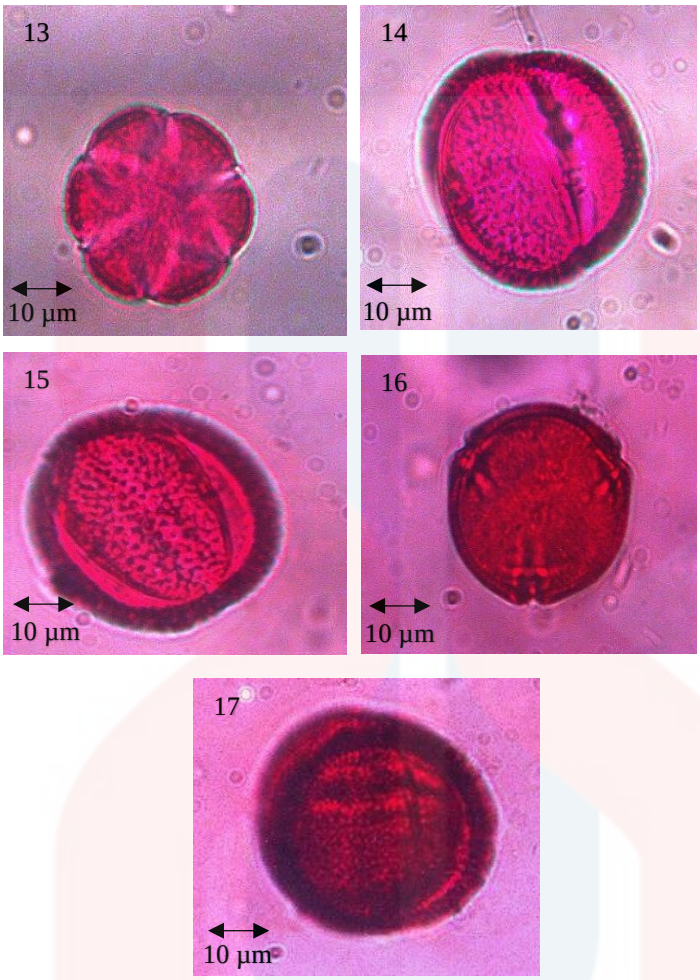

Figure 4.6 (d): The morphology of different pollen under 40x magnification. 13-*Melastoma malabathricum* (Melastomataceae); 14-*Cyperus brevifolius* (Cyperaceae); 15-*Antigonan leptopus* (Polygonaceae); 16-*Pterocaarpus indicus* (Fabaceae); 17-*Manihot esculenta* (Euphorbiaceae).

From the result obtained in the honey samples collected from Guar Sanji, pollen from *Mutingia calabura* from Mutingiaceae family was found to be predominant in the honey with abundance of 43.70%. Therefore, it can be clarified that the types of honey were unifloral honey. Since taxon of dominancy for a particular species of plant exceeded 40% of the overall pollen count. Altogether there were 17 different types of pollen from this honey samples. This reveals that bees in Guar Sanji have access to a diverse range of flora for their own nutritional needs. Kelulut honeybee is known to be a good pollinator in agriculture and natural habitats at different times of the year (Upadhyay et al., 2014). The amount of pollen found from honey collected from Guar Sanji was far more accumulated compared to honey collected from Padang Pauh. The total number of pollens found in this sample was 778 pollens. However, both had different pollen who dominate their samples which is *Mutingia calabura* from Mutingiaceae family dominates honey sample from Guar Sanji and the least was *Ipomea pes-caprae* vice versa in Padang Pauh whereas the species *Veptorium odoratum* from Asteraceae family dominates the honey samples and the least collected was *Mimusops elangi* from Sapotaceae family.

Mutingia calabura from Mutingiaceae family dominates this pollen with the highest percentage (43.70%), thus this type of sample was unifloral honey. The common name for this species was 'Malayan Cherry Tree'. It is a tiny tree native to tropical America that grows up to 30 meters tall and has an umbrella-shaped crown. It was once customary to plant a shade tree in a community and let it grow wild on the waste area. It blooms at all times of the year. For bees, the white flowers with many stamens provide a good source of nectar and pollen. The petals of the flowers fall in the afternoon after they open in the morning. Bees pollinated the flower.

The secondary pollen discovered in the sample had 1 species only which was *Cyperus brevifolius* with percentage abundance of 23.14%. The important minor pollen from this region that been observed were *Gliricidia sepium* (3.08%), *Combretum indicum* (6.04%), *Tecomaria capensis* (4.88%) and *Antigonan leptopus* (5.78%). The minor pollen discovered in this sample were *Cyperus aromaticus* (1.67%), *Veitchia merrillii* (0.90%), *Ipomea pes-caprae* (0.39%), *Neomarica longifolia* (1.41%), *Ischaemum muticum* (1.93%), *Artocarpus heterophyllus* (2.83%), *Sporobolum indicum* (0.90%), *Anacardium accidentale* (0.64%), *Melastoma malabathricum* (1.16%), *Pterocarpus indicus* (0.51%) and *Manihot esculenta* (1.03%).

According to some studies, bees are the world's principal pollinators and perform critical roles in wild farmed plants where insect pollination is critical. Guar Sanji honey was unifloral in nature. Because they are made mostly from the nectar of one variety of flower, unifloral honeys are thought to be more desirable (Spanik et al., 2014). Each type of unifloral honey has its distinct physico-chemical qualities, which influence customer preference (Agashe & Caulton, 2009).

4.7 Honey samples from Sanglang, Perlis

Table 4.7: The number of pollen and percentage of abundance of pollen types in the honey samples from Sanglang.

Plant species	**Family**	**No. of pollen**	**Percentage of abundance (%)**
Combretum indica	Combretacea	421	43.72%
Capsicum fructescenes	Solanceae	38	3.95%
Tecomaria capensis	Bignoniaceae	180	18.69%
Mimosa invisa	Fabaceae	25	2.60%
Moringa pterygosperma	Moringaceae	70	7.27%
Antigonan leptopus	Polygonaceae	150	15.58%
Veitchia merrillii	Areceae	17	1.77%
Cocos nucifera	Arecaceae	12	1.25%
Melastoma malabathricum	Melastomataceae	3	0.31%
Solanum torvum	Solanaceae	13	1.35%
Dillenia suffructocosa	Dilleniaceae	6	0.62%
Amaranthus lividus	Amaranthaceae	7	0.73%
Anacardium occidentale	Anacardiaceae	15	1.56%
Asystasia gangetica	Acanthaceae	4	0.42%
Bougainvillea sp.	Nyctaginoceae	2	0.21%
TOTAL NUMBER OF POLLEN		963	

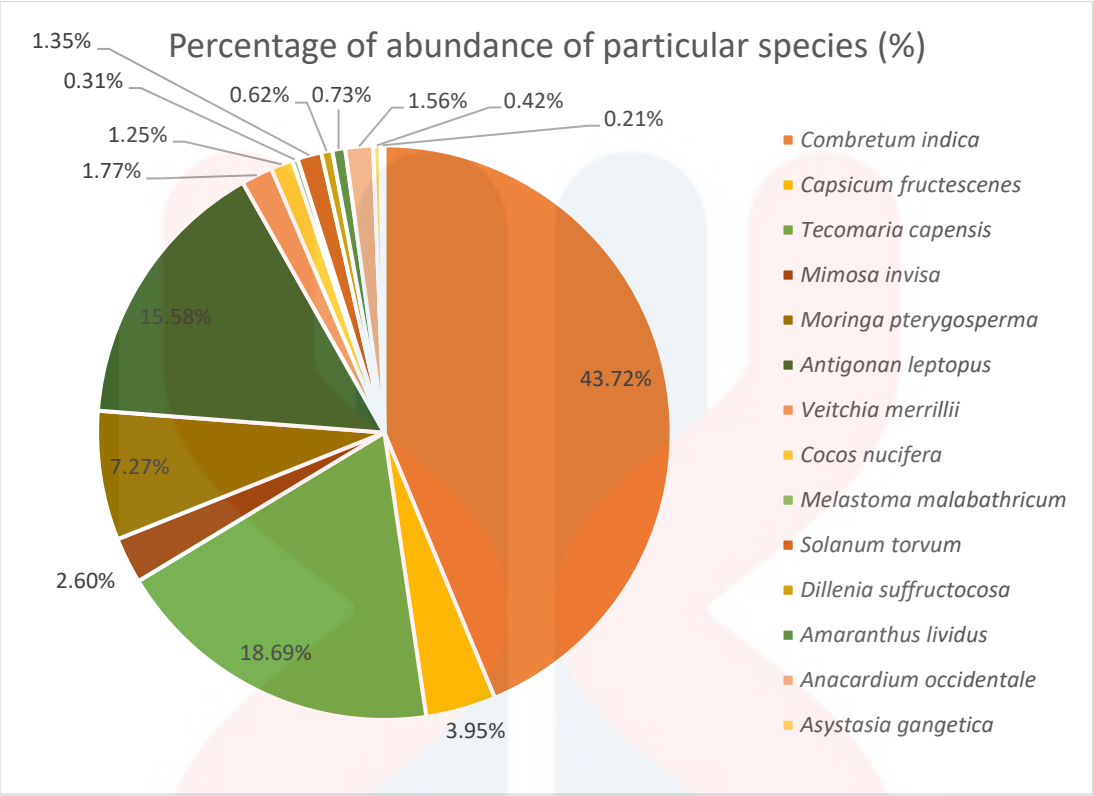

Figure 4.7: Percentage abundance of honey sample from Sanglang

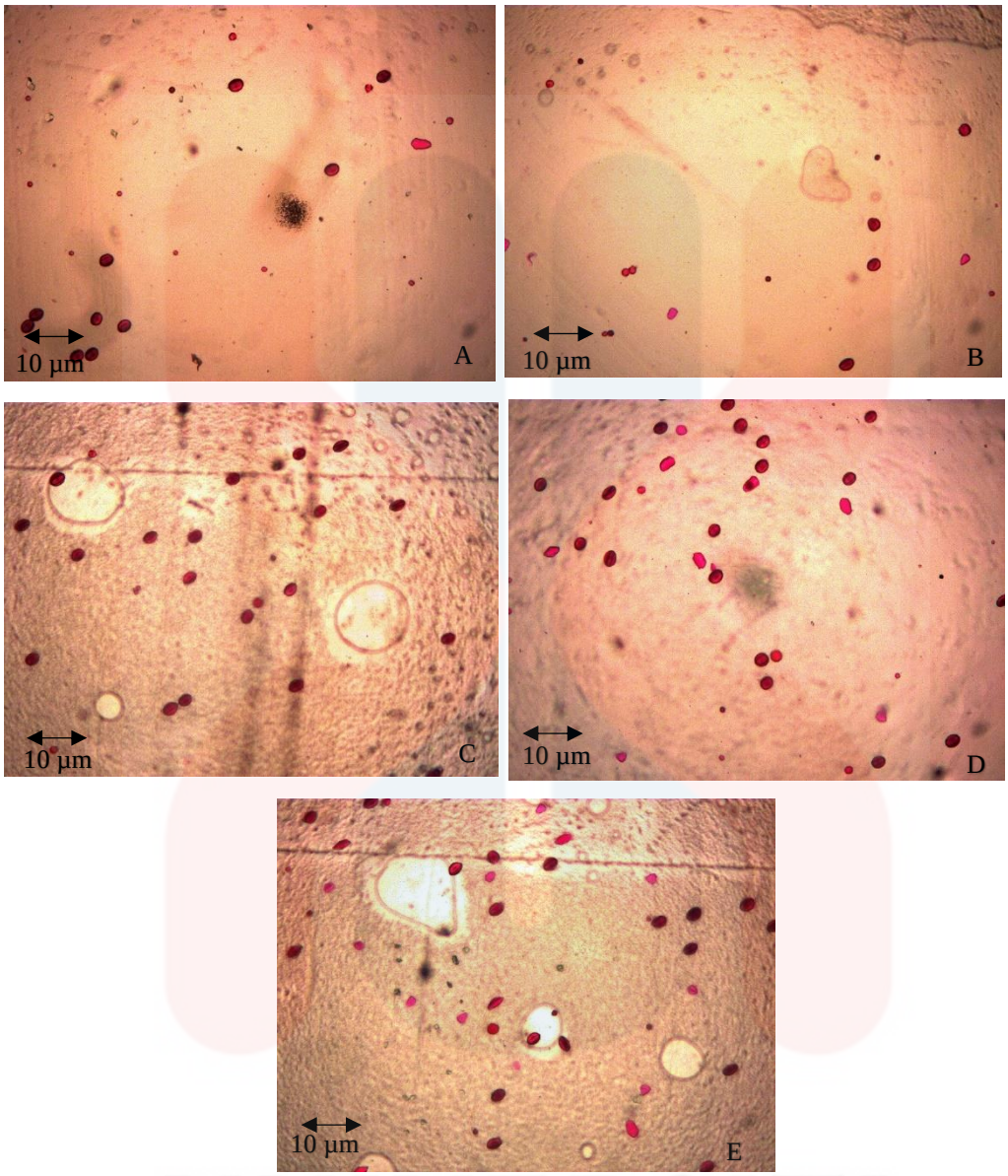

Figure 4.7 (a): Microscopic overviews of pollen density of honey sample from Sanglang under 10x magnification. Overviews of A- left edge of cover slip (top); B- right edge of cover slip (top); C- left edge of the cover slip (bottom); D- right edge of the cover slip (bottom); E-middle of the cover slip

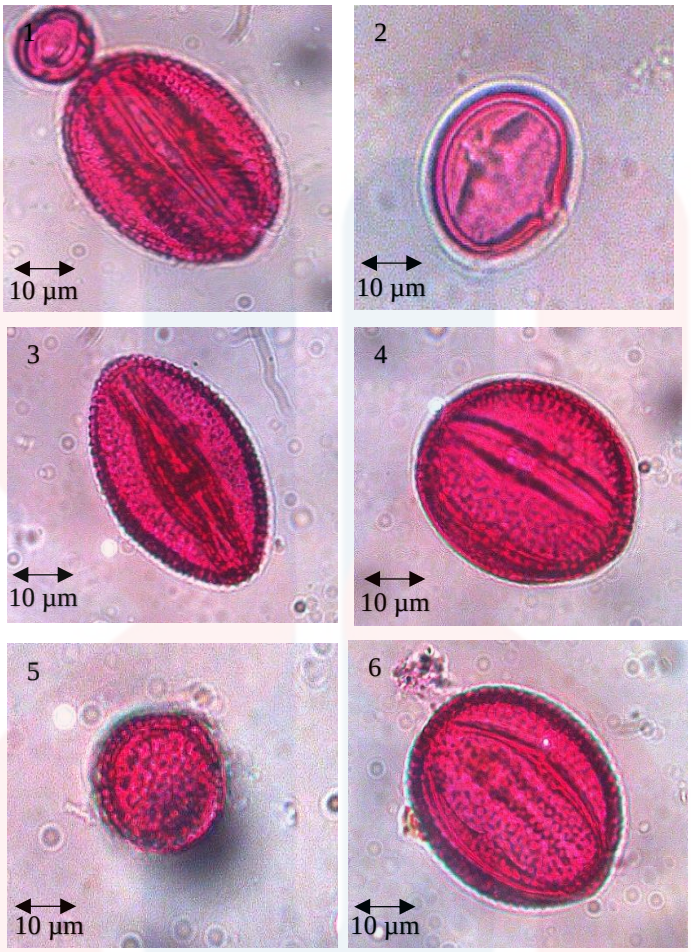

Figure 4.7 (b): The morphology of different pollen under 40x magnification.1-*Combretum indicum* (Combretaceae); 2-*Capsicum fructescenes* (Solanaceae); 3-*Tecomaria capensis* (Bignoniaceae); 4-*Mimosa invisa* (Fabaceae); 5-*Moringa pterygosperma* (Moringaceae); 6-*Antigonan leptopus* (Polygonaceae).

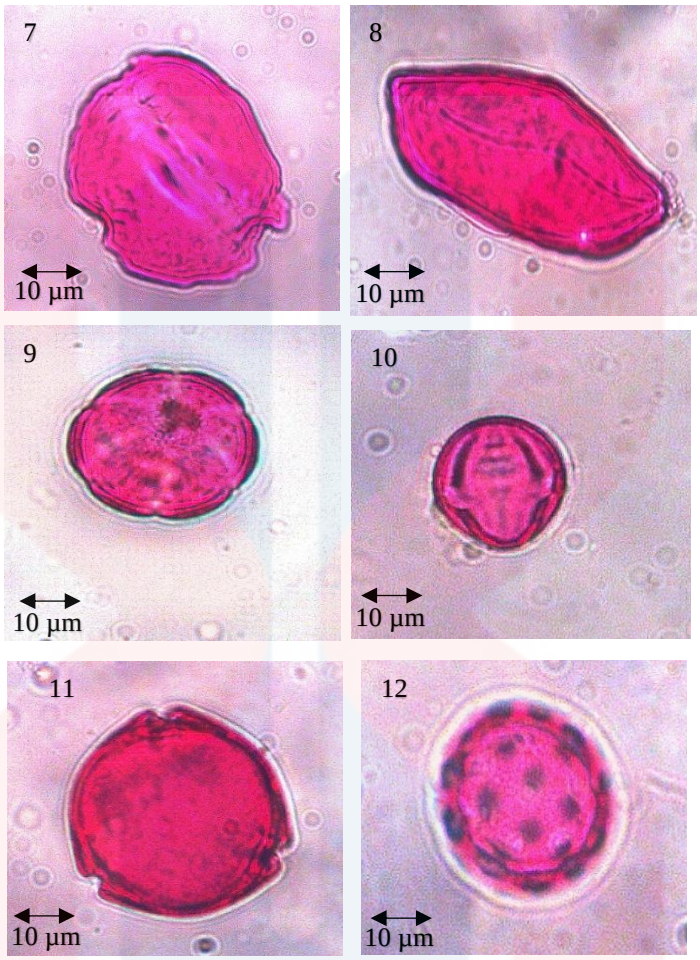

Figure 4.7 (c): The morphology of different pollen under 40x magnification. 7-*Veitchia merrillii* (Arecaceae); 8-*Cocos nucifera* (Arecaceae); 9-*Melastoma malabathricum* (Melastomataceae); 10-*Solanum torvum* (Solanaceae); 11-*Dillenia suffructocosa* (Dilleniaceae); 12-*Amaranthus lividus* (Amaranthaceae).

Figure 4.7 (d): The morphology of different pollen under 40x magnification. 13-Anacardium accidentale (Anacardiaceae); 14-Asystasia gangetica (Acanthaceae); 15-Bougainvillea sp. (Nyctaginoceae).

Sanglang had 15 species in the sample and the total number of pollens was 963. The highest percentage abundance in this sample belonged to *Combretum indicum* (43.72%). Therefore, type of honey sample from Sanglang was unifloral honey. This species also found in previous sample but it was categorized as secondary pollen The species plant was dominates by stingless bee preference to forage nectar and pollen. The lowest percentage abundance of pollen belonged to species *Bougainvillea sp.* from Nytaginoceae family. Species Bougainvillea sp. had present in previous sample from Padang Pauh, Tambun Tulang , Sena and thus become minor pollen in honey sample.

The secondary pollen was with percentage more than 16% contributed to only 1 species such as *Tecomaria capensis*. Then, followed by important minor pollen with percentage abundance between 3% to 16% such as *Capsicum fructescenes*, *Moringa pterygosperma*, and *Antigonan leptopus.* Last but not least were species from *Mimosa invisa*, *Veitchia merrillii, Cocos nucifera, Melastoma malabathricum, Solanum torvum, Dillenia suffructocosa, Amaranthus lividus, Anacardium occidentale, Asystasia gangetica* and *Bougainvillea sp.* which is only got less than 3% from the pollen spectrum.

The species *Combretum indica* common name was 'Double Rangoon Creeper'. A climber who prefers the woods. Hooks are used to help it climb. The leaves are elliptic to elliptic-oblong in shape and are abaxially pilose and adaxially glorious save for the brown and pilose mid-vein. As a climbing aid, the leaf petiole is transformed into a thorny grappling hook. The white double-petaled blossoms are carried on a suspended raceme, and the upper surface of the flower develops pink and eventually crimson over time.

The cashew nut, scientifically known as Anacardium, is a primary source of nectar for stingless bee honey. This cashew pollen has a pleasant scent and a light colour. Pollen is a key protein food source for honey bees in a given colony, and honey bees play an important role in the secretion of bee nutrition (Ismail et al., 2013).

4.8 Honey samples from Tambun Tulang, Perlis

Table 4.8: The number of pollen and percentage of abundance of pollen types in the honey samples from Tambun Tulang.

Plant species	Family	No. of pollen	Percentage of abundance (%)
Combretum indica	Combretaceae	237	12.74%
Citrus aurantifolium	Rutaceae	260	13.98%
Antigonan leptopus	Polygonaceae	288	15.48%
Capsicum fructescenes	Solanaceae	92	4.95%
Mutingia calabura	Mutingiaceae	217	11.67%
Pterocarpus indicus	Fabaceae	180	9.68%
Melastoma malabathricum	Melastomataceae	50	2.69%
Areca catechu	Arecaceae	111	5.97%
Avicennia alba	Verbenaceae	118	6.34%
Fragraea fragrans	Gentianaceae	190	10.22%
Amaranthus spinulosus	Amaranthaceae	87	4.68%
Bougainvillea sp.	Nyctoginoceae	30	1.61%
TOTAL NUMBER OF POLLEN		1860	

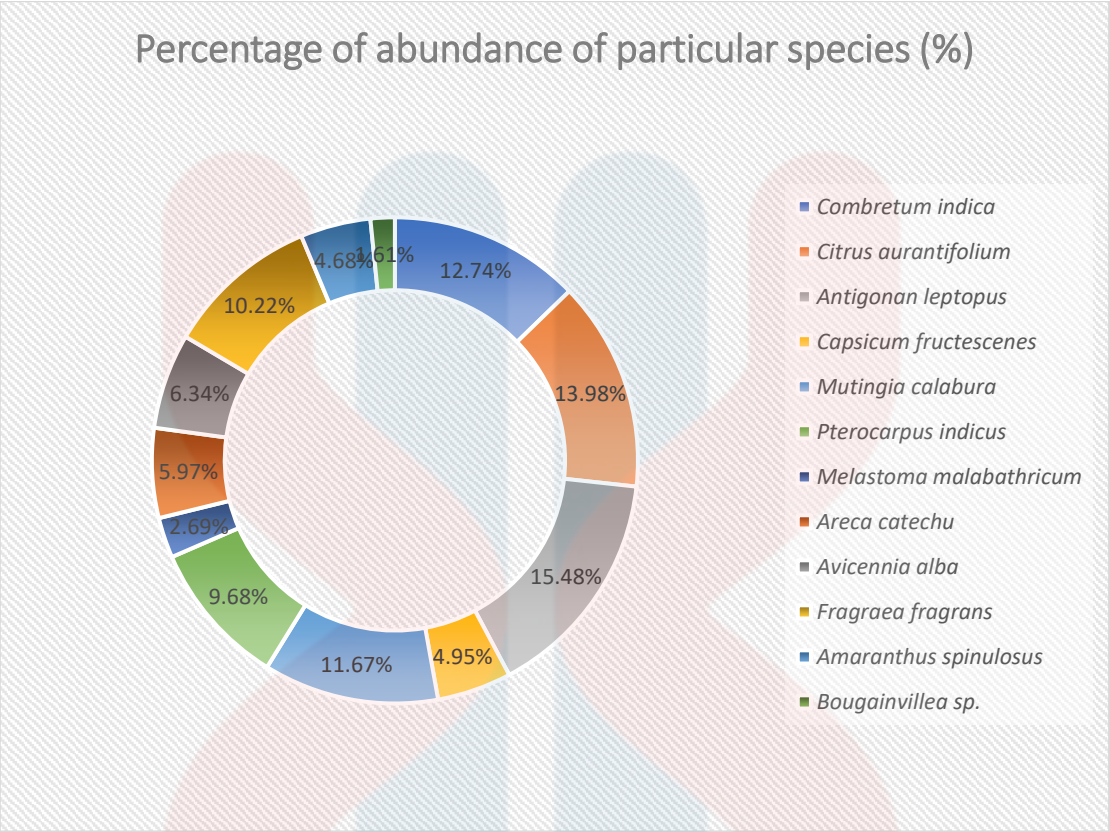

Figure 4.8: Percentage abundance of honey sample from Tambun Tulang

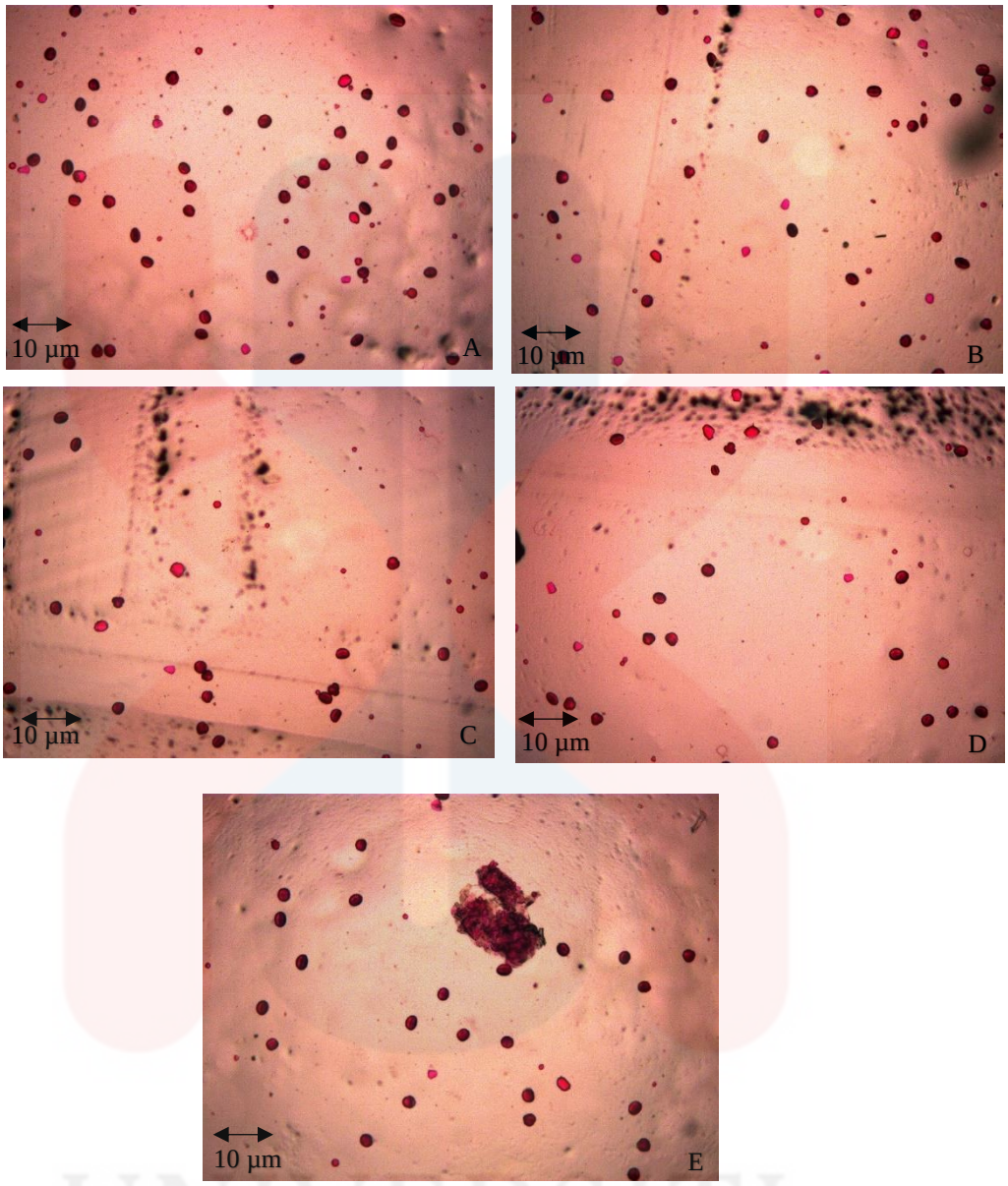

Figure 4.8 (a): Microscopic overviews of pollen density of honey sample from Tambun Tulang under 10x magnification. Overviews of A- left edge of cover slip (top); B- right edge of cover slip (top); C- left edge of the cover slip (bottom); D- right edge of the cover slip (bottom); E-middle of the cover slip

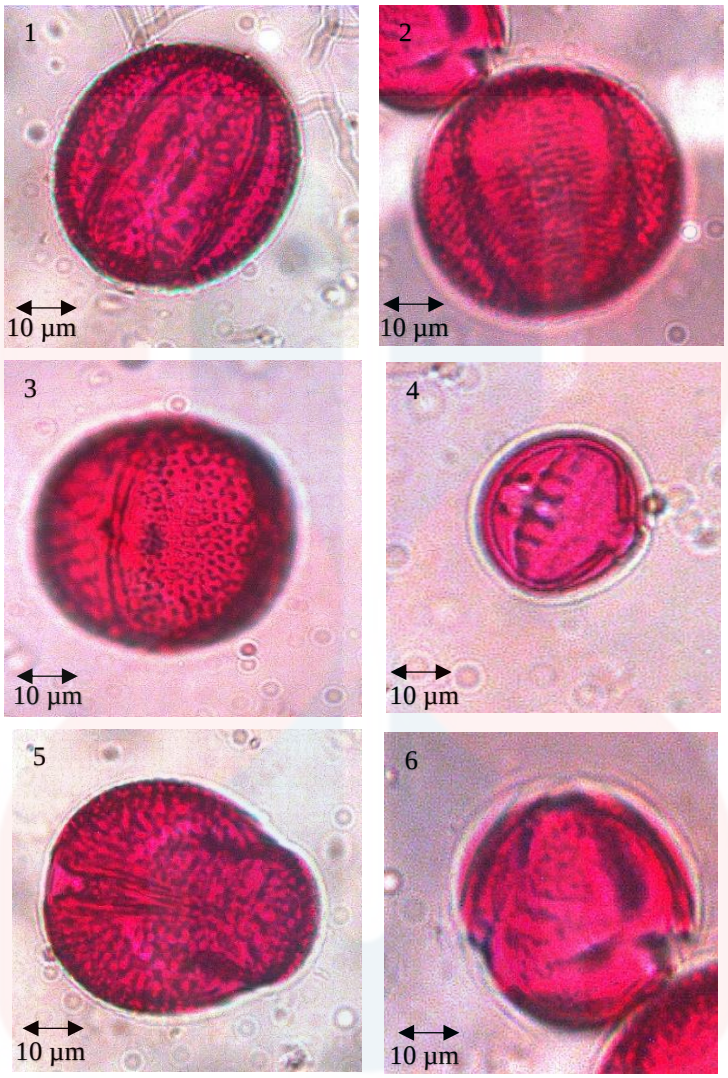

Figure 4.8 (b): The morphology of different pollen under 40x magnification. 1-*Combretum indicum* (Combretaceae); 2-*Citrus aurantifolium* (Rutaceae); 3-*Antigonan leptopus* (Polygonaceae); 4-*Capsicum fructescenes* (Solanaceae); 5-*Mutingia calabura* (Mutingiaceae); 6-*Pterocarpus indicus* (Fabaceae).

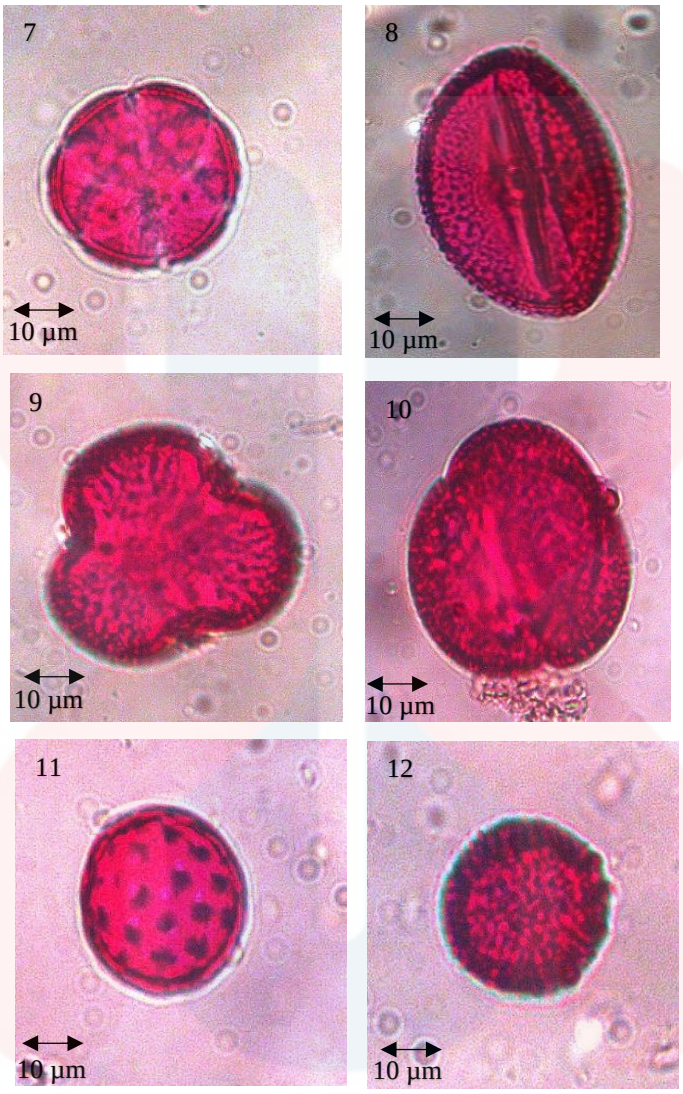

Figure 4.8 (c): The morphology of different pollen under 40x magnification. 7-*Melastomata malabathricum* (Melastomataceae); 8-*Areca catechu* (Arecaceae); 9-*Avicennia alba* (Verbenaceae); 10-*Fragraea fragrans* (Gentianaceae); 11-*Amaranthus lividus* (Amaranthaceae); 12-*Bougainvillea sp.* (Nyctaginaceae).

12 different types of pollen species were present in this honey sample from Tambun Tulang. The total number of pollens counted in this sample was 1860 and became the highest pollen counted in all sample collected. Analysis had been done which showed that there was no predominant taxon of pollen found in Tambun Tulang. Thus, the sample from Tambun Tulang was the multifloral honey. The most abundance pollen was *Antigonan leptopus* plant species accounting up to 15.48% of pollen abundance count. There was none secondary pollen found in the sample. *Combretum indica* (12.74%), *Citrus aurantifolium* (13.98%), *Antigonan leptopus* (15.48%), *Capsicum fructescenes* (4.95%), *Mutingia calabura* (11.67%), *Pterocarpus indicus* (9.68%), *Areca catechu* (5.97%), *Avicennia alba* (6.34%), *Fragraea fragrans* (10.22%), and *Amaranthus spinulosus* (4.68%). were classified as important minor pollen. The next category, minor pollen also found in the sample namely *Melastoma malabathricum* (2.69%), and *Bougainvillea sp.* (1.61%). The lowest abundance of pollen was *Bougainvillea sp.* comes from Nytaginoceae family and the highest percentage abundance was *Antigonan leptopus* comes from Polygonaceae family.

The common name of Antigonan leptopus was called as coral vine. The Coral Vine (Antigonon leptopus), which is adorned with beautiful pink blossoms, is a prolific bloomer that is always flowering under bright sunlight. The flowers provide a lot of nectar, so bees and butterflies can eat them. The heart-shaped leaves of this lush climber drape over trellises, providing a dense canopy that provides shade from the sun. The Coral Vine has cultivated forms that either white or dark crimson blossoms. Herbaceous slender-stemmed vine with tendrils growing from inflorescence ends that climb.

When established, it is heat and drought resistant. Fast-growing, with rich foliage that soon covers walls, trellises, and arbours. In Malaysia, plants may only develop blossoms near the apex of their growth. Flowers are brilliant pink and fragrant, and they bloom in cascading racemes that attract bees and butterflies (Rajput, 2015).

Honey bee often collect pollen from flowers nearest to their surroundings. Another 3 different types of pollen had been identified as secondary pollen in this multifloral honey. Among them were Cassia biflora (Leguminosae), Cocos nucifera (Arecaceae), and Manihot esculenta (Euphorbiaceae). No important minor pollens were present in this sample.

4.9 Honey samples from Sena, Perlis

Table 4.9: The number of pollen and percentage of abundance of pollen types in the honey samples from Sena.

Plant species	**Family**	**No. of pollen**	**Percentage of abundance (%)**
Areca catechu	Arecaceae	165	11.19%
Artocarpus heterophyllus	Moraceae	170	11.53%
Brassica chinensis	Brassicaceae	70	4.75%
Pterocarpus indicus	Fabaceae	130	8.82%
Bougainvillea sp.	Nytaginoceae	45	3.05%
Amaranthus lividus	Amaranthaceae	32	2.17%
Turmera sp.	Passifloraceae	88	5.97%
Leucaena leucocephala	Leguminosae	180	12.21%
Tamarindus indica	Fabaceae	132	8.96%
Vitex pinnata	Verbenaceae	65	4.41%
Antigonan leptopus	Polygonaceae	210	14.25%
Combretum indica	Combretaceae	187	12.69%
TOTAL NUMBER OF POLLEN		1474	

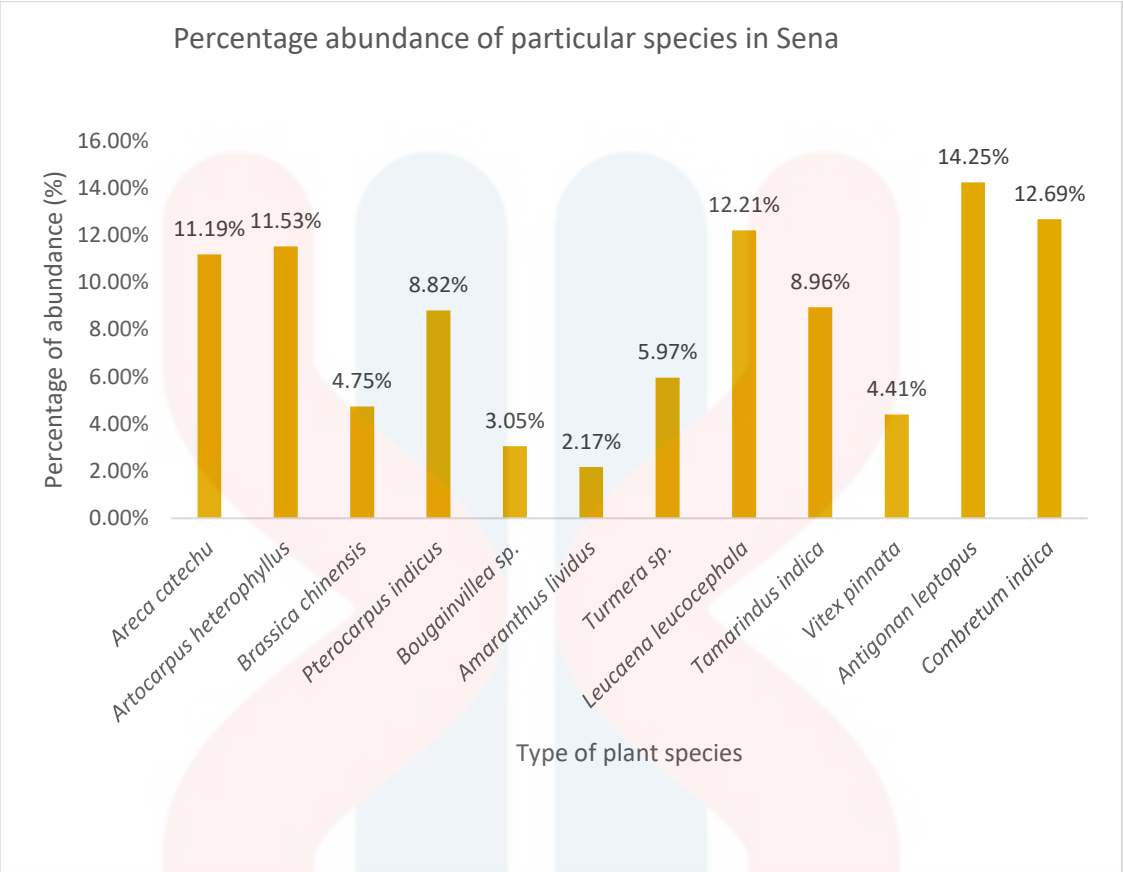

Figure 4.9: Percentage abundance of honey sample from Sena

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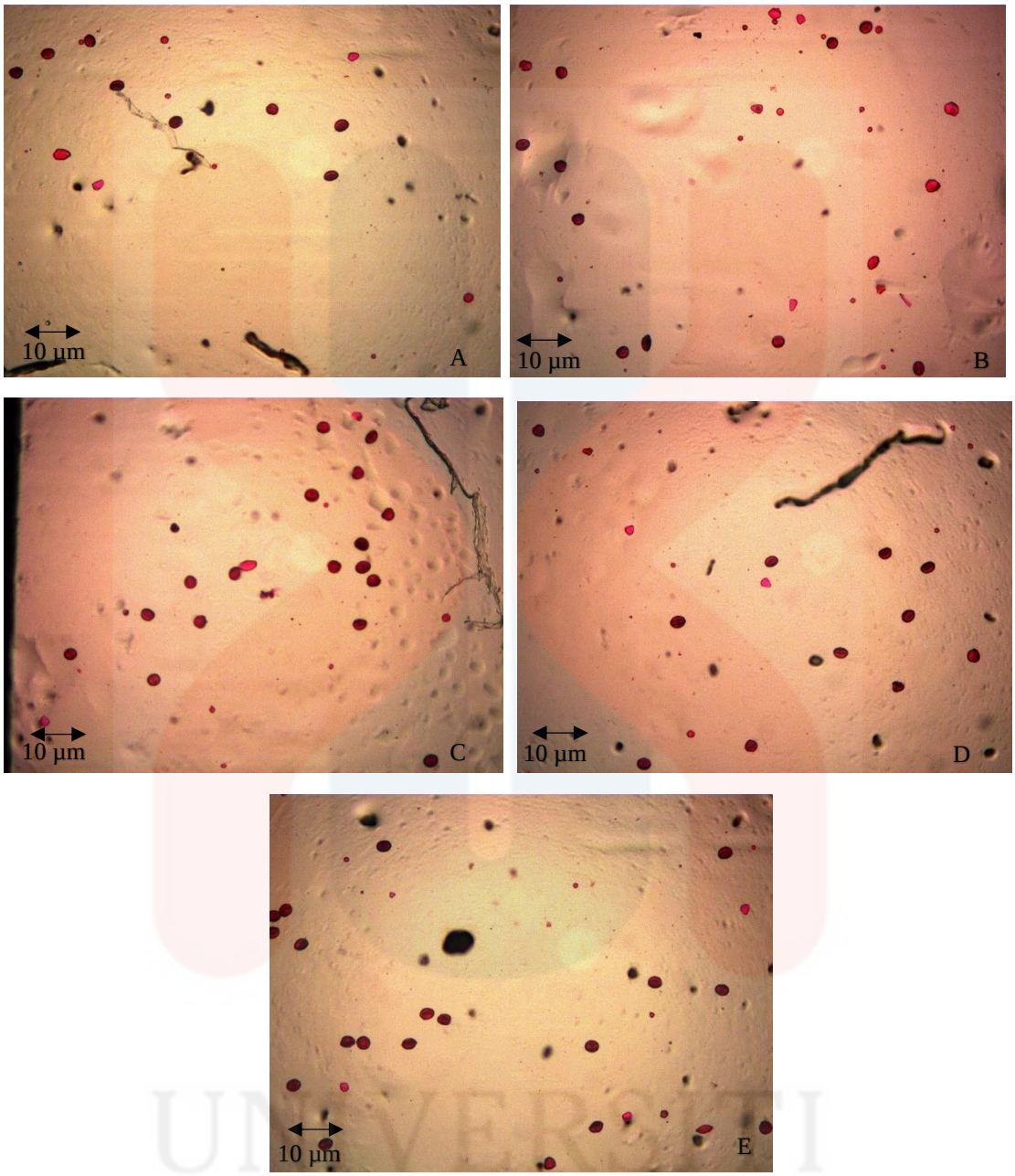

Figure 4.9 (a): Microscopic overviews of pollen density of honey sample from Sena under 10x magnification. Overviews of A- left edge of cover slip (top); B- right edge of cover slip (top); C- left edge of the cover slip (bottom); D- right edge of the cover slip (bottom); E-middle of the cover slip

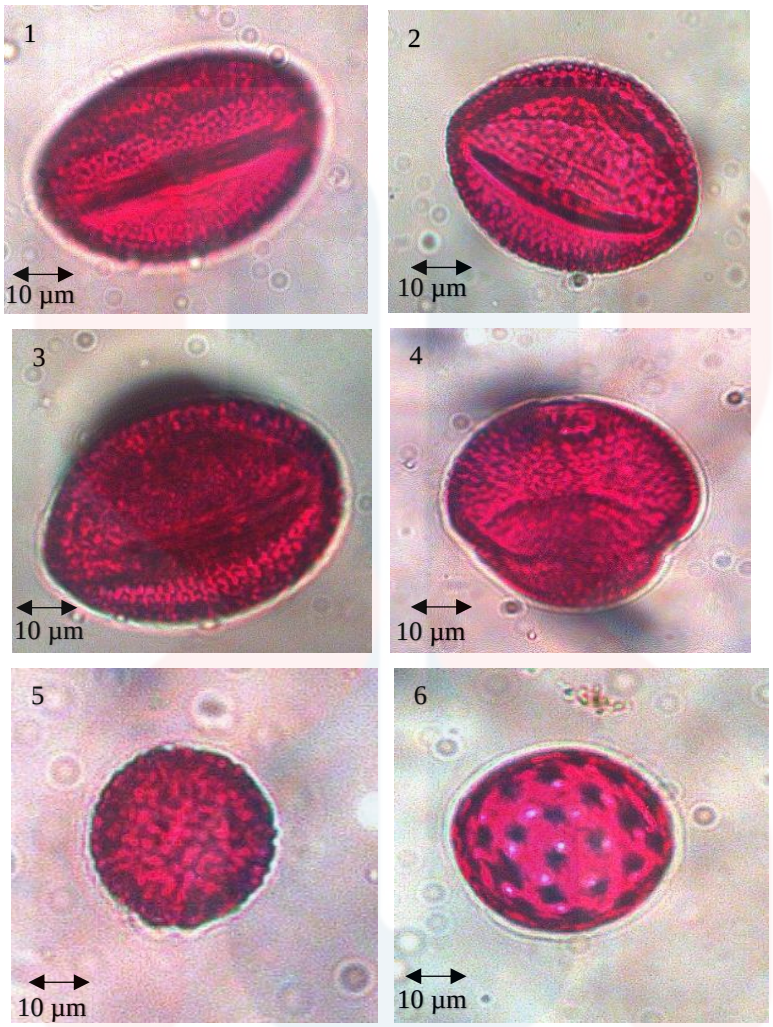

Figure 4.9 (b): The morphology of different pollen under 40x magnification. 1-*Areca catechu* (Arecaceae); 2-*Artocarpus heterophyllus* (Moraceae); 3-*Brassica chinensis* (Brassicaceae); 4-*Pterocarpus indicus* (Leguminosae); 5-*Bougainvillea sp.* (Nyctaginoceae); 6-*Amaranthus lividus* (Amaranthaceae).

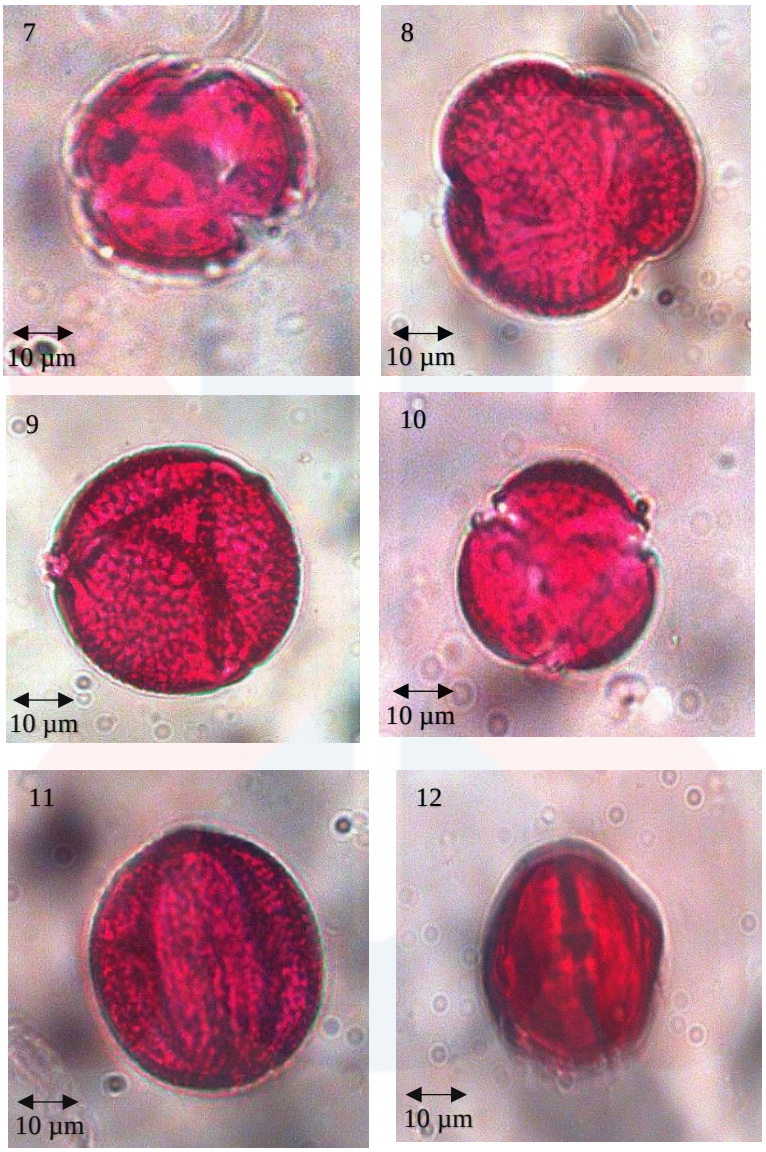

Figure 4.9 (c): The morphology of different pollen under 40x magnification. 7-*Turmera sp.* (Passifloraceae); 8-*Leucaena leucocephala* (Leguminosae); 9-*Tamarindus indica* (Fabaceae); 10-*Vitex pinnata* (Verbenaceae); 11-*Antigonan leptopus* (Polygonaceae); 12-*Combretum indica* (Combretaceae).

Analysis on the honey samples from Sena showed the occurrence of 12 different types of pollen, namely *Areca catechu* (Arecaceae), *Artocarpus heterophyllus* (Moraceae), *Brassica chinensis* (Brassicaceae), *Pterocarpus indicus* (Leguminosae), *Bougainvillea sp.* (Nytaginoceae), *Amaranthus lividus* (Amaranthaceae), *Turmera sp*. (Passfloraceae), *Leucaena leucocephala* (Leguminosae), *Tamarindus indica* (Fabaceae), *Vitex pinnata* (Verbenaceae), *Antigonan leptopus* (Polygonaceae) and *Combretum indica* (Combretaceae). The total number of pollens counted in this sample was 1474 and contributed to second highest of pollen counted. The most abundant pollen species collected in Sena was *Antigonan leptopus* produced by flower with 14.25% of abundancy. In this sample only *Amaranthus lividus* (2.17%) was categorised under the minor pollen of pollen group due to its percentage abundance less than 3%. The secondary pollen in the sample was not detected.Meanwhile, the rest of plant species such as *Areca catechu* (Arecaceae), *Artocarpus heterophyllus* (Moraceae), *Brassica chinensis* (Brassicaceae), *Pterocarpus indicus* (Leguminosae), *Bougainvillea sp.* (Nytaginoceae), *Turmera sp*. (Passfloraceae), *Leucaena leucocephala* (Leguminosae), *Tamarindus indica* (Fabaceae), *Vitex pinnata* (Verbenaceae), *Antigonan leptopus* (Polygonaceae) and *Combretum indica* (Combretaceae).were categorised as important minor pollen because of their pollen count were range from 3-16%. The similarity from previous sample Tambun Tulang and this sample Sena were both contain plant species *Antigonan leptopus* as the highest pollen abundance in the sample.

This clearly demonstrated that honey bees less prefer to forage on the *Amaranthus lividus* species, whose blossoms may be found all year in Sena. Common name for *Amaranthus lividus* species was ‘Bayam itik’ This frequent plant of roadsides and open waste ground has modest pale-yellow blooms. It will continue to flower once it has reached maturity. Because bees constantly visit its blossom, it is a good supply of pollen. Pollen from this plant can be discovered in honey from mixed woodland regions. The unarmed *Amaranthus lividus* can be recognised from the spiny *Amaranthus spinulosus* Melissopalynology is particularly beneficial in beekeeping operation and can also be used to predict when unifloral honey will be produced (Upadhyay et al., 2014).

4.10 Honey samples from Kayang, Perlis

Table 4.10: The number of pollen and percentage of abundance of pollen types in the honey samples from Kayang.

Plant species	Family	No. of pollen	Percentage of abundance (%)
Ananas cosmosus	Bromeliaceae	4	1.48%
Cucumis sativum	Cucurbitaceae	7	2.58%
Citrus aurantifolium	Rutaceae	21	7.75%
Combretum indicum	Combretaceae	16	5.90%
Sporobolus indicum	Poaceae	32	11.81%
Areca catechu	Arecaceae	40	14.76%
Artocarpus heterophyllus	Moraceae	35	12.92%
Oryza sativa	Poaceae	14	5.17%
Zea mays	Poaceae	9	3.32%
Veitchia merrillii	Arecaceae	13	4.80%
Acacia auriculiformis	Fabaceae	45	16.61%
Fragraea fragrans	Gentianaceae	35	12.92%
TOTAL NUMBER OF POLLEN		271	

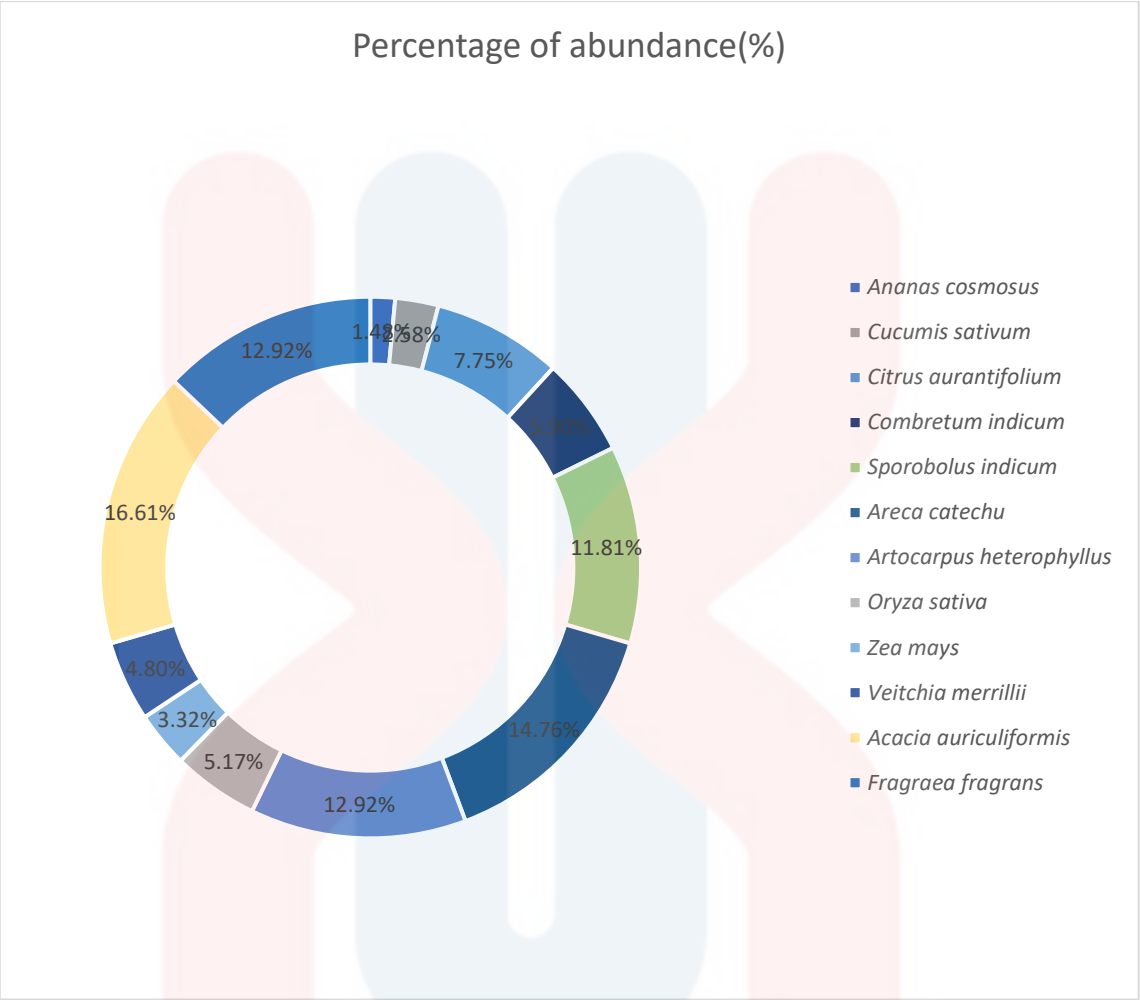

Figure 4.10: Percentage abundance of honey sample from Kayang

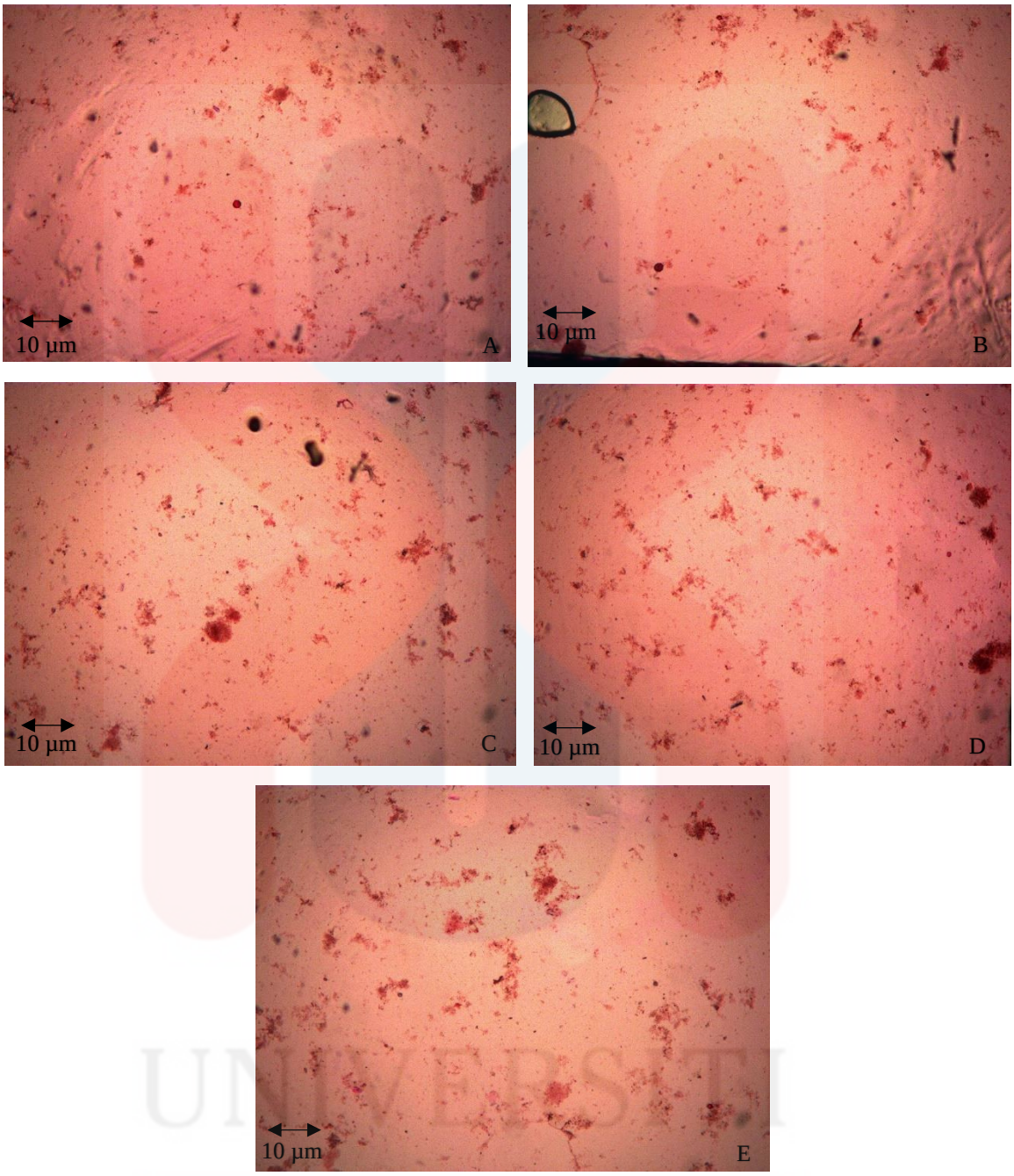

Figure 4.10 (a): Microscopic overviews of pollen density of honey sample from Kayang under 10x magnification. Overviews of A- left edge of cover slip (top); B- right edge of cover slip (top); C- left edge of the cover slip (bottom); D- right edge of the cover slip (bottom); E-middle of the cover slip

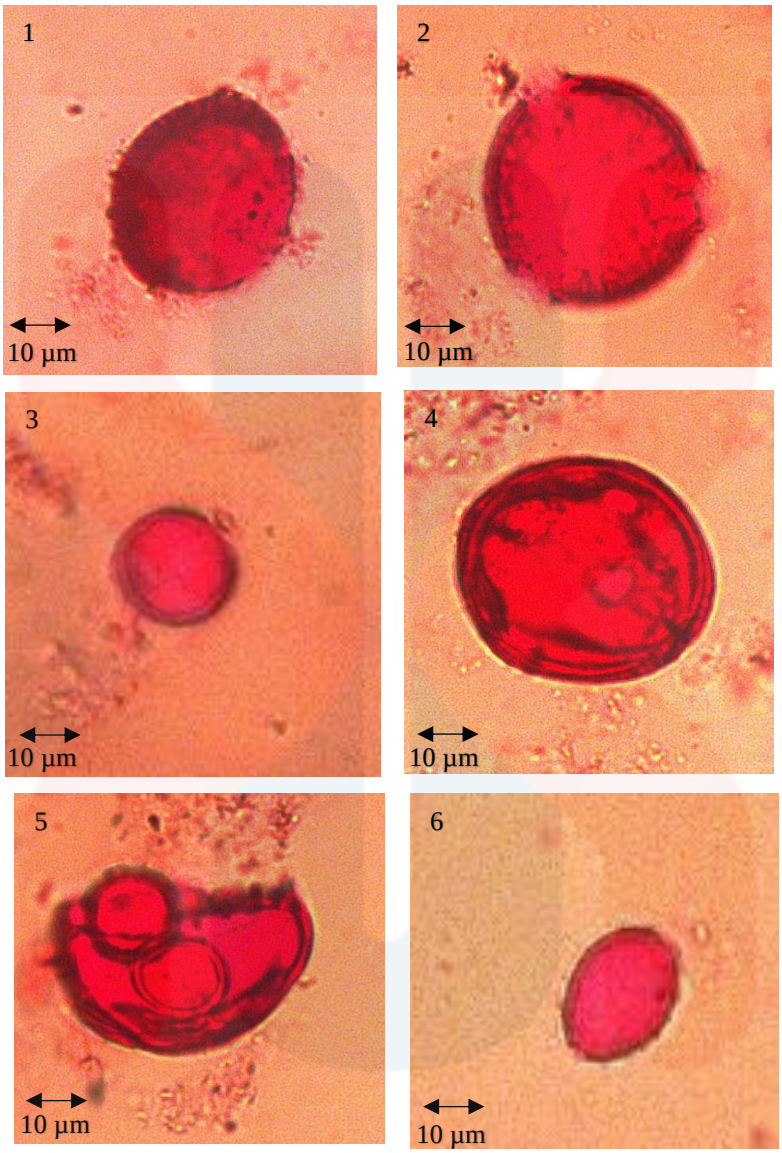

Figure 4.10 (b): The morphology of different pollen under 40x magnification. 1-*Ananas cosmosus* (Bromeliaceae); 2-*Cucumis sativus* (Cucrbitaceae); 3-Citrus aurantifolium (Rutaceae); 4- *Combretum indicum* (Combretaceae); 5- *Sporobolus indicum* (Gramineae); 6- *Areca catechu* (Palmae).

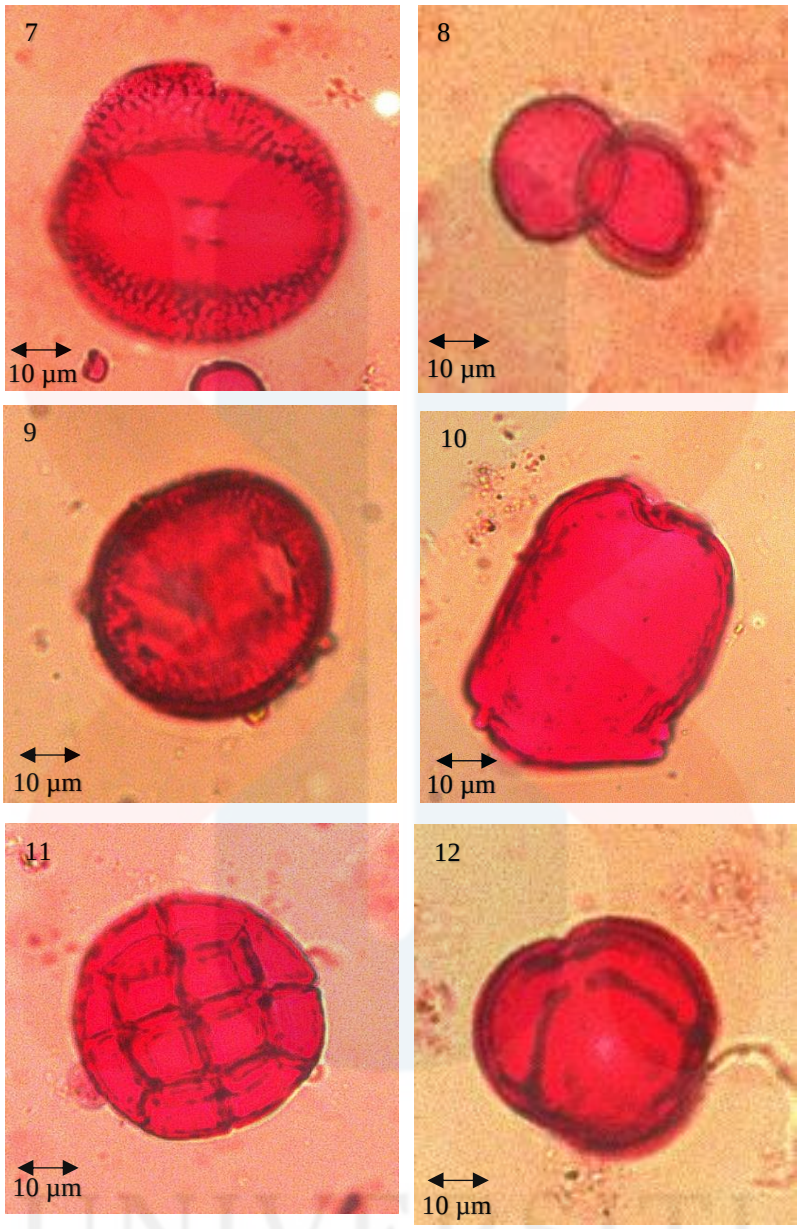

Figure 4.10 (c): The morphology of different pollen under 40x magnification. 7-*Artocarpus heterophyllus (Moraceae); 8- Oryza sativa* (Bramineae); 9- *Zea mays (Poaceae)*; 10-*Veitchia merrillii* (Arecaceae); 11-*Acacia auriculiformis* (Fabaceae); 12-*Fragraea fragrans* (Gentianaceae).

Based on the observation, pollen staining with Safranin was successful, giving the pollen a reddish colour under a compound microscope with camera. Due to the presence of debris and dirt on the microscopic slides, images of a few pollens did not appear as visible under microscope viewing.

Analysis on the honey samples collected from Kayang depicted the presence of 12 different species of pollen. There were total number of pollens counted to 271 in the sample. There was none present of premilinary pollen, so types of honey from sample from Kayang was multifloral honey. Secondary pollen in this sample was *Acacia auriculiformis* (Leguminosae) with highest percentage of abundance 16.61%. *Acacia auriculiformis* was a squarish tree with a reticulate exine surface that was packed tightly together like bricks (polyad). Because the most abundant species were present, bees in Kayang regions preferred to scavenge blooms from those species.

Acacia auriculiformis was a medium-sized tree with distinctive crescent-shaped leaves that's widely planted along roadsides, and it's originally from tropical Australia. Its golden, mildly fragrant flowers bloom on and off throughout the year. It grows swiftly from seed and establishes itself in sandy soil. It is an important pollen source in non-coconut locations, and it is highly recommended for planting in apiaries as a protein supply during dearth seasons, especially December to February. Its blossoming frequency provides it a superior pollen supply than bees (Ruth & Muhammad, 1991).

The important minor pollen with percentage between 3% to 16% were belonged to 9 species of plants such as *Citrus aurantifolium, Combretum indicum, Sporobolus indicum, Areca catechu, Artocarpus heterophyllus, Oryza sativa, Zea mays, Veitchia merrillii* and *Fragraea fragrans* Lime is the common name for Citrus aurantifolium, a plant native to Malaysia that belongs to the Rutaceae family. Lime blooms have a strong fragrance and are a good nectar provider, attracting honey bees. *Ananas cosmosus* and Cucumis sativum were classified as minor pollen due to percentage abundance less than 3%.

4.11 Honey samples from Bintong, Perlis

Table 4.11: The number of pollen and percentage of abundance of pollen types in the honey samples from Bintong.

Plant species	**Family**	**No. of pollen**	**Percentage of abundance (%)**
Antigonan leptopus	Polygonaceae	75	41.90%
Mussaenda philippica	Rubiaceae	3	1.68%
Melastoma malabathricum	Melastomataceae	6	3.35%
Durio zibenthus	Bombacaceae	11	6.15%
Mimosa pudina	Fabaceae	14	7.82%
Brassica chinensis	Brassicaceae	27	15.08%
Biden pilosa	Asteraceae	9	5.03%
Capsicum sp.	Solanaceae	20	11.17%
Averrhoa carambola	Oxalidaceae	8	4.47%
Cosmos caudatus	Asteraceae	4	2.23%
Bruiguiera cylindrica	Rhizophoraceae	2	1.12%
TOTAL NUMBER OF POLLEN		179	

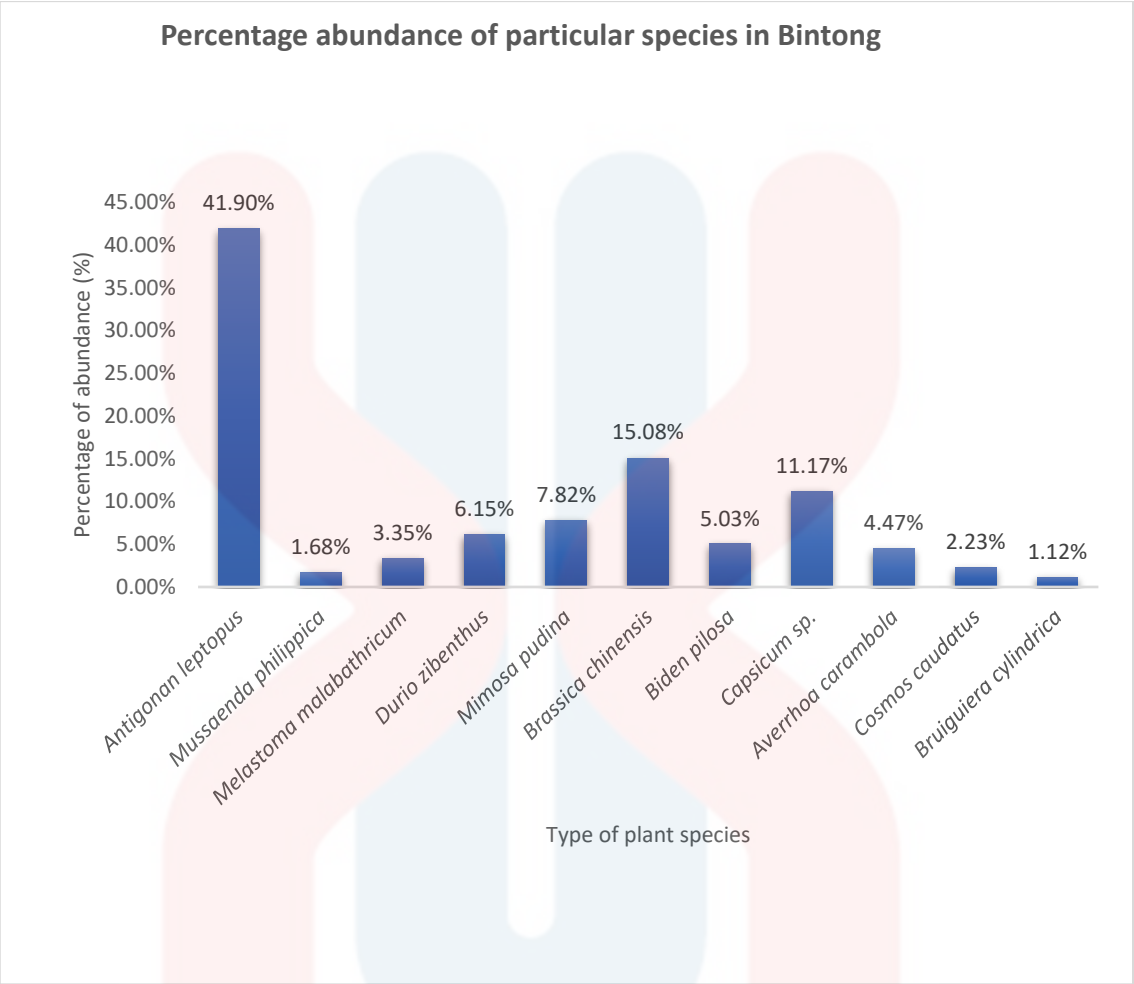

Figure 4.11: Percentage abundance of honey sample from Bintong

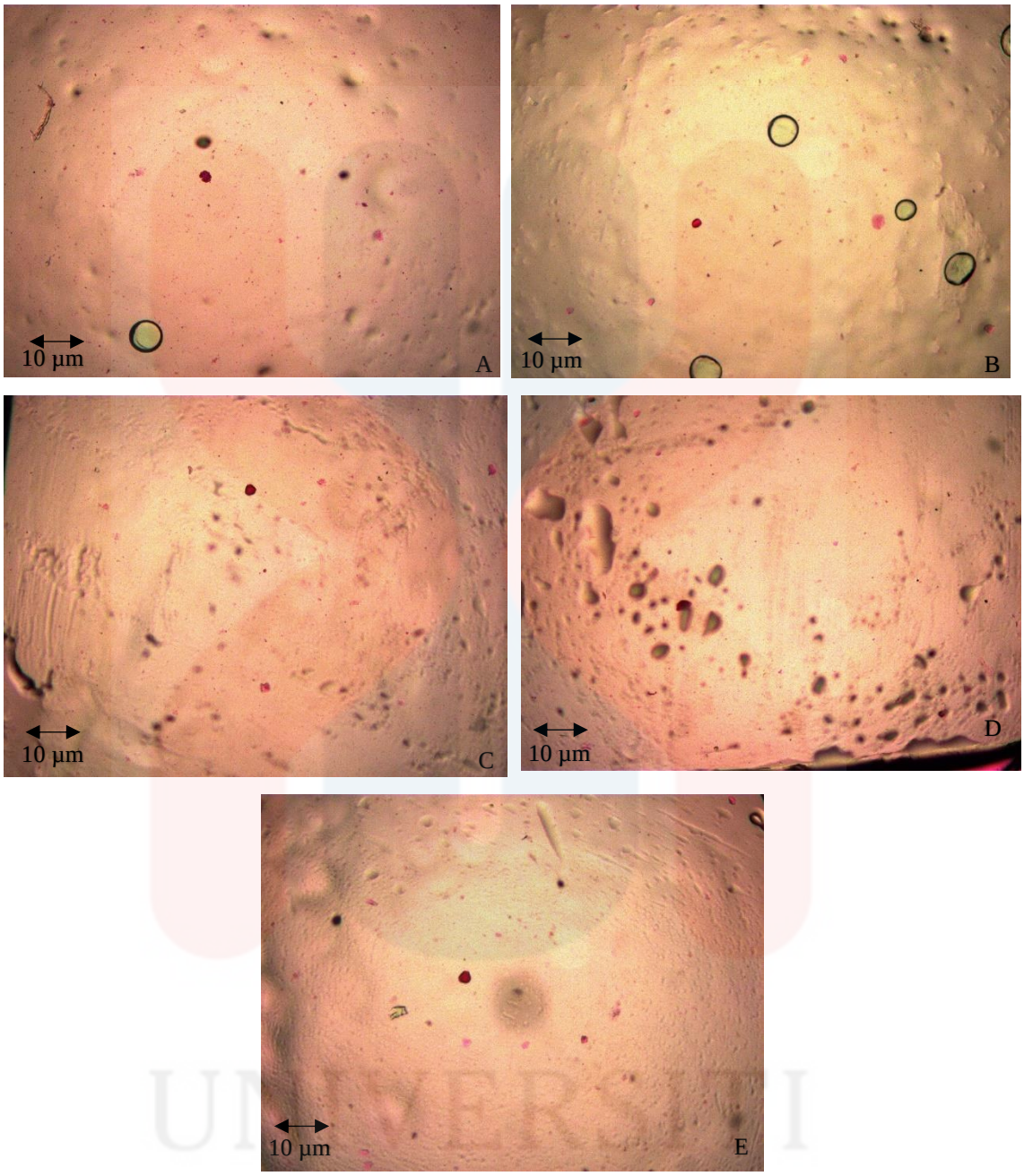

Figure 4.11 (a): Microscopic overviews of pollen density of honey sample from Bintong under 10x magnification. Overviews of A- left edge of cover slip (top); B- right edge of cover slip (top); C- left edge of the cover slip (bottom); D- right edge of the cover slip (bottom); E-middle of the cover slip

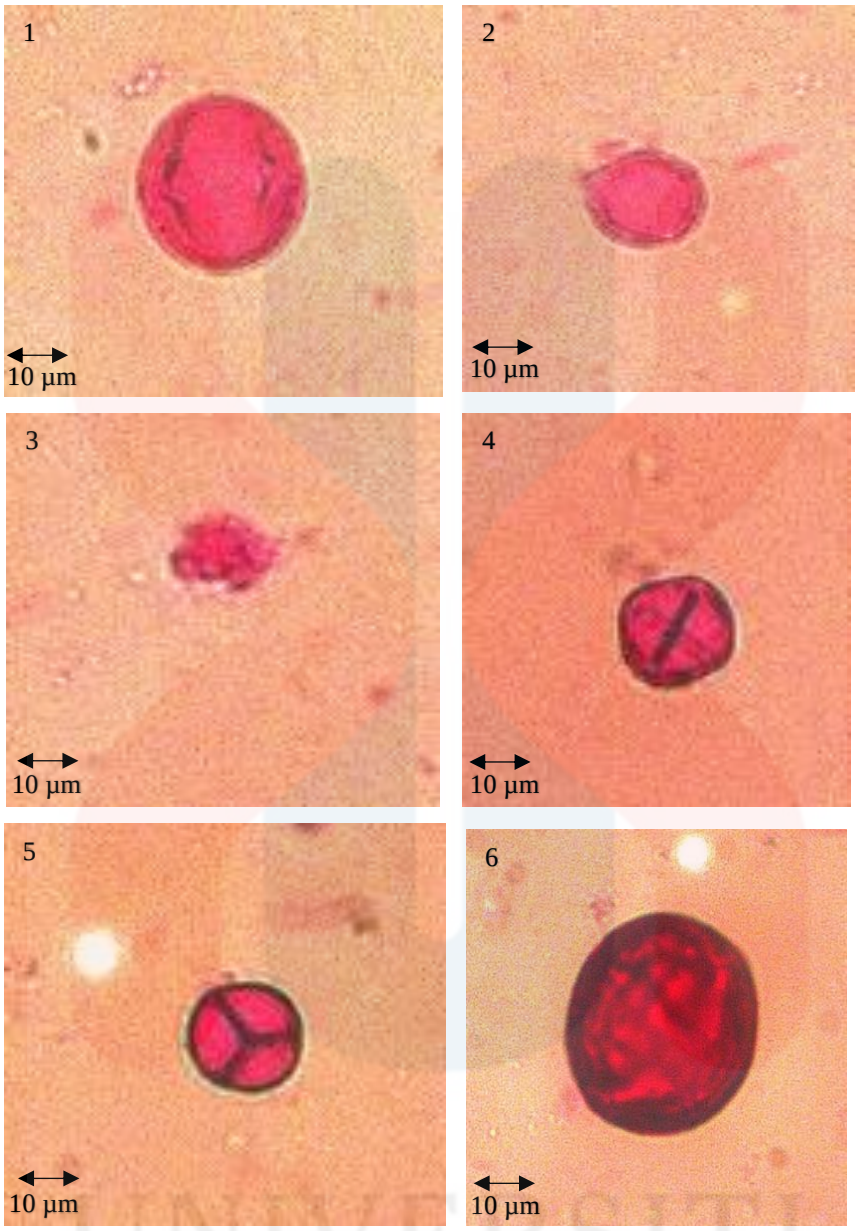

Figure 4.11 (b): The morphology of different pollen under 40x magnification. *1- Antigonon Leptopus* (Polygonaceae); 2- *Mussaenda philippica (Rubiceae)*; 3- *Melastoma malabathricum* (Melastomataceae); 4- *Durio zibethinus (Bombacaceae);* 5- *Mimosa Pudina* (Fabaceae); 6- *Brassica chinensis* (Brassicaceae).

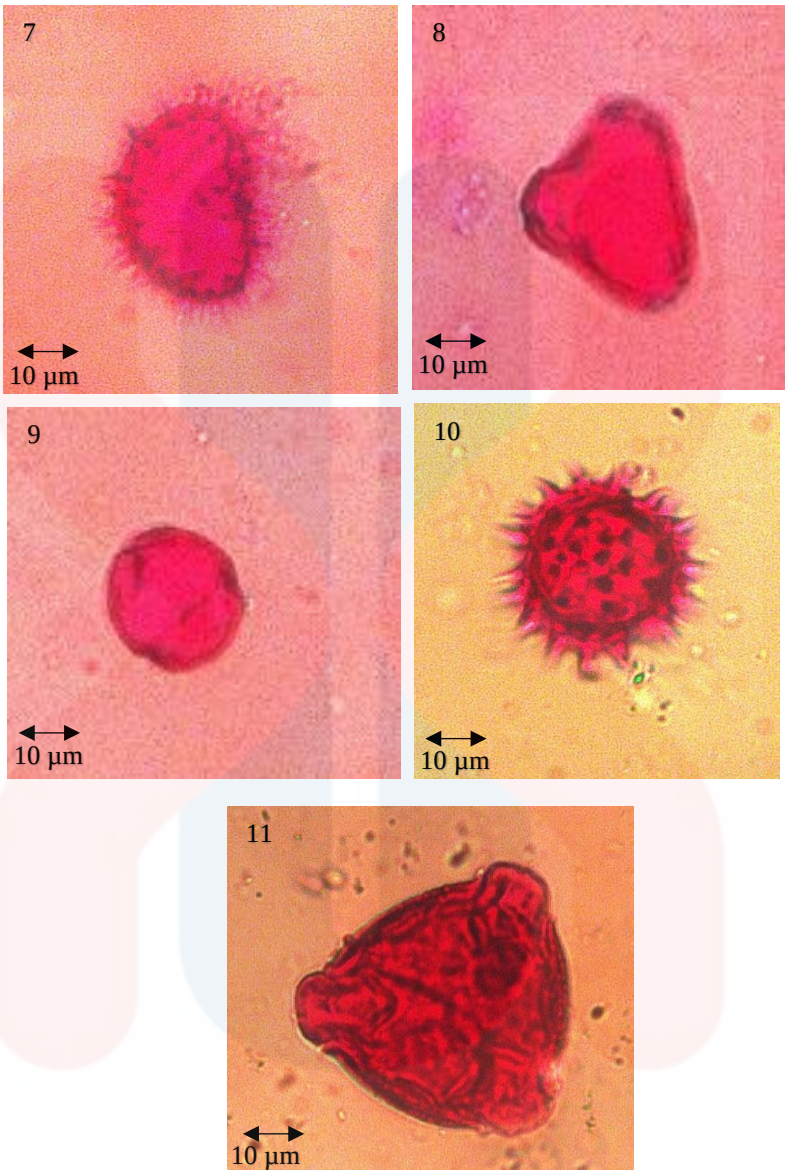

Figure 4.11 (c): The morphology of different pollen under 40x magnification. 7- *Bidens pilosa* (compositae); 8- *Capsicum sp.* (Solanaceae); 9- *Averrhoa carambola* (Oxalidaceae); 10- *Cosmos caudatus* (Asteraceae); 11-*Bruiguiera cylindrica* (Rhizophoraceae).

Furthermore, under a compound microscope with camera, pollen staining with Safranin was successful, resulting in a pinkish colour for the pollen. Due to debris and dirt on the microscopic slides, images of a few pollens did not appear as visible under microscope observation. Besides that, to the presence of distinct pollen species, honey samples from Bintong had a diverse floral diversity. Important minor pollen and minor pollen were also present, resulting in a greater variety of honey samples. (Fatin, 2019). *Antigonan leptopus* had the highest percentage of abundance in sample from Bintong with percentage of 41.90%. thus, this sample was a unifloral honey because the for consider honey as uniforal, the pollen need exceed more than 40%. *Antigonon leptopus* is a sturdy creeper native to Mexico that thrives with little care once established. It continues to bloom. It provides a superb overhead canopy and nectar source for recovering colonies when grown on supports. Pink scentless flowers were common in the Malaysian variety. The white version is extremely unusual. Cuttings from the stem can be used to reproduce it, but cuttings from the underground rhizome are more successful. It does not seem to thrive on moist coastal soils. Because it produces a lot of nectar, this was the most useful plant. This species, it was planted near the hives on fences (Ruth & Muhammad, 1991). This species also found in many of the sample from Padang Pauh, Guar Sanji, Sanglang, Sena, Tambun Tulang as one of their favourite plants for collecting nectar.

For important minor pollen in the percentage between 3% to 16% namely *Melastoma malabathricum, Durio zibenthus, Mimosa pudina, Brassica chinensis, Biden Pilosa, Capsicum sp.,* and *Averrhoa carambola.* For minor pollen with percentage not more than 3% namely *Mussaenda philippica, Cosmos caudatus*, and *Bruiguiera cylindrica*. As an outcome, Melissopalynological plays a very important role in determining the botanical and geographical origin of honeys through pollen analysis. The presence of additional pollen species such as *Durio zibethinus* and others in the Bintong showed the existence of coconut and other native plants.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

Melissopalynological analysis on the honey samples from 10 different region in Perlis. The presence of 64 types of pollen from 39 distinct plant families was discovered in honey samples from ten different locations in Malaysia's Perlis region. Three of the honey samples were unifloral, whereas the other seven were multifloral. As a result, each of the sample locations has its own type of pollen honey. The predominant pollen will define the honey as unifloral if the pollen abundance percentage is greater than 40%. Most of the samples had one prominent pollen, notably in unifloral samples, whereas multifloral samples had no predominant pollen, resulting in them being classified as multifloral pollen. There are 3 types of pollen that are very famous which continuously or repeated be found on this pollen analysis which are Antigonon leptopus, from Polygonaceae family, Combretum indicum from Combretacea family and Veitchia merrillii from Arecaceae family. Most of the pollen taxa were herbaceous or member of the gramineous types. The predominant and secondary pollen types were mainly from trees and shrubs. The main source of nitrogen and protein for most stingless bees is pollen, which is collected in huge quantities by stingless bee workers. Pollen research has gotten a lot of attention in recent years.

Melissopalynological analysis has become one of the most important approaches in post-glacial vegetation research. Partly due to the requirement of researching such geological data in terms of current processes, and partly due to the desire to learn more about plant allergies. For acquire a better result from pollen honey samples, conduct lab activities carefully, especially during the acetolysis process, as a tip for future research. It is hoped that this study will help strengthen meliponiculture in Malaysia.

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APPENDICES

Location and collecting of honey sample:

Equipment and apparatus used in the lab work: