Pollen Analysis of Airborne Pollen Samples Collected From Different Locations of Pahang, Malaysia.

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DECLARATION

I hereby declare that the work embodied in this report is the result of the original research and has not been submitted for a higher degree to any universities or institutions.

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Date:

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LIST OF SYMBOLS

rpm	Rotary per minute
°C	Degree Celsius
%	Percentage
Mm	micrometer
µL	microliter
mL	mililitre
g	gram
ft	feet
min	minute
m	meter
“	inch
>	greater than
<	less than

Analisis Debunga Pada Sampel Udara Yang Diperolehi Daripada Lokasi Yang Berbeza Di Pahang, Malaysia

ABSTRAK

Penyiasatan ke atas analisis debunga sampel udara yang dikumpul telah dijalankan dengan menggunakan sebuah helikopter kawalan jauh di enam lokasi berbeza di Pahang, Malaysia. Debunga dalam udara adalah penyumbang peratusan utama reaksi alahan berlaku. Objektif kajian ini adalah untuk menilai bilangan debunga yang hadir dalam sampel udara yang dikumpul dari lokasi yang berbeza di Pahang, untuk mengenal pasti jenis-jenis debunga berdasarkan morfologi debunga di dalam sampel udara yang diambil dari lokasi berbeza di Pahang dan mengenal pasti debunga alahan yang hadir dalam debunga sampel udara yang dikumpul. Tempat pilihan adalah Kuantan, Bera, Jerantut, Temerloh, Raub dan Kuala Lipis. Kiraan debunga dan kaedah debunga acetolysis telah dijalankan untuk menentukan bilangan debunga di dalam atmosfera. Sebanyak 183 butir-butir debunga telah direkodkan yang terdiri daripada 19 spesies daripada enam lokasi. Kuantan mempunyai jumlah debunga tertinggi (24.5%) manakala Raub merekod jumlah debunga terendah (8.2%) disebabkan oleh faktor-faktor iklim dan serta faktor-faktor geografi. *Elaeis guineensis* daripada keluarga Arecacea direkodkan sebagai bijirin debunga yang paling biasa dijumpai di Pahang dan ia dikenal pasti sebelum ini sebagai alahan debunga.

POLLEN ANALYSIS OF AIR POLLEN SAMPLES COLLECTED FROM DIFFERENT LOCATIONS OF PAHANG, MALAYSIA

ABSTRACT

The investigation on the analysis of air pollen samples was carried out by using a remote control helicopter in the atmosphere at six different locations in Pahang, Malaysia. Pollen in air is the main causal of allergic reactions. The objective of this research is to evaluate the number of pollen present in the air samples collected from different locations of Pahang, to identify the types of pollen based on pollen's morphology in the air samples collected from different locations of Pahang and to identify the allergenic pollen present in the air samples collected. The chosen places were Kuantan, Bera, Jerantut, Temerloh, Raub and Kuala Lipis. Pollen count and pollen acetolysis method was carried out in order to determine the number of pollen loads in the atmosphere. A total number of 183 pollen grains were recorded in which consisted of 19 species from the six locations. Kuantan has the highest abundance of pollen loads (24.5 %) while Raub recorded the lowest abundance of pollen loads (8.2%) due to its climatic factors as well as geographical factors. *Elaeis guineensis* from Arecacea family was recorded as the most common pollen grains found in Pahang and it was previously identified as allergenic pollen.

CHAPTER 1

INTRODUCTION

1.1 Research Background

All things at the atmosphere can cause allergy towards the human's immune system. The most common things is pollen. Pollen is a fine powdery substance, typically pink colour, come from tree, shrub and weed. Consisting microscopic grains discharged from the male part of a flower or from a male cone. This pollen travel by the wind, insects and other animals. Therefore, pollen grains are known as the important cause of pollinosis and respiratory system which is direct target organ when the airborne pollen is inhaled by the human (Sigh, 2014). Airborne pollen is any substance light enough to be carried by air currents and capable of evoking an immune system. For example are pollens, fungal spores and algae.

Allergic disease has been increasing rapidly especially in developing countries. Various adverse health outcomes such as allergic disease can be attributed to rapidly

increasing air pollution levels. Human body is exposed to the increased quantities of ambient air pollution.

Air pollution potentially trigger asthma exacerbation. Furthermore, exposure to particulate matter, ozone and nitrogen dioxide contributes to the increased susceptibility to respiratory infections. Effects of air pollutants such as asthma, allergic rhinitis and eczema (Suh.Y.L., Yoon. S.C., Song. H.C., 2013).This is logical as pollen grains are extremely tiny particles comparable to dust particles which cannot be seen by the naked eye. Pollen is ubiquitous in nature unlike other plant parts. It occurs buried deep in rocks, ground, on water and in air, indoor or outdoor including the upper atmosphere. Furthermore, this pollen finds its way through the nasal and oral cavities to the digestive tract of humans and animals. The pollen also can causing the different degrees of discomfort (Agashe and Coulton 2009). The pollen is not just producing from the plant but also their position with respect to distance from the pollen sampler. Some pollen may travel long distances and in large quantities (Tyldesly, 1997), so knowledge of occurrence over regions beyond the local area is desirable. U.S Department of Health and Human Services (2012) stated, most of the pollen from plants like trees, weeds and grasses. It causes the most to allergic reaction. This is because the plant produced small, light and dry pollen are made to carry by the wind. Allergens are known as the substances that cause allergic reaction. Allergens are commonly proteins or glycoprotein. It is shows that protein is a major allergen due to the allergic reaction in at least 50% of individuals sensitive to the particular species from which the allergen is derived (Weed, Bhalla and Singh, 2002).

1.2 Problem Statement

Pollen in air is the main causal for allergic reactions such as bronchial asthma, conjunctivitis, rhinitis, sneezing, runny nose and watery eyes. These symptoms are collectively known as 'hay fever'. In addition, hay fever is misnomer since it is not necessarily caused by hay. It can rise in body temperature. Through this pollen analysis and pollen identification of airborne pollen samples collected, it can help allergic patients in order to take precautions step to avoid the allergy symptoms. This research study is about airborne pollen samples collected from different locations of Pahang, Malaysia.

1.3 Research Objectives

This study is to achieve the following objectives:

1. To evaluate the number of pollen present in the airborne pollen samples collected from different locations of Pahang.
2. To identify the type of pollen based of pollen's morphology in the airborne pollen samples collected from different locations of Pahang.
3. To identify the allergenic pollen present in the airborne pollen samples collected.

1.4 Hypothesis of the study

Kuantan have the highest abundance of pollen loads while Raub have the lowest abundance of pollen loads due to its climatic factors as well as geographical factors. *Elaeis guineensis* is probably the most common pollen grains found as allergenic pollen among the pollen samples collected in Pahang.

1.5 Significance of the study

The significance of study is to determine the type of pollen and distribution of allergenic pollen in Pahang by using two analysis methods which are pollen count and pollen acetolysis. The significance of the study for individual are to help people to be aware of the dust, pollen and small particle that can cause hay fever and allergy symptoms. Next, the significance of study for community, it can reduce the health impacts and risks of the allergy reaction. For government benefit, it will increase the knowledge and research about pollen and the side effect to human and animals.

CHAPTER 2

LITERATURE REVIEW

2.1 Pollen as Allergens

Allergens are antigens that stimulate the allergy reaction. The list of structures that have been identified as allergens represents a tiny subset of antigenic universe to which human are routinely exposed. Probably the most important process that takes place during the pollen transport is the release of allergen from the pollen grains. Furthermore, "in a dry atmosphere, the pollen is very stable and can keep its content over years" (Stanley & Linskens, 1974). The pollen grains also can be release in specific conditions within minutes: (i) a high relative air humidity; (ii) thunderstorms and heavy rain; (iii) high concentrations of air pollutants.

Pollen allergens are generally glycoproteins. In allergy, immunity has gone awry and the system reacts to substances that are respiratory have problem. For example are sneezing, runny nose, and watery eyes. This is because inhaled allergens from growing weeds, trees and grasses or moulds, house dusts and mites. This symptoms are called 'hay fever'.

Even though many individuals outgrow allergies over time, allergies also can be develop at any age including during adulthood. The environment plays main role in the development of allergy. There is a greater risk of developing allergic conditions if a person has a family history of allergy especially in parents or siblings. According to Cardiff, Hyde (1952), the magnitude of annual catches of individual pollen types varied considerably and attributed this mainly to variation in pollen productivity.

Pollen productivity due to the rainfall season especially for pollen from seasonal fruit such as durian, rambutan, papaya, watermelon, mango and others. The duration for pollen travel in atmosphere is high during July until September. This is because that month is the peak season for mostly seasonal fruit in Malaysia. Seasonal, location and weather have the correlation of aeroallergens.

Parthenium hysterophorus pollen is one of allergenic pollen. This pollen already found in Kedah, Perlis, Perak, Selangor, Melaka, Johor, Negeri Sembilan and Pahang, Malaysia. Pollen can travel in long distanced. So, this research is to avoid or increase precautions for individual that has low body's immunity towards the allergens. It has harmful effect on human and animal health, crop production (40%), pasture production (80%), biodiversity and soil ecosystem

(Maszura, C.M., Karim, S.M.R., Norhafizah, M.Z., Kayat, .F., Arifullah, M.,2018).

2.2 Pollen Morphology and Pollen Identification

Pollen morphology is an important and fundamental branches of palynology. It cannot be exaggeration if pollen morphology is referred mother of palynological studies. Furthermore, it is the principle tool that been used for correct pollen identification. Any error during identification can cause an erroneous conclusion. For example is the identification of airborne pollen in Bangalore. Because of the lack of proper background knowledge of pollen morphology, *Cassia* pollen were shown to be the second most abundant pollen in Bangalore's atmosphere. After carried out by sound pollen morphological knowledge, *Casuarina equisetifolia* pollen is the correct result (Agashe et al., 1994) So, the pollen morphology is very important and must be studied carefully to avoid any mistake due to the result.

The pollen morphology can be measured due to the range of size, shape and even colour. The pollen grains is looks like round, round, long or triangular, semicircular and boat shaped. Some of the pollen may have the several sides which is flat or rounded (Agashe and Coulton 2009). Pollen can be group into porate, netted, spheroidal inaperturate, triaperturate and irregularly shaped. Pollen group

based on the shaped and size. Every pollen has exine, furrows, pores, intine, interior as the pollen description.

In some spores, there is a covering outside the exine which in many cases is loose and separated by a cavity from the exine. The spore can be list out as radial triradiate spore, trilete spore, bilateral monolete spore and sporangium spore.

2.3 Airborne Pollen Studies Previously

2.3.1 Airborne Pollen Studies Previously in Malaysia.

Airborne pollen studies previously in Malaysia to find the allergenic pollen and identified the various type of pollen in the atmosphere. According Suhana. M.A (2016), there are 205 pollen grains have been identified at Johor, Malaysia. The most common pollen species at Johor is *Decaspermum fruticosum* from Myrtaceae family. The Myrtaceae family known as Eucalyptus, Clove or Guava family. This family is eighth largest family of flowering plants, both economic and ecological importance. A large number of Myrtaceae species are found in the wet tropics such as in Tropical Asia (Angel,2015).

The other research done by Agus, D.P.,Budi, P., Bandung, S., Ramadhani, E.P., Ida, K. (2017) stated that *Elaeis guineensis* is a common pollen in Indonesia and Malaysia. This is due to the number of plantation in Indonesia and Malaysia are highest and one of economic income for that country. Allergic rhinitis is

chronic allergic affecting an estimated four million people in Malaysia. In the basic statistic by the previous research at Klang Valley, the results of an aerobiological survey, twelve local extracts of molds and two local extracts of grass pollen prepared by the Institute for Medical Research for this study. A total of 85 allergic rhinitis patients recorded for this studies. All molds and grass pollens extract tested, elicited positive response to SPT (Ishlah, L. W., & Gendeh, B. S. (2005).

2.3.2 Airborne Pollen Studies Previously in Bangalore, India

Bangalore, India is of place that has unique distinction of gaining many adjectives including ‘ Air Conditioned City of India’. Bangalore also known as ‘Allergy City’. This is because in fact it has highest atmospheric pollution next to Delhi which is responsible for a large proportion of the population suffering from various types of allergies. Allergenic pollen come from weeds, grass, tree and atmospheric pollen. This abundance can causes allergy symptoms for sensitive individuals. So, netizen in Bangalore must take extra careful and precautions by undergoing desensitization. It also proper pharmacotherapy for their own health. Bangalore also the biggest place that contain Parthenium hysterophorus 43%. Parthenium hysterophorus is a species of flowering plant in the aster family, Asteraceae. This weed causing disastrous loss of yield, affecting livestock and crop production, affecting human health. This is because this weed produces

Allelopathic chemicals that suppress crop and pasture plants and allergens. Frequently causes pollen allergies.

Bangalore has Pollen Cryobank at the Indian Institute of Horticultural Research (IIHR), Bangalore, India. Because of this place have many issues regarding the pollen and human health, so there are institute that doing research on 600 types of pollen belonging to 45 species and 15 families under cryogenic conditions. Long term cold storage (cryopreservations) of nuclear genetic diversity in the form of pollen in several plants of horticultural, medicinal and forest importance can be successfully achieved (Ganeshan et al., 2005).

2.4 Pollen Count

Pollen count is one of the technique that use for quantitative in pollen identification. Pollen count and weather have correlation each other. This is because, the windy or dry weather bring a lot of pollen dispersal especially by the wind. But some of pollen is bring by the bee for honey production and some is bring by the pollinator for plants pollination. Pollen can travel for a long distance. It can be absent, low and high according a conditions. The number of population also relate to the effect of pollen allergy towards human. Pollen count needs aerobiology and immunotherapy to cure human allergy. Pollen count is the measurement of the number of grains of pollen in a cubic meter of air. The high pollen count would be lead to the increased rates of an allergic reaction for those

with allergic disorders. Pollen count also needed to know how much the pollen load for each places and each species. Mild winters with warmer days lead to an increase in pollen count. Furthermore, the pollen decrease in colder winters.

2.5 Pollen Acetolysis

Gurnnar Erdtman is the person that introduced pollen acetolysis method in his studied on pollen morphology (Nilsson & Praglowski, 1992). Fresh pollen grains when observed under a light microscope appear as dense objects and reveal only the colour, size, shape and faint outline of sculpture patterns and pollen wall excrescenses. So, by using microscope can diagnose pollen morphology character and easy to identified the type of pollen. Pollen is a mass of microspores in a seed plant appearing usually as a fine dust. Acetolysis is a the breakdown of organic compound using either acetic acid or acetic anhydride. Acetic acid or acetic anhydride is a chemical compound (CH3CO)2O. It is commonly abbreviated Ac2O, the simplest isolable of a carboxylic acid. This acid used as a reagent in organic synthesis. It is a colourless liquid and have strong smell. This chemical is first class chemical and hazardous. It react with moisture in the air.

2.6 Analysis of Airborne Pollen Samples

The purpose of pollen analysis is to analyse the pollen to determine the type of pollen based on the morphology. This pollen analysis is used microscope. Two main microscopes that can be used are light microscope (LM) and scanning electron microscope (SEM). Each type of microscope has its own pluses minuses. Light microscopy is more frequently used for pollen analyses regardless of the discipline. Because LM is the main microscope used for pollen analyses, there are many atlases, books, micrographs of pollen and pollen reference collections available to prove it.

Unfortunately, identification of the pollen would be more difficult by using LM. LM is the lack of resolution compared to a SEM. Jones and Bryant (2007) found differences in the pollen diversity when the same sample was examined with LM and SEM. For the first count, they found 22 taxa with LM and 40 with SEM. This difference in the number of taxa was due in part to the increased resolution of SEM. But when using SEM, multiple types of taxa that were not found when using LM was appear using the SEM. In addition, the differences in the taxa were carefully examined. Some of the differences could also be seen when they re-examined the samples with LM. Some of the differences could not be seen with LM, and could only be seen with SEM (Twiddle, 2012).

2.7 Pollen Identification

2.7.1 Pollen Size and Shape

Size is important to know the structural of one thing. Sometimes inadequate for distinguishing species and sizes becomes a reliable criterion. For example in *Picea*, the measurements have aided species identification. Pollen grains of angiosperms range from 5-200 µm in diameter. But mostly the grain's range is from 25 -100 µm in living angiosperms. The shape of pollen is varieties in different views. The outline in polar view is circular, triangular, square, pentagonal, rounded, three lobed or in other geometrical shapes. The description of the pollen's shape is based on the polar axis ratio.

Walker and Doyle (1975) have simplified the following six classes of size of pollen grains based on diameter or length of the longest axis. Firstly, minute grains less than 10 µm. Second, the small grains which is 10 -24 µm. Next, medium sized grains which is 24- 29 µm. Then, large grains which is 50-99 µm. Fifth, very large grains which is 100-199 µm. Lastly, gigantic grains which is 200 µm. The pollen wall is one of important structures in plants. the pollen grain wall has two layers, the outer (sculptured) layer is called exine and inner layer is the intine. The exine is a composed of a complex substances collectively known as sporopollenin. Sporopollenin functional as protective internal contents from the harmful effects of the environment such as radiation hazards due to its ability to absorb UV

radiation. The ultimate meiotic divisions to produce tetrads of haploids cells and the development of the sporopollenous exine wall prior to the development of the intine. Pollen grain ontogeny in both gymnosperms and angiosperms have features in common, the initiation of the pollen mother cells from sporogenous central tissue.

2.7.2 Pollen Analysis Technique

In previous study, acetolysis method is done to remove cellulosic particles around the pollen. Moreover, cellulose can be removed most effectively by acid hydrolysis. This technique is very useful in pollen analysis (Dartmouth.edu, 2015). Jones (2012a) finds difficulty in finding pollen and its identification without using acetolysis method. This is because the pollen grains already mixed with pollinator's tissue and lipids on the grains. Jones (2012b) has described the pollen recovery techniques using acetolysis and it be written for palynologists who know and understand all the steps for recovering pollen. It is easier to diagnose the characteristics of the pollen grains because it is more visible, thus accurate pollen identification can be done. By using a proper technique, a better data also can be obtained (Jones, 2014). The acetolysis method must be conducted under a fume hood and specialized equipment is needed. Acetolysis also takes a longer time due to its repeated steps (Jones, 2014).

CHAPTER 3

MATERIALS AND METHODS

3.1 List of chemicals and reagents

3.1.1 Chemicals for Acetolysis Mixture

Chemicals that used for acetolysis mixture process were 1ml sulphuric acid and 9ml acetic anhydride.

3.1.2 Chemicals for Pollen Analysis

Chemicals that used for pollen analysis process were 1000µL ethanol, 1000µL glacial acetic acid and 500µL acetolysis mixture.

3.1.3 Chemicals for Preparation Glycerine Jelly

Chemicals that used for preparation glycerine jelly were 25g gelatin powder, 75ml glycerine, 0.1g safranin powder, 3.5g phenol crystals and 87.5ml distilled water.

3.1.4 Apparatus

The apparatus that used in the experiments were centrifuge machines, vortex, water bath, fume- hood cabinet and Leica microscope with camera (DM750-ICC50HD), cover slips, glass slide, micropipette, oak ridge tubes, microcentrifudge tube, beaker, glass rod and petri dish.

3.1.5 Airborne Pollen Samples Collected

The airborne pollen samples was collected in six different locations in Pahang. The location of airborne pollen sample collected was summarised in Table 3.1.5. The airborne pollen samples collected in controlled condition and same parameter such as the helicopter must flying in the air around 30 minutes for each time taken recorded at the average of human height. Average of human height was 178.3 cm. The location selected must be close to the housing area. The sample was taken for 3 time specifically 9 o'clock in morning, 2 o'clock in afternoon and 6 o'clock in evening.

Table 3.1.5: Location of Airborne Pollen Sample Collected

Locations	Collection date
Kampung Jeram Beserah, Kuantan	7 September 2018
Kampung Durian Tawar, Bera	8 September 2018
Felda Sungai Tekam, Jerantut	9 September 2018
Jalan Merdeka, Temerloh	11 September 2018
Felda Tersang 2, Raub	12 September 2018
Felda Sungai Koyan,Kuala Lipis	13 September 2018

3.2 Site of sampling

All the airborne sample was collected at six different sites in Pahang which were Kuantan, Bera, Jerantut, Temerloh, Raub, and Kuala Lipis. The research sites was as indicated in Figure 3.2. The samples collected were performed in an open area. The location was selected based on the highest number of residential and housing area. This is because previously it was recorded that the higher the number of residential, the higher the percentage of abundance of pollen collected (Hasnain, S.M., Alqasim, A., Al-Modaish, A.S., Mahjoub, M.O., Al-Frayh, A., 2016).

Figure 3.2: The research sites in Pahang map (Source :Google map)

3.3 Collection of Sample

Airborne pollen samples was collected by using a remote control helicopter. The helicopter is fixed on a pole and fly in the open field with no obstruction in front of the wing movement. The spores in the atmosphere was obtained by using a sticky cellophanes tape that has been stuck at each blades of the helicopter. The helicopter was flying for 30 minutes in each location. The estimation durations to take the samples in around May until September 2018. The airborne samples must be done for 3 times at each site location for better results.

3.4 Preservation of Sample

The cellophane tape with the spores taken from each blade was transfer to the microscopic slide as each one microscopic slide was contained sample from each blade. The microscopic slides was placed in a petri dish and the petri dish must be sealed using a paraffin for the preservation.

3.5 Sample Analysis

The sample analysis process was summarised in Figure 3.5. The process involved were pollen acetolysis, pollen count and pollen identification of types and species.

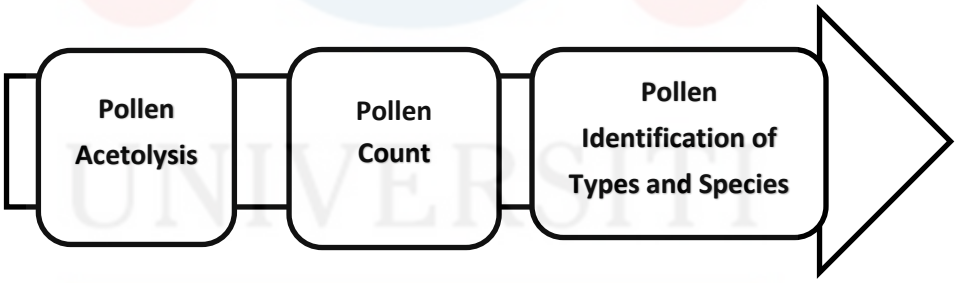

Figure 3.5: The process flow of Sample Analysis

3.5.1 Pollen Acetolysis

The cellophane tapes that contain the air pollen were placed into an oak ridge centrifuge tube which contain 95% ethanol and undergo a scrubbing process by using a vortex for 10 minutes. The purpose of adding the ethanol was to remove the gums of the cellophane tape from the samples. The cellophane tapes were taken out from the oak ridge centrifuge tube and the oak ridge centrifuge placed inside the Centrifuge machine to undergo centrifugation for 8 minutes at 5000 rpm with the temperature of 25°C. The supernatant formed after the centrifuge process was carefully removed again. Then, 500 µL of Glacial acetic acid was transferred into the microcentrifuge tube and undergoes the same centrifugation process for 8 minutes 5000 rpm. Since the pollen contains water, the structure of pollen would not be able to be observed under the microscope. Thus, the role of the Glacial acetic acid was to dehydrate the pollen for better observation later. The supernatant formed was removed, and 500 µL of Acetolysis mixture was carefully added into the same microcentrifuge tube. Acetolysis mixture was the mixture of 1mL concentrated Sulphuric acid, H_2SO_4 and 9 mL Acetic anhydride. The purpose of adding the Acetolysis mixture was to digest the pollen's cytoplasm in order to expose the exine of the pollen in outer surface. The microcentrifuge that contains the acetolysis mixture was boiled inside the water bath at the temperature of 65°C for 8 minutes. It was then centrifuged for 8 minutes at 5000 rpm. The supernatant formed from the acetolysis mixture was removed carefully and 500 µL of Glacial acetic acid was added. It undergoes the centrifugation process for 8 minutes at 5000 rpm. The supernatant was removed again and the Glycerine jelly was added into the microcentrifuge tube. The microcentrifuge tube

which contains the pallet and the Glycerine jelly was heated for 2 minutes at the temperature of 65°C in order to melt the Glycerine jelly. Lastly, the Glycerine jelly was dropped onto the microscopic slide and was covered with the cover slip. The process flow of pollen acetolysis was summarised in Figure 3.5.1.

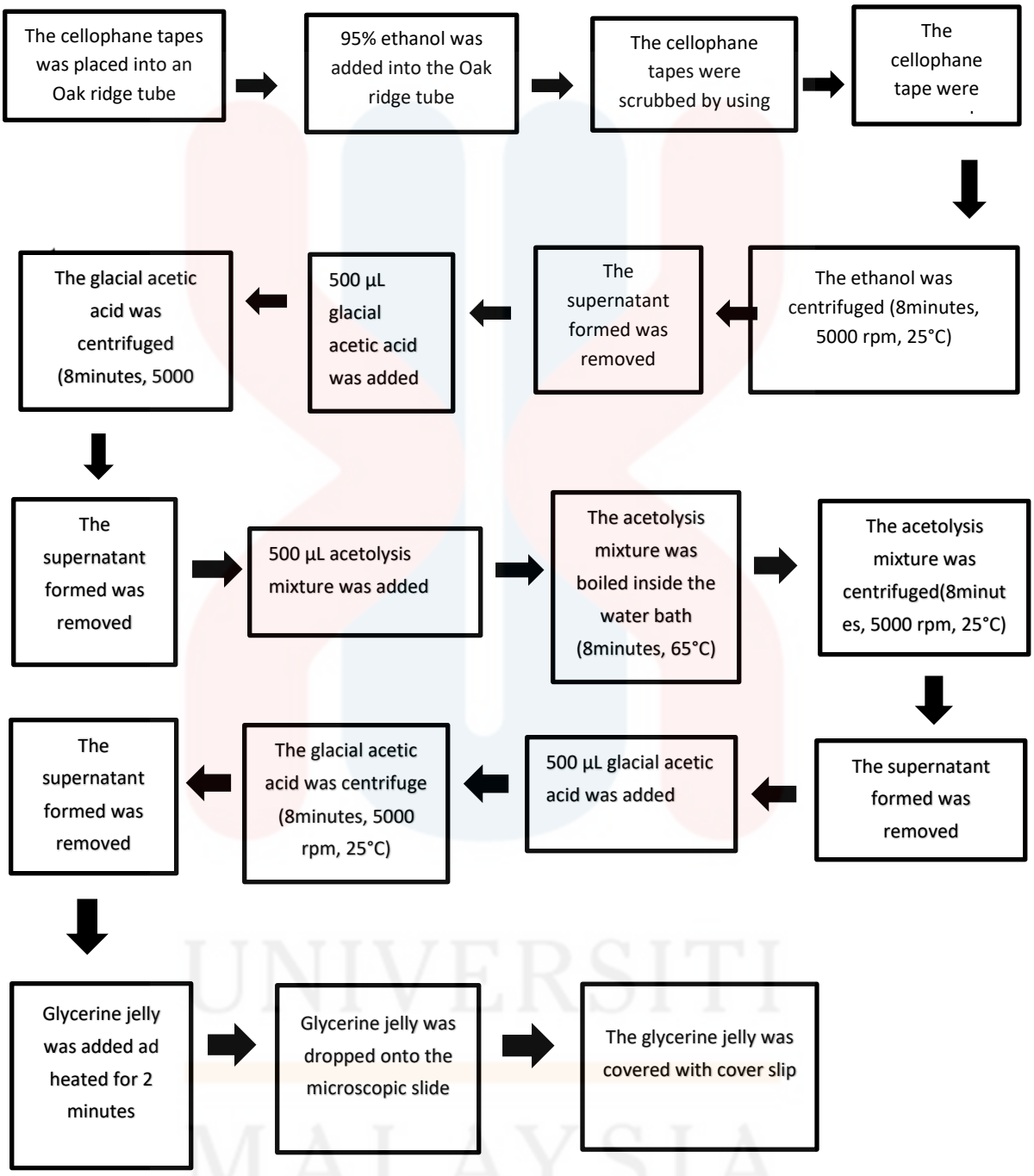

Figure 3.5.1: The process flow of Pollen Acetolysis

3.5.1.1 Glycerine Jelly Preparation

The ingredients involved in the glycerine jelly preparation was gelatin powder, distilled water, glycerine, safranin powder and phenol crystal. 25 g of gelatin powder in a beaker was dissolved with 87.5 ml of distilled water. The solution was stirred to make sure it was homogenous. Next, 75ml of glycerine was added and mixed together. Then, 0.1 g of safranin powder was added. The addition of safranin to stain the jelly to become pinkish colour. The purposed of that pinkish colour was to make it clear image and easier to observed the pollen under microscope observation. The solution was boiling continuously. 3.5g of phenol crystal was added. These phenol crystal was harmful and hazardous. The solution was removed from the hot plate once it turn transparent. The jelly was filtered by using muslin cloth to make sure it smoothly and was transferred into a petri dish. Solidification of the jelly was further enhanced by storing the petri dish in refrigerator. The jelly prepared used in order to mount the pollen material for observation of pollen morphology under compound microscope. Normally, Kisser's method of glycerine jelly prepared was followed by the palynologists in the studies of pollen (Agashe & Caulton, 2009). The process flow of glycerine jelly preparation was summarised in Figure 3.5.1.1.

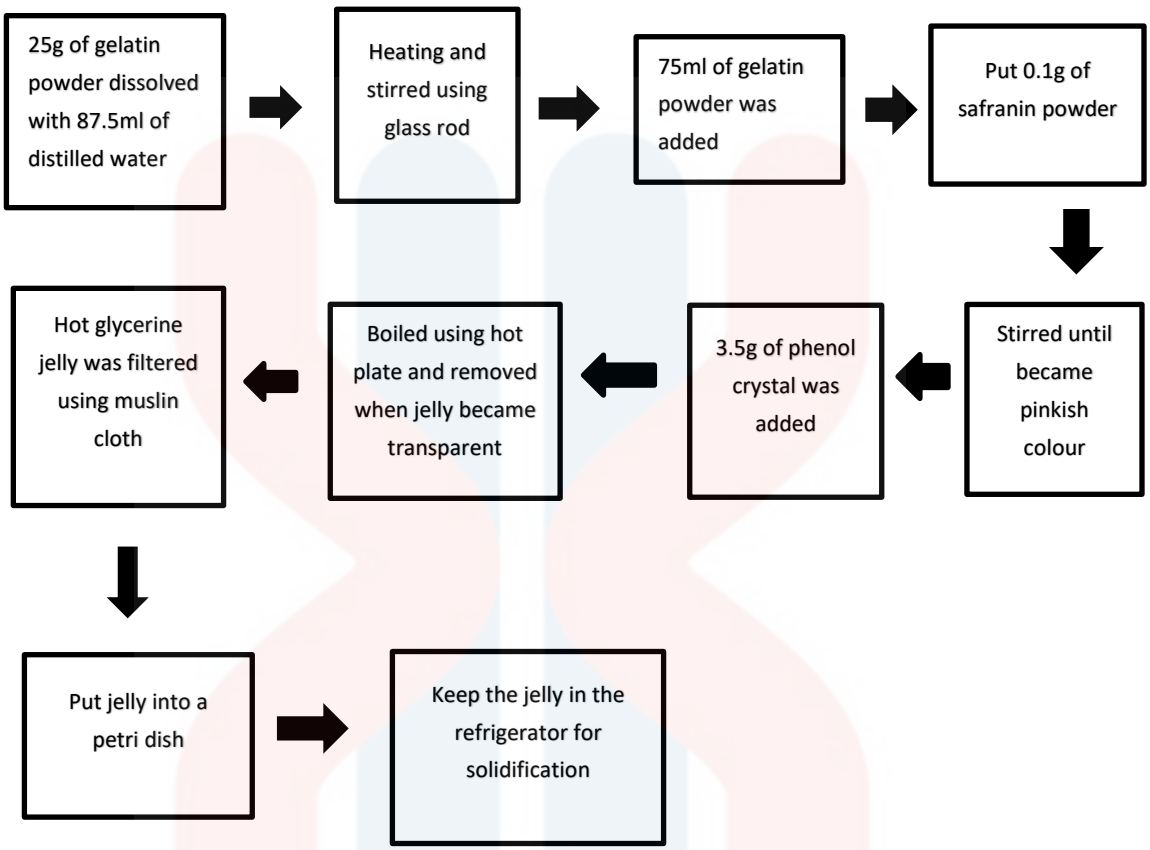

Figure 3.5.1.1: The process flow of Glycerine Jelly Preparation

3.5.2 Preparation of Pollen Slides for Microscopic Observation

The preparation of pollen slides done for microscopic observation. The image appeared on the slide easy to observe and identified by using compound microscope. The purpose of observation was to know the type of pollen and pollen morphology. In pollen studies, a small amount of glycerine jelly was added in centrifudge tube together with pollen pellet. The tube was boiling or heated in the ewater bath. Then, the mixture was placed on the slide. The slide was covered with a cover slip. Then, the glass slide was observed and the result was recorded. The microscope was undergo by 4x magnification, 10x magnification, 40x magnification and 100x magnification.

3.5.3 Pollen Count

Pollen count was important procedure to calculated the pollen that found in airborne pollen sample. Pollen count was done to know the frequency and amount of pollen for each location collected in Pahang. It can be used to know how much the pollen and allergy frequency in that place.

3.5.4 Pollen Identification of Types and Species

Pollen is a tiny object that cannot be seen using naked eyes. The pollen just can be seen under compound microscope. The pollen had their own characteristics and morphology. The outer layer, walls, inner layer were different between others. According to the previous studies by Richard, W.W., 1998, the pollen characteristics can used for identified the type of pollen and the species. By applying a visual utilizing grain number, size, shape, surface and internal details of botanical taxanomic level. Identification may be possible only to the family or order but most frequently to the genus and occasionally to the species. The data collected from the pollen identification were compared with published or existing pollen catalogue, magazines, books or journal in order to identify the pollen types and species.

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 Results and Discussions

4.1.1 Pollen Count of Airborne Pollen Samples

Pollen in the airborne pollen samples from Pahang had been identified. The pollen was collected from Kuantan, Bera, Jerantut, Temerloh, Raub and Kuala Lipis. The total number of pollen that had been found in the slide was 150 pollen grains. The pollen was observed under electronic microscopic and being captured by the camera to identify the pollen type from different plant in a particular area. The number of pollen in every slide was counted by using Leica DM 750 microscope described by (Ohe *et al*., 2004) in Harmonized Methods of Melissopalynology. Then the pollen abundance was counted for every species in a location. The total number of pollen grains for each location was

recorded in the Table 4.1 and the percentages of pollen collected from different locations was shown in Figure 4.1.

Table 4.1 : The airborne pollen count for six different locations in Pahang.

Locations	Pollen Count
Kuantan	45
Bera	41
Jerantut	38
Temerloh	21
Raub	15
Kuala Lipis	23
Total	**183**

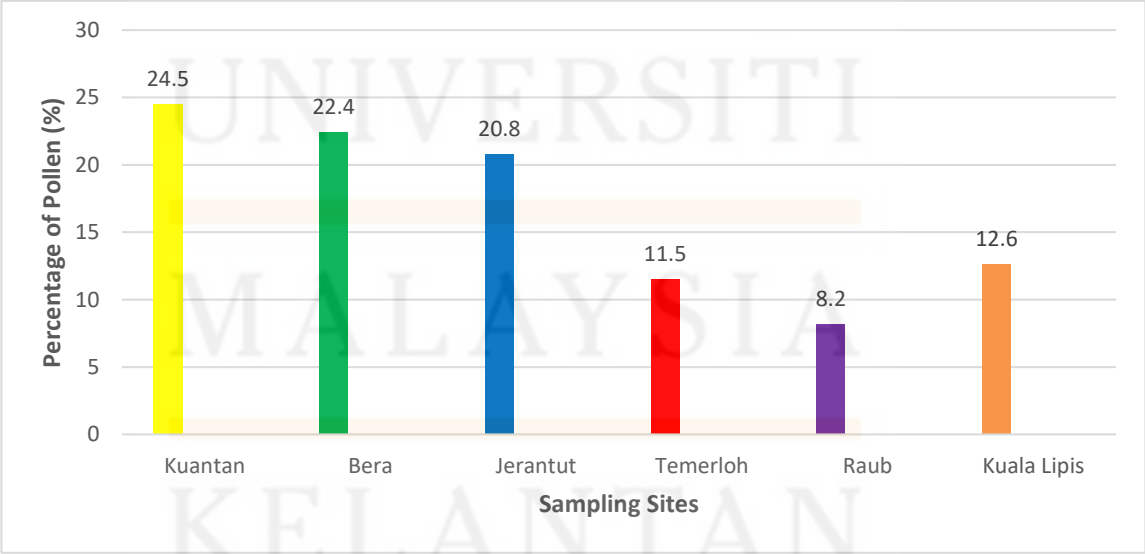

Figure 4.1 : Percentage of pollen collected from different locations in Pahang.

4.1.2 Airborne Pollen Samples from Kampung Jeram Beserah, Kuantan, Pahang

Data for total pollen load in airborne pollen samples was collected in Kampung Jeram Beserah, Kuantan, Pahang. The result recorded four different species of pollen (Table 4.1.2.1). The total pollen load was 45 pollens (24.5%). Percentage abundance of pollen collected from Kampung Jeram Beserah,Kuantan, Pahang was summarised in Figure 4.1.2.2.

Table 4.1.2.1: The number of pollen in airborne pollen sample collected from Kampung Jeram Beserah, Kuantan, Pahang.

Plant species	**Family**	**No. of Pollen**
Elaeis guineensis	Arecaceae	24
Durio zibethinus	Bombacaceae	12
Rhynchospora breviseta	Cyperaceae	10
Syzygium caryophyllatum	Myrtaceae	9
Total no. of pollen		**45**

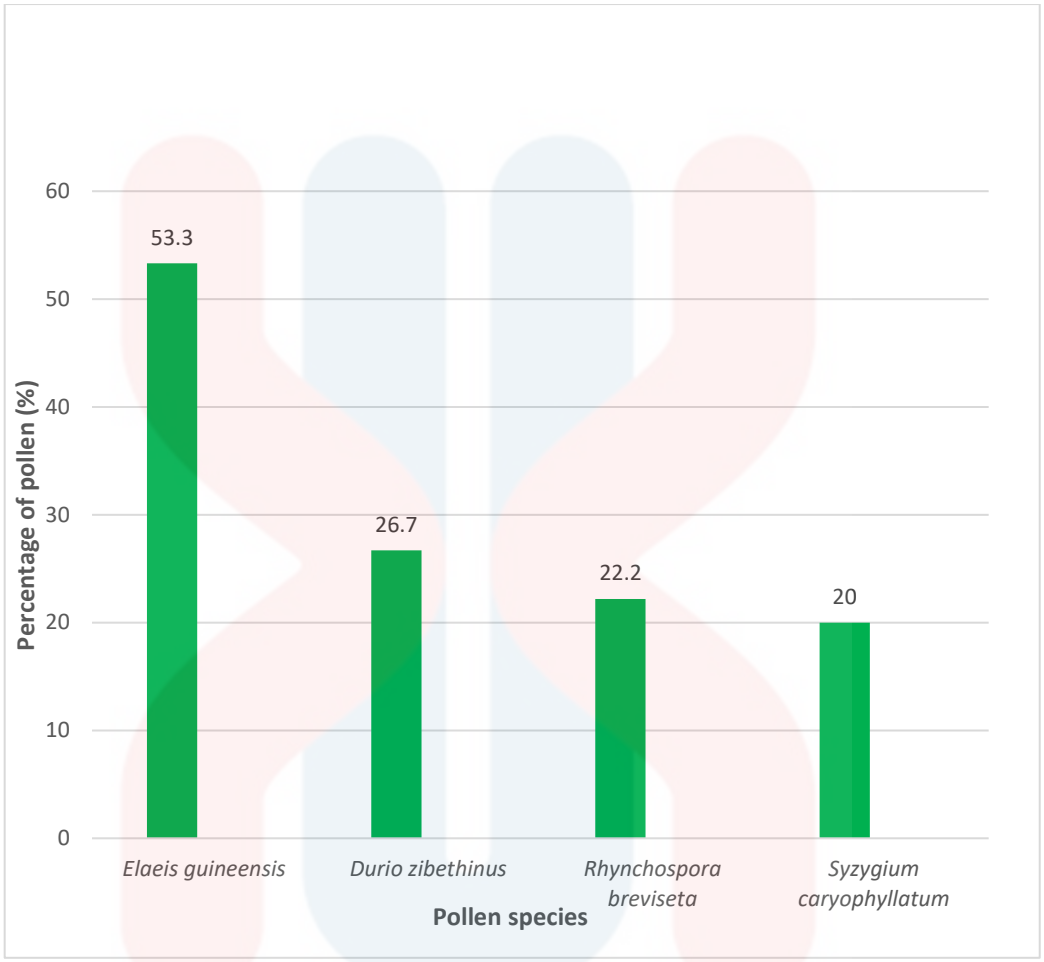

Figure 4.1.2.2: Percentage abundance of pollen collected from Kampung Jeram Beserah,Kuantan, Pahang.

First location in Pahang for collecting sample was Kuantan. Different pollen species in airborne pollen samples from Kuantan, Pahang was summarised as in Figure 4.1.2.3.In these location, there were four type of pollen species such as *Elaeis guineensis* (Arecaceae), *Durio zibethinus* (Bombacaceae), *Rhynchospora breviseta* (Cyperaceae) and *Syzygium caryophyllatum* (Myrtaceae). Kuantan had the highest percentage of pollen, 24.5 % with 45 pollen in the sample. The pollen of a particular plant species was said to be predominant if its occurrence in sample was highest in pollen count (Agashe &Caulton, 2009). The other pollen type that differ can be found by analysing the differences in the pollen abundance in it (Upadhyay et al., 2014). *Rhynchospora breviseta* (Cyperaceae) was the pollen species that had the lowest pollen amount in Kuantan, Pahang. These species was a major species in group Angiosperm. It also flowering plant species. Also known as sedges or grasses that flowering. It can be categorised as herb. The morphology was 1-aperturate grain, poorly defined pore, tectate exine and indistinct columellae. *Elaeis guineensis* (Arecaceae) known as palm oil tree. These plant had species name guinensis referred to the name for the area, Guinea at the southwest Africa. The type of *Elais gueineensis* was a tree. The morphology, it had 2-colporate grain and pores. It had tectate exine. These plant also normally planted in the plantation area. Weevil was a pollinator pollen for these *Elaeis guineensis* plant. Kuantan had 4 large palm oil plantation such as Far East Holding, Kurnia Setia Bhd, Ladang Sungai Mars Sdn Bhd and Kema Development Sdn Bhd. That's why Kuantan had highest pollen loads for these species. Second pollen species was *Durio zibethinus* (Bombacaceae). These species was commonly found because of Pahang famous with Durian fruit season. *Durio zibethinus* (Herbarium) known as tree type. The morphology was 3-colporate grain, pores circular and colpi short lalongate. Apertures are both costate and marginate. There was a

pronounced thickening around the nexine and changed in shape of the sexine, which extends over in the nexine. It had tectate-perforate exine, distinct columellae and scabrate pattern. Next pollen grain species was *Syzygium caryophyllatum* (Myrtaceae). These plant had white flower and edible fruit. The common name for these species was “kelat jambu”. These species come from Sri Lanka, India. Commonly found near forest and wild plant. It can be categorised as shrub and tree. The morphology was 3- parasyncolporate grain with lalongate endoapertures symmetrical, apocolpia field not present, tectate exine, indistinct columellae, and granulate pattern.

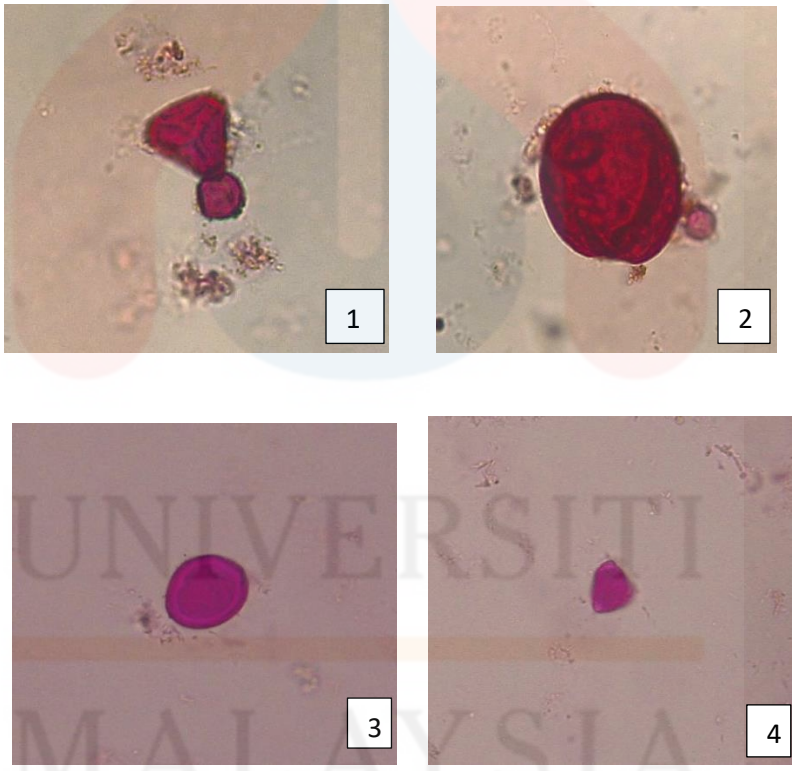

Figure 4.1.2.3: Different pollen species in airborne pollen samples from Kuantan, Pahang under 400x magnification. **1-** *Elaeis guineensis* (Arecaceae), **2-***Durio zibethinus* (Bombacaceae), **3-** *Rhynchospora* (Cyperaceae), **4-***Syzygium caryophyllatum* (Myrtaceae)

4.1.3 Air Pollen Samples from Kampung Durian Tawar, Bera, Pahang

Data for total pollen load in airborne pollen samples collected in Kampung Durian Tawar, Bera, Pahang. The result recorded five different species of pollen (Table 4.1.3.1). The total pollen load was 41 pollens (22.4%). Percentage abundance of pollen collected from Kampung Durian Tawar, Bera, Pahang was summarised in Figure 4.1.3.2.

Table 4.1.3.1: The number of pollen in airborne pollen sample collected from Kampung Durian Tawar, Bera, Pahang.

Plant species	Family	No. of Pollen
Lorus nobilis	Lauraceae	8
Cocus nucifera	Arecaceae	17
Artocopus heterophyllus	Moraceae	5
Tilia cordata	Malvaceae	7
Unidentified 1	-	4
Total no. of pollen		**41**

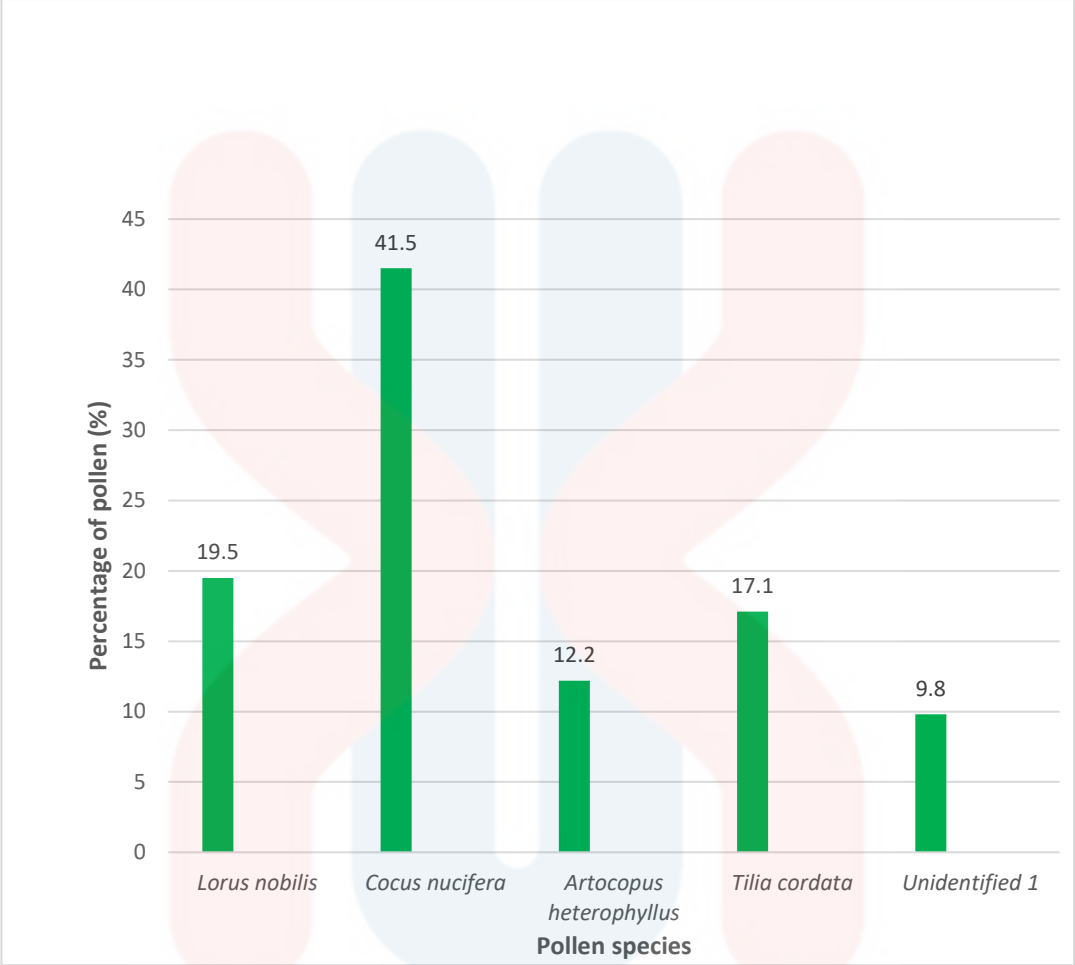

Figure 4.1.3.2: Percentage abundance of pollen collected from Kampung Durian Tawar, Bera, Pahang.

Different pollen species in airborne pollen samples from Bera, Pahang was summarised as in Figure 4.1.3.3. Bera was the second highest location that contained 41 pollen amount with 5 pollen species type such as *Lorus nobilis* (Lauraceae), *Cocus nucifera* (Arecaceae), *Artocopus heterophyllus* (Moraceae), *Tilia cordata* (Malvaceae) and Unidentified 1. In these location, there was one type of pollen species that unidentified the species name so labelled with Unidentified 1. The pollen cannot be identified due to irregular shape and not founded the source of structure belonging. Bera was had the huge Bera Lakeside known as "Tasik Bera". *Tilia cordata* (Malvaceae) had 7 pollen load in Bera, Pahang sample collected. These species commonly called as lime tree. It can be huge leaved tree or common lime tree in Malaysia due to the species. *Tilia cordata* (Malvaceae) had about 30 species tree or bushes. Next pollen was *Lorus nobilis* (Lauraceae) had 8 of pollen load at Bera, Pahang. *Lorus nobilis* was aromatic evergreen tree or large shrub with green, glabrous and flowering plant. Commonly called as bay leaves. The morphology was 1-sulcate grain, tectate exine, thick exine wall and circle pattern. Then, *Cocus nucifera* (Arecaceae). These species come from coconut tree. These species had 17 pollen load in Bera sample collected. Easily found these pollen species because it was not a rare pollen. Because of Bera had lake so coconut was a plant or crop that usually planted near the lake housing area. *Cocus nucifera* (Herbarium) was categorised as shrub. The morphology was 1- sulcate grain. It had tectate exine, both sexine and nexine are visible. Columellae short and also almost indistinct. The pattern was granulate. The lowest amount of pollen load at Bera was Unidentified 1 species that had 4 pollen amount in Bera sample collected. The structure of pollen little bit rare because it had 3 combined pollen like conjointed twins. The pollen was circle in shape but had 3 in one same time. In the circle shape had another small structure like irregular shape.

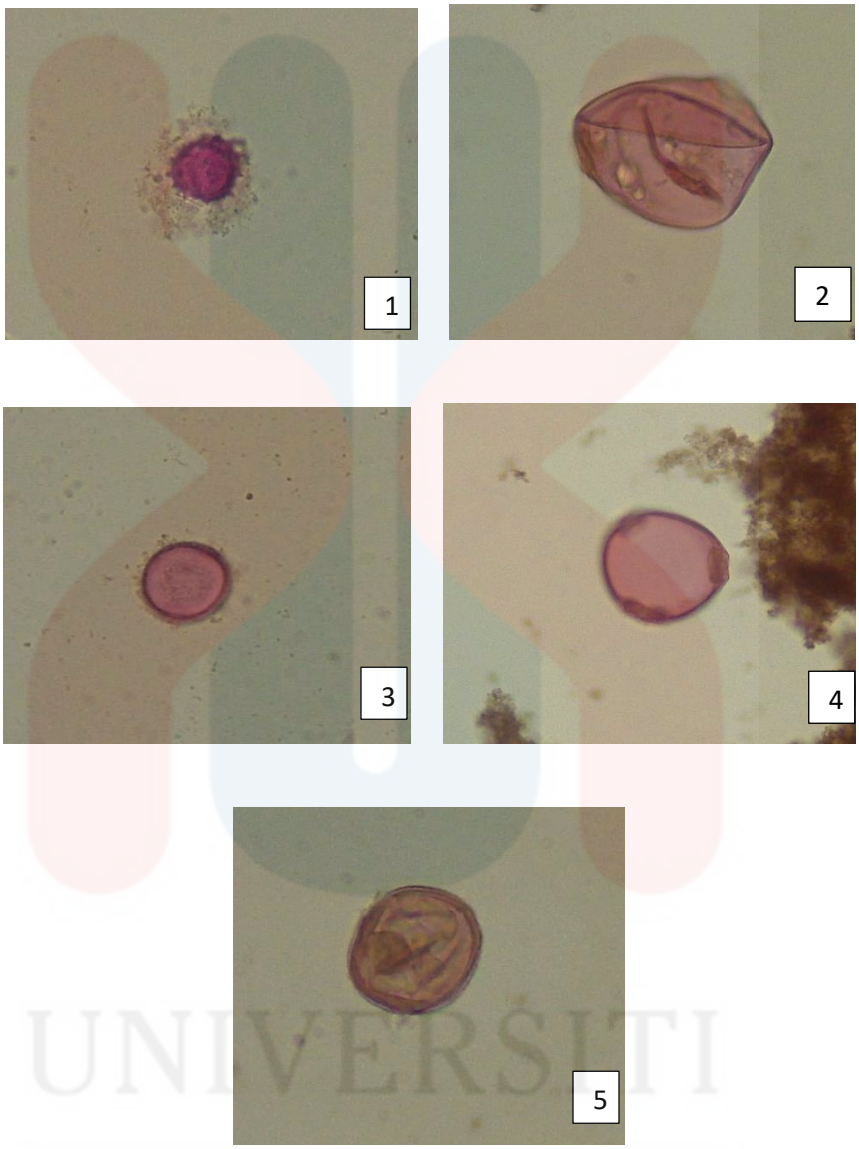

Figure 4.1.3.3 : Different pollen species in airborne pollen samples from Bera, Pahang under 400x magnification. **1-** *Lorus nobilis* (Lauraceae), **2-***Cocus nucifera* (Arecaceae), **3-***Artocopus heterophyllus* (Moraceae), **4-** Unidentified 1, **5-***Tilia cordata* (Malvaceae) **6-** Unidentified 2.

4.1.4 Air Pollen Samples from Felda Sungai Tekam, Jerantut, Pahang

Data for total pollen load in airborne pollen samples collected in Felda Sungai Tekam, Jerantut, Pahang. The result recorded 4 different species of pollen (Table 4.1.4.1). The total pollen load was 38 pollens (20.8%). Percentage abundance of pollen collected from Kampung Durian Tawar, Bera, Pahang was summarised in Figure 4.1.4.2.

Table 4.1.4.1: The number of pollen in airborne pollen sample collected from Felda Sungai Tekam,Jerantut, Pahang.

Plant species	**Family**	**No. of Pollen**
Veitchia merrilli	Areraceae	10
Garcinia mangostana	Clusiaceae	15
Betula albosinensis	Betulaceae	6
Unidentified 3	-	7
Total no. of pollen		**38**

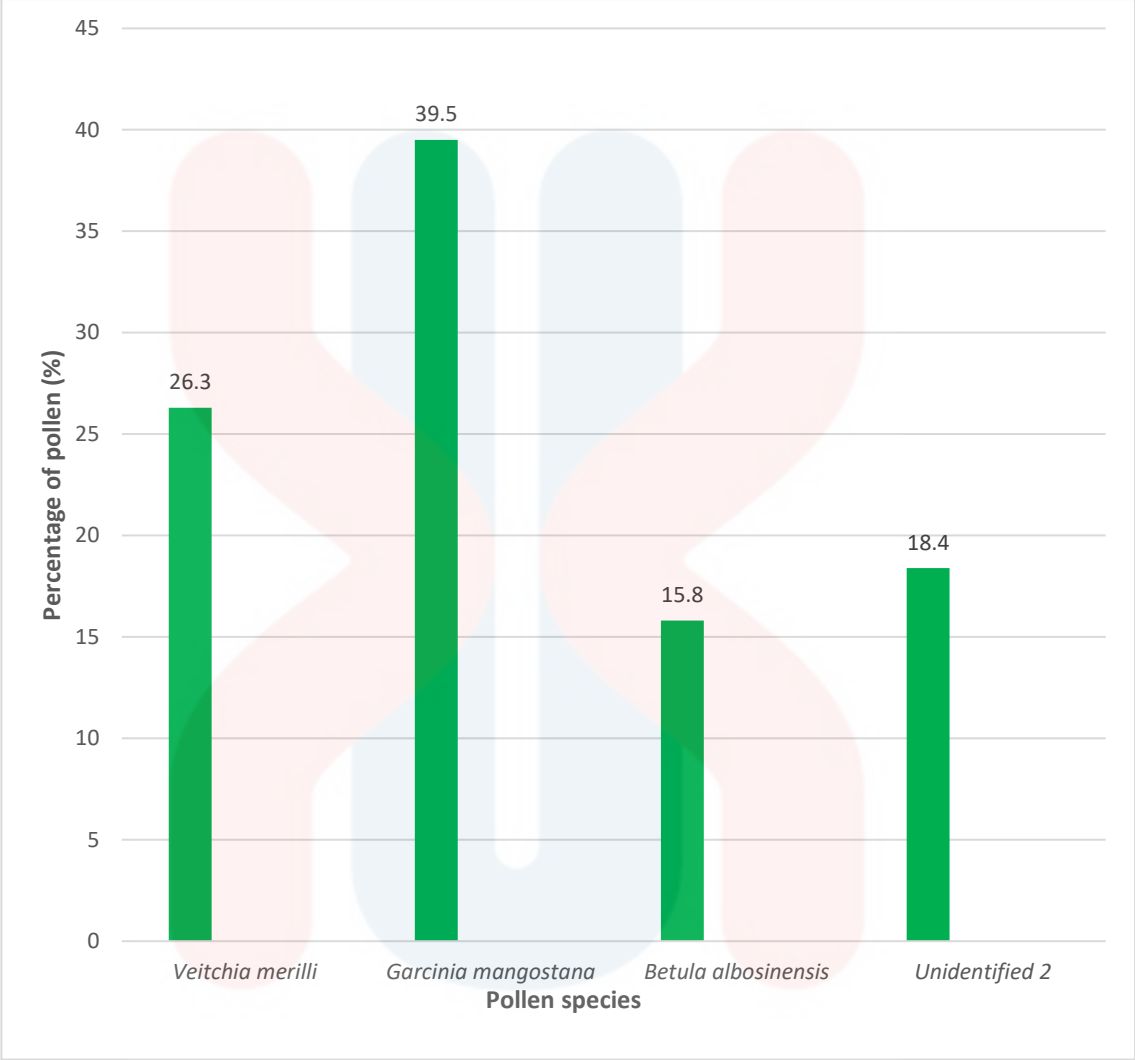

Figure 4.1.4.2: Percentage abundance of pollen collected from Felda Sungai Tekam,Jerantut, Pahang.

Jerantut was a third location sample collected in Pahang. Different pollen species in airborne pollen samples from Bera, Pahang was summarised as in Figure 4.1.4.3. Jerantut location was near Bera and Temerloh. In these location, there were 38 pollen load amount that found in the sample collected. These location consist 4 pollen species type such as *Veitchia merilli* (Arecaceae), *Garcinia mangostana* (Clusiaceae), *Betula albosinensis* (Betulaceae) and Unidentified 2. *Veitchia merilli* (Arecaceae) had 10 pollen load, *Garcinia mangostana* (Clusiaceae) had 15 pollen load, *Betula albosinensis* (Betulaceae) had 6 pollen load and Unidentified 3 had 7 pollen load. *Veitchia merilli* (Arecaceae) was known as Christmas palm. The tree looks like a Christmas tree with triangular shape. Other international name were adonidia palm, dwarf royal palm and manila palm. These tree commonly planted by people in front of their house for landscaping purpose. It also usually planted at field or beach for protective strong wind because had strong stem and root. *Betula albosinensis* (Betulaceae) was third highest pollen load in Bera. *Birch* known as silver birch or beech oak tree. A thin leaved deciduous hardwood tree, flowering and fruiting tree. *Betula albosinensis* (Herbarium) known as tree type. The morphology was 3-colporate grain, pores evenly spaced around equator, circular to slightly elliptical. It had thin exine, thickened a little round pores and ectexine thicker than exine. And also had subtriangular or sub-oblate, porate and aspidate. *Garcinia mangostana* (Clusiaceae) had the highest number of pollen load. *Garcinia mangostana* was called mangosteen tree. These tree consist of purple fruit that had white flash. These was a seasonal fruit like durian. These fruit known as queen of fruit. The morphology was 3-colporate grain with lalongate., rectangularly endoapertunes, long ectocolpi, tectate exine and indistinct columellae. Lastly was Unidentified 2 pollen species. The shape looks like King crab "belangkas". It cannot be identified in which group of pollen species.

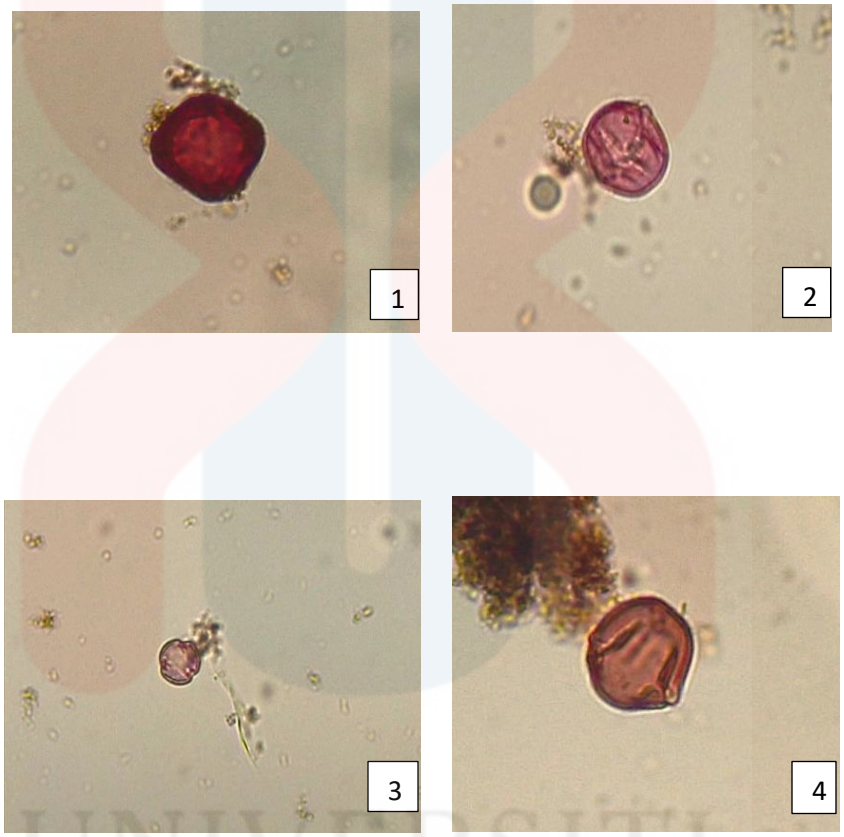

Figure 4.1.4.3 : Different pollen species in airborne samples from Jerantut, Pahang under 400x magnification. **1-** *Veitchia merilli* (Arecaceae), **2-***Garcinia mangostana* (Clusiaceae), **3-***Birch* (Betulaceae), **4-** Unidentified 3.

4.1.5 Air Pollen Samples from Jalan Merdeka, Temerloh, Pahang

Data for total pollen load in airborne pollen samples collected in Kampung Durian Tawar, Bera, Pahang. The result recorded 2 different species of pollen (Table 4.1.5.1). The total pollen load was 21 pollens (8.2%). Percentage abundance of pollen collected from Kampung Durian Tawar, Bera, Pahang was summarised in Figure 4.1.5.2.

Table 4.1.5.1: The number of pollen in airborne sample collected from Jalan Merdeka, Temerloh, Pahang.

Plant species	Family	No. of pollen
Unidentified 3	-	8
Piper umbellatum	Piperaceae	13
Total		**21**

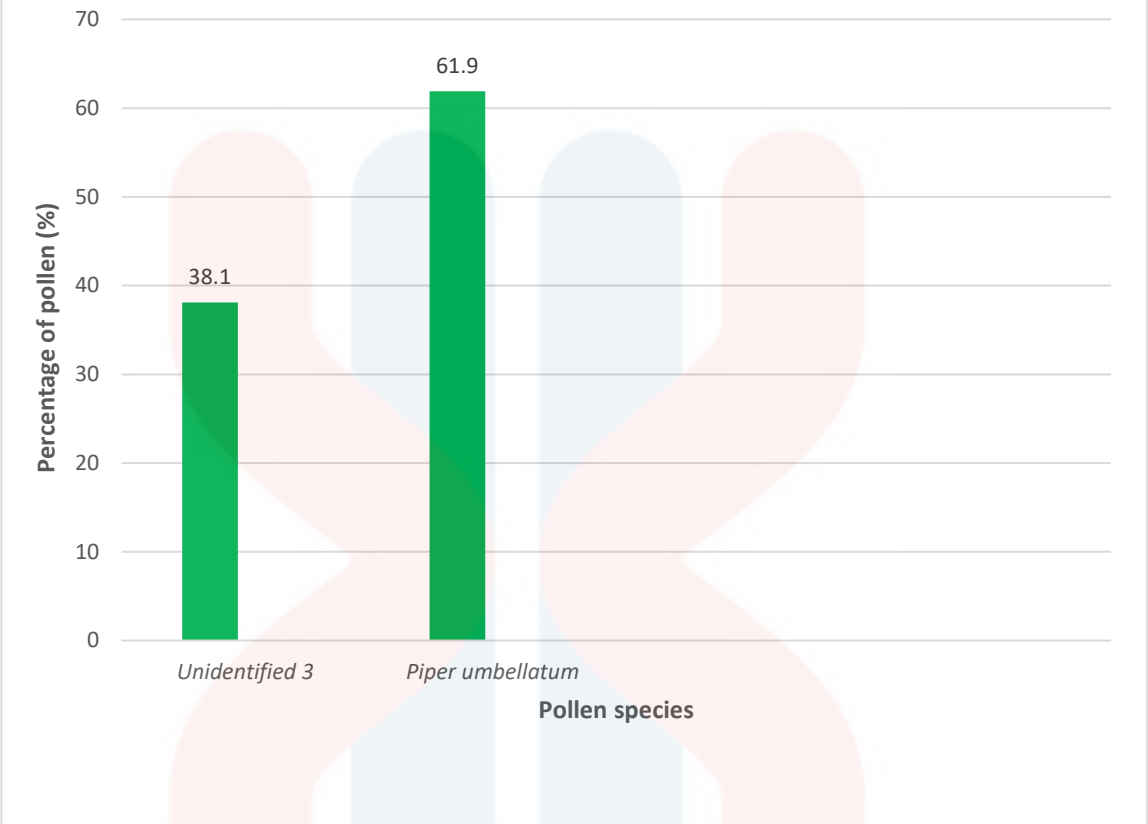

Figure 4.1.5.2: Percentage abundance of pollen collected from Jalan Merdeka, Temerloh, Pahang.

Temerloh was fifth location for airborne sample collected in Pahang. Different pollen species in airborne pollen samples from Bera, Pahang was summarised as in Figure 4.1.5.3. Temerloh had 21 pollen load with 2 species of pollen type. These location was fourth highest after Kuantan, Bera and Jerantut. Temerloh consist 11.5% overall from Pahang airborne sample collected. The species was Unidentified 3 and *Piper umbellatum* (Piperaceae). Even though these location had quite more amount of pollen but mostly from same pollen species. Just *Piper umbellatum* (Piperaceae) can be recognized and categorized belonging to which pollen group species. The English name was "false kava" or pepper plants. it can be categorized under shrub, herb and lianas. It also can be black pepper, white pepper and green pepper according to their species and colour. It was flowering and fruiting plant. Other unidentified species, the structure looks like a crystal, twin crystal, moving fungi and euglena or paramecium form. The structure was different from a common pollen found. *Piper umbellatum* was shrub type. The morphology was 1-2 sulcate grain ends with straight. It had tectate exine, indistinct columellae, thin exine, inseparable walls and scabrate pattern.

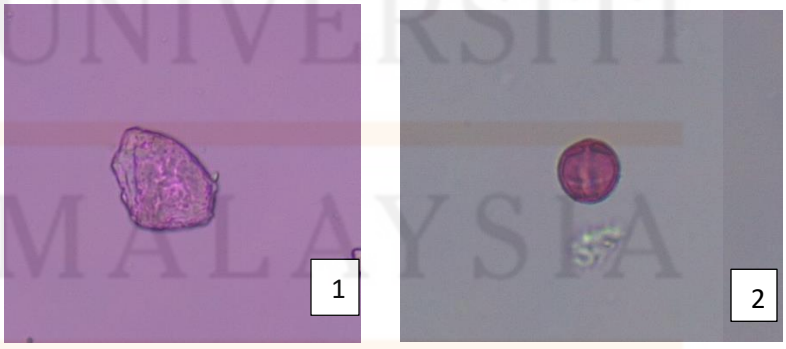

Figure 4.1.5.3 : Different pollen species in airborne pollen samples from Temerloh, Pahang under 40x magnification. **1-** Unidentified 3, **2-** *Piper umbellatum* (Piperaceae).

4.1.6 Air Pollen Samples from Felda Tersang 2, Raub, Pahang

Data for total pollen load in airborne pollen samples collected in Felda Tersang 2, Raub, Pahang. The result recorded two different species of pollen (Table 4.1.6.1). The total pollen load was 15 pollens (8.2%). Percentage abundance of pollen collected from Kampung Durian Tawar, Bera, Pahang was summarised in Figure 4.1.6.2.

Table 4.1.6.1: The number of pollen in airborne pollen sample collected from Felda Tersang 2,Raub, Pahang

Plant species	Family	No. of pollen
Grass	Poaceae	10
Veitchia merrilli	Arecacea	5
Total		**15**

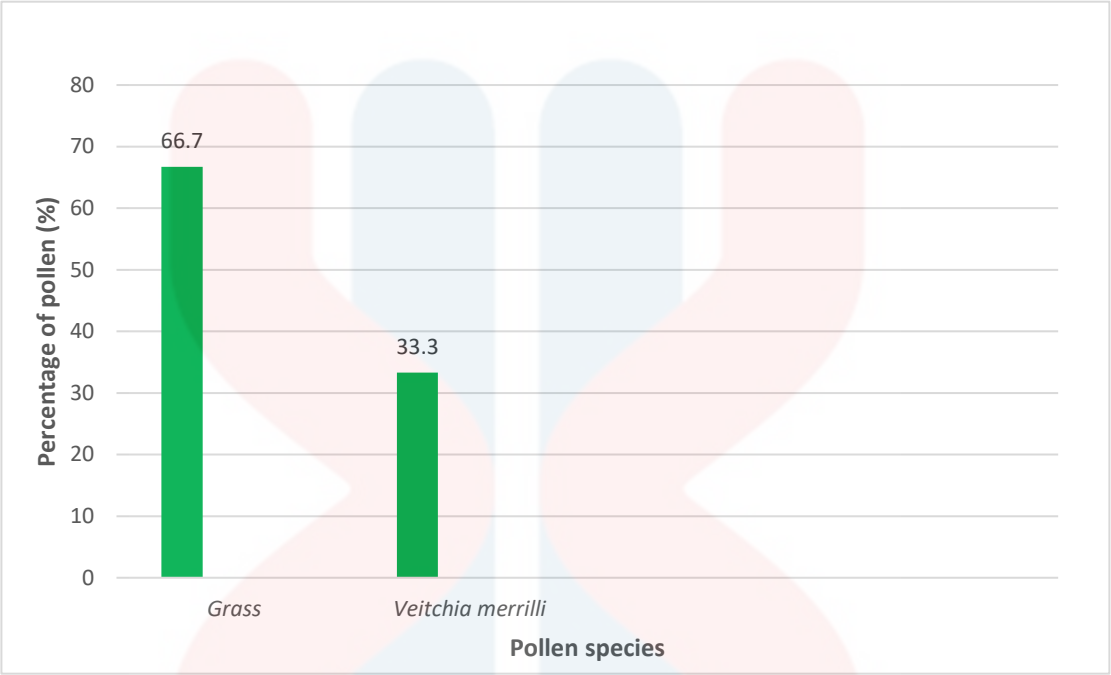

Figure 4.1.6.2 : Percentage abundance of pollen collected from Felda Tersang 2,Raub, Pahang.

Raub was second last location for airborne pollen sample collected. Different pollen species in airborne pollen samples from Bera, Pahang was summarised as in Figure 4.1.6.3. Raub had 10 pollen load with 6 species pollen type. These location consist 9% from 100% pollen in Pahang's sample. Raub location near Kuala Lipis area. Pollen species in these location were *Grass* (Poaceae) and *Veitchia merrilli* (Arecaceae). Only two pollen species identified in Raub. *Grass* (Poaceae) had the highest pollen load amount with 10 pollen. Grass was a common pollen found due to the weather and environment surrounding. Every place had grass either the wild grass or can called as weed and also turf grass as landscape and beauty. Grass was allergy pollen to human. Lot of people was allergic to the pollen that come from grasses. It would bring symptoms like a runny or stuffy nose, itchy eyes and cough. There were hundreds type of grasses. The usually trigger allergic was Bermuda grass, Kentucky grass, Johnson grass, Rye grass, Sweet vernal grass and orchard. The others pollen structure quite rare but still in pinkish colour looks like a pollen species due to the characteristics. The other pollen species was *Veitchia merrilli* (Arecaceae) with 5 pollen load. *Veitchia merrilli* can be categorised as tree and shurbs. The morphology was 1-sulcate grains. It had thin exine and also nexine thicker than exine.

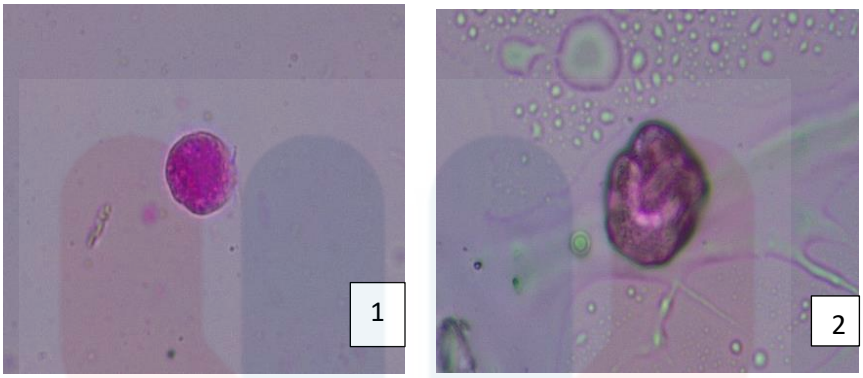

Figure 4.1.6.3 : Different pollen species in airborne samples from Raub, Pahang under 400x magnification. **1-** *Grass* (Poaceae), **2-** *Veitchia merrilli* (Arecaceae).

4.1.7 Air Pollen Samples from Felda Sungai Koyan, Kuala Lipis, Pahang

Data for total pollen load in airborne pollen samples collected in Felda Sungai Koyan, Kuala Lipis, Pahang. The result recorded three different species of pollen (Table 4.1.7.1). The total pollen load was 23 pollens (12.6%). Percentage abundance of pollen collected from Kampung Durian Tawar, Bera, Pahang was summarised in Figure 4.1.7.2.

Table 4.1.7.1 : The number of pollen in airborne pollen sample collected from Felda Sungai Koyan, Kuala Lipis, Pahang.

Plant species	Family	No. of pollen
Artocopus heterophyllus	Moraceae	11
Alnus glutinosa	Betulaceae	5
Manihot esculenta	Euphorbiaceae	7
Total		**23**

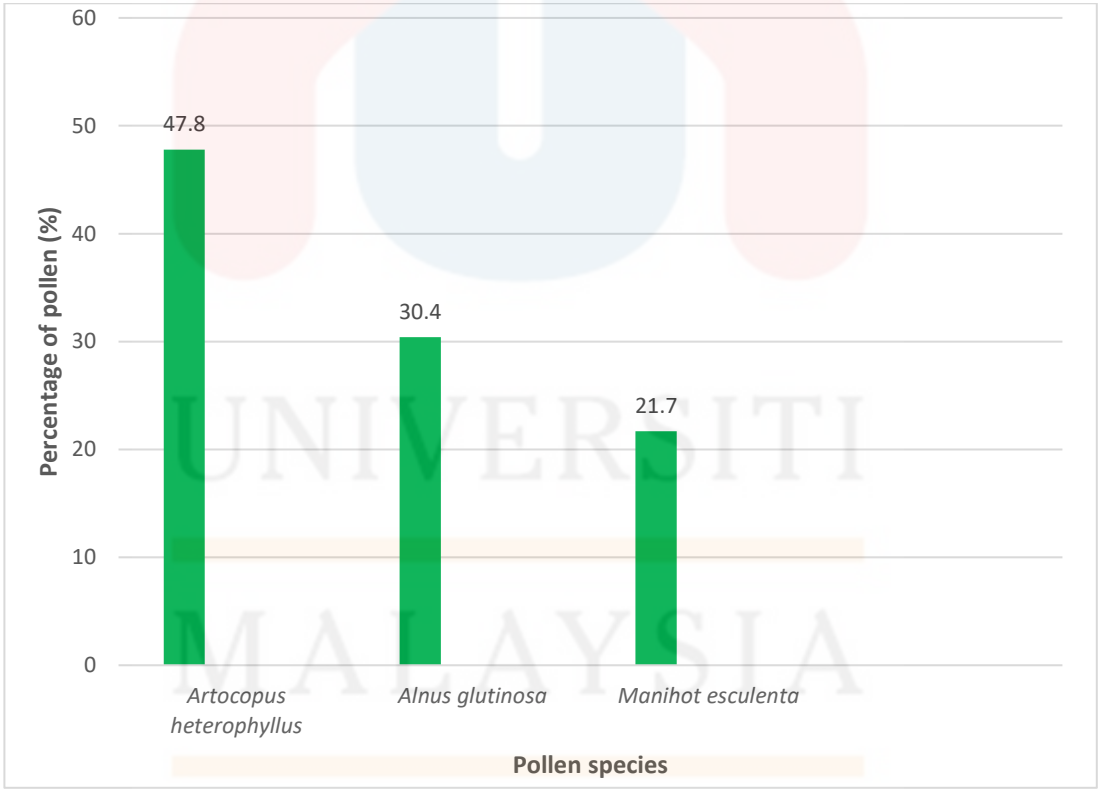

Figure 4.1.7.2: Percentage abundance of pollen collected from Felda Sungai Koyan, Kuala Lipis, Pahang.

Last location was Kuala Lipis. Different pollen species in airborne pollen samples from Bera, Pahang was summarised as in Figure 4.1.7.3. Kuala Lipis consist 23 pollen load with 12.6 %, the third lowest before Temerloh and Raub. In these location, there was 3 species of pollen type. The pollen was *Artocopus heterophyllus* (Moraceae), *Alnus glutinosa* (Betulaceae) and *Manihot esculenta* (Euphorbiaceae). The highest pollen in Kuala Lipis was *Artocopus heterophyllus* (Moraceae). There were 11 pollen for these species pollen type. *Artocopus heterophyllus* was a jackfruit tree pollen. Jackfruit tree is nonseasonal fruit. Jackfruit was planted by people and not a wild plant. The pollen was circle in shape and easy to recognized. The morphology was 3-porate grain, very small apertures, pores circular, tectate-perforate exine, indistinct columellae and granulate pattern. Next was *Alnus glutinosa* (Betulaceae) that had 7 pollen load in these Kuala Lipis collected sample. *Alnus glutinosa* called as common alder or black alder. Common alder provided food and shelter to wildlife, with a number of insect, liches and fungi being completely depend on these tree. These tree available in forest and riverside. It can be categorised as tree type. The morphology was 3-colporate, pores, sub-oblate to spheroidal and triporate with small onci. Next was *Manihot esculenta* (Euphorbiaceae). *Manihot esculenta* was tropical tree that known as Cassava or tapioca plant. It was shrub that only 3 metre tall. Cultivated tropical food. The shape was circle but has a lot small dotted or blackspot inside the huge circle. Had the small thorns shape outside layer. It can be categorised as shrub type. The morphology was polyporate, pantoporate, spheroidal, large poricircular, thick exine and sexine thicker than nexine. The pattern was sculpturing croton and triangular pegs.

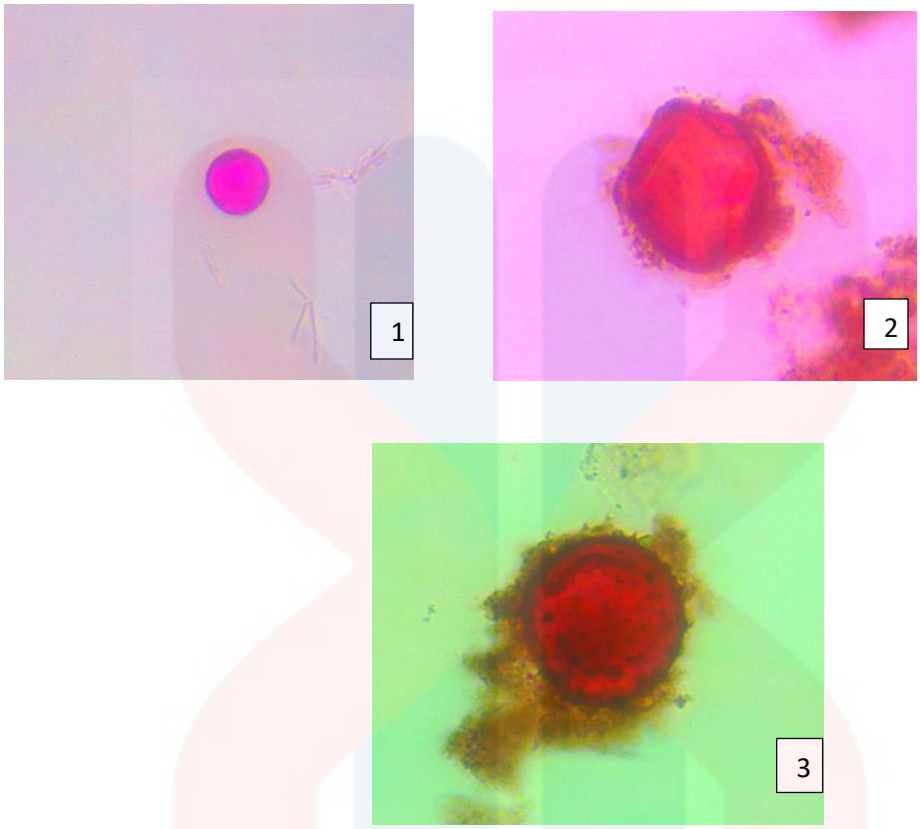

Figure 4.1.7.3: Different pollen species in airborne pollen samples from Kuala Lipis, Pahang under 400x magnification. **1-** *Artocopus heterophyllus* (Moraceae), **2-** *Alnus glutinosa* (Betulaceae), **3-***Manihot esculenta* (Euphorbiaceae).

DISCUSSION

Inhalation allergy is common disorders where at least 10% of the world population suffers from the manifestation of hypersensitivity (Bicakci *et al.*, 2004). There is correlation between disease symptoms and pollen occurrence of pollen grains in the air. It is important for medical treatment and prophylaxis. The pollen studies have been carried

out in many countries such as India, Indonesia as well as Malaysia for example in Kuala Lumpur and Kedah.

In these research, pollen analysis of airborne samples was done in six different location of Pahang, Malaysia. A total number of 183 pollen grains and 19 different pollen species have been successfully identified by using the pollen acetolysis and pollen count method. These method were used in order to identify the morphological structure of pollen and also the number of pollen loads in the atmosphere. Pollen morphology is the principle tool used for the correct identification. The features of pollen are studied in the pollen morphology. The parameters that are used in the pollen morphological study are symmetry, shape, size and pollen wall (Agashe, 2009).

There is pollen for each location airborne sample collected even though some are very little just contain 15 pollen load only. From airborne sample collected, the pollen mostly can be categorized as allergic pollen. Kuantan 45 pollen count, Bera 41 pollen count, Jerantut 38 pollen count, Temerloh 21 pollen count, Raub 15 pollen count, Kuala Lipis 23 pollen count. *Elaeis guineensis* has the highest amount of pollen species found in airborne sample collected with 24 pollen. Next is *Cocus nucifera* has second highest amount of pollen species found in airborne sample collected with 17 pollen. The lowest pollen species is Unidentified 1 with 4 pollen count. *Veitchia merrilli* also has lowest pollen species which is 5 pollen count.

This project is successfully done with a remote helicopter that spin at the airborne with same speed level in 178.3 cm height. This is the average height for both male and female. Male's height is 185.6 cm while female's height is 171.0 cm. The end of this

project, already get to evaluate the number of pollen present in the airborne sampled collected from different locations of Pahang. Successfully identified the type of pollen based of pollen's morphology in the airborne samples collected. Nasal allergy to a particular things such as pollen known as Pollinosis. Allergy to grass pollen is known as Hay fever. This study purposely to know the human allergy level towards what kind of pollen type and species already done.

From this research, the most common pollen grains that found can be identified as allergic pollen. *Elaeis guineensis and Cocus nucifera* are the allergic pollen. These pollen can effect human's immunity due to the pollen sensitivity. These pollen may commonly induce symptoms of allergic rhinitis and asthma in sensitized individuals especially in Malaysia and Singapore (Chew *et al.,* 2000). Extract of oil palm were observed to have the highest frequency of positive reactions (40%). Positive responses to these extracts correlated with the total serum IgE levels of the subjects and were significantly associated with the presence of atopic disease (Kimura *et al*., 2002). So, the allergy to the fruit of oil palm is probably possible. In these study also identified the Coconut as sensitivity pollen. PIMA CONTY Department of Environmental Quality (2015) has classified pollen into two major groups, the bigger particle and smaller particle. People with asthma record should avoid any outdoor activity and everyone else especially the elderly and children should limit outdoor exertion. Everyone would in hazardous conditions if the Air Quality Index in range 301-500. Therefore, everyone should avoid outdoor exertion or avoid deal with people that have respiratory disease such as asthma and rhinitis.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

As a conclusion, from airborne pollen sample collected, the pollen mostly can be categorized as allergenic pollen. There was 183 pollen grains from airborne pollen samples collected in Pahang. Kuantan had the highest abundance of pollen while Raub had the lowest abundance of pollen. *Elaeis guineensis* was the highest amount of pollen species found in airborne pollen sample collected with 24 pollen count. Next, *Cocus nucifera* was the second highest amount of pollen species found in airborne pollen sample collected with 17 pollen count. *Elaeis guineensis* and *Cocus nucifera* was known as allergenic pollen.

5.2 RECOMMENDATIONS

As a recommendation for further studies, the selection of site for collecting airborne pollen samples can be expanded to the city area because of more tourism, higher number of netizen, higher air pollution due to the environmental of city, temperature and humidity factor. Based on the result, it is recommended that individuals with high pollen hypersensitivity should be cautious when outside, especially on Kuantan area, where there were high pollen loads of allergenic pollen. Allowing them to adjust their daily activities to minimize contact towards allergens. In addition, for further studies also, the result of airborne pollen collected can be summarised and recorded in a pollen catalogue for easier pollen identification.

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Figure 5.1: Scrubbing process using vortex

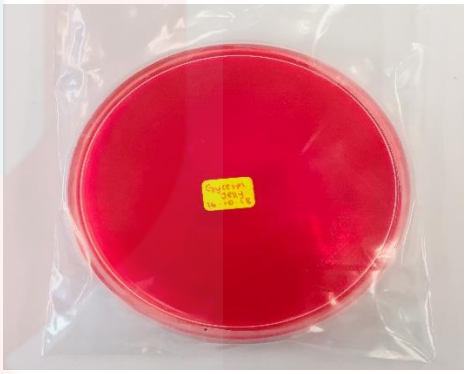

Figure 5.2: Glycerine Jelly

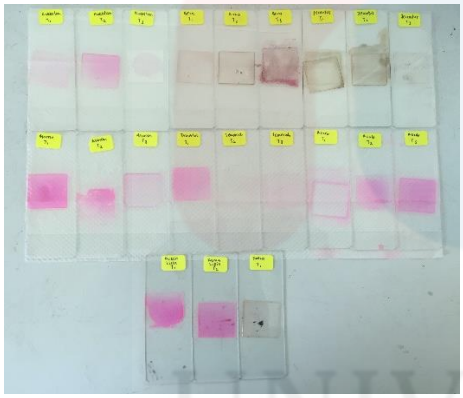

Figure 5.3: Slide for observation

Figure 5.4: Prepare Glycerine Jelly