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Pollen Analysis of Selected Malaysian Honey

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Abstract: Pollen analysis or also known as melissopalynology is a valuable tool for identification of the botanical and geographical origin of honey samples. The aim of the present study is to determine the botanical origin of four selected Malaysian honey namely Tualang (*Koompassia excelsa*), Gelam (*Melaleuca cajuputi*), Acacia (Robinia pseudoacacia) and Nenas (*Ananas comosus*) honey. The morphology of pollen contributing as nectar resources were observed with environmental scanning electron microscope (ESEM) and total pollen counts were expressed in pollen percentage frequency (P.P.F). Based on the qualitative analysis, Tualang honey was classified as multifloral honey and the remaining three local honeys were unifloral. This study has identified foraging plant sources for honeybees and demonstrated adequate potential for expanding and sustaining beekeeping in Malaysia.

Key words: Pollen Analysis · Malaysian Honey · Multifloral Honey · Unifloral Honey · Morphology · Esem

INTRODUCTION

Honey is a natural and easily digested food produced by honey bees. Bees obtain pollen from flowers and chemically converted by process of regurgitation and evaporation into honey. In this study, four selected Malaysian honey namely Tualang (*Koompassia excelsa*), Gelam (*Melaleuca cajuputi*), Acacia (Robinia pseudoacacia) and Nenas (*Ananas comosus*) honey were selected to observe the botanical origin [1, 2]. Honey roughly contains 80% of carbohydrates (35% glucose, 40% fructose and 5% sucrose) and 20% waters [3]. The composition of honey mainly depends on the flowers consumed by the honey bees and the climatic factors [4-6]. Hence, knowing the origin of the nectar flower of the honey bee consumed is important.

Pollen is the male spore plasma of the plant, produced on the flower's anthers, each of which is at the outer end of a stamen [7]. Certain external factors such as environmental and seasonal factors influence the production of honey itself [8]. During foraging, the honeybees will suck the nectar of flower and at the same time, the pollen of the flower will also stick to the bee's leg. Different types of pollen are used to indicate flora nectar sources utilized by bees to produce honey owing to the distinctive appearance of pollen grains produced by different species [9].

Pollen analysis or Melissopalynology deals with the quantitative and qualitative study of pollen grains in honey [9]. Pollen is an essential tool in the analysis of honey as it indicates the major and minor plant taxa utilized by honeybees. The presence of dominant pollen is used to characterize unifloral honey while if none is dominant, it is of mixed floral [10]. The analyses of pollen contents in honey provide reliable information of the floral components of honey thus identifying the source plants used by bees in the production of honey [11]. This method is not only useful in the determination of the geographical and botanical origin of a particular type of honey but also provides great importance for quality control and helps to ascertain whether honey is adulterated or not Ebenezer and Olugbenga [11] and Bibi *et al.* [12]. The pollen analysis in a honey sample can be used as a basis for determining the floral sources, as well as assessing claims made by apiarist as to the purity of the product [13]. Furthermore, the detailed

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characterization of the different honey type's existent is important, to avoid the occurrence of adulterations and important for the commercial valorisation of honey [14].

A pollen analytical study of Malaysian honey provides a basis for identifying the origins of honey in terms of locality and floral source. Identification of the pollen found in honey shows which plants honeybees visit to obtain the nectar to make honey. Thus, management strategies can be implemented to maintain the strength of the colony as well as maximize honey yields. Therefore in this study, the identification of pollen using environmental scanning electron microscopy (ESEM) technique was carried out to determine the botanical source of selected Malaysian honey either from unifloral or multifloral honeys.

MATERIALS AND METHODS

Materials: Four types of selected Malaysian Honey were collected from different states of Malaysia, Gelam Honey (Agricultural Department of Terengganu), Nenas honey (Agricultural Department of Johor), Acacia (Agricultural Department of Terengganu) and Tualang honey (Bee hunter in Kedah). Deionized water was provided by Reservoir® (Elga Water System, UK).

Preparation of Slides Without Acetolysis: One ml of each honey was dissolved in 5ml of deionized water in Eppendorf tube at 37°C. The solutions were centrifuged for 5 min at 10000 rpm before the supernatant was aspirated out. Following that, 5 ml of deionized water was added into the Eppendorf tube containing sediment and centrifuged again for 5 min at 10000 rpm. The sediment was dropped on an aluminium stub and covered with an aluminium tape. Each of the honey sample was placed on a petri dish, respectively. Each of the sample was left dry at room temperature for 1 day. Each of the sample was viewed by using environmental SEM [15].

Quantification of Pollen: The following terms have been used in estimating of pollen grain frequencies. "Vary frequent" describes grains constituting more than 45% of the total count, "Sporadic" describes grains constituting less than 3% and the following terms used for frequency classes: "Predominant pollen" (More than 45% of the pollen grains counted), Secondary Pollen (16-45%), important minor pollen (3-15%), Minor pollen (Less than 3%) [16].

Identifying the Pollen in Each Honey: dentification of each pollen grains in the treated honey sample was done with the aid of pollen atlas and other published floras which gave brief descriptions of the observed pollen in the each honey. Most of the pollen identification was taken from Ibrahim [17], Song *et al.* [18] and pollen atlas of Roubik and Moreno [19].

RESULTS AND DISCUSSION

Total Pollen Count and Species in Selected Malaysian and Manuka Honey: Table 1 summarizes the number of pollen count and species found in each honey.

Table 1: Number of pollen count and species found in Tualang, Gelam, Nenas and Acacia honey

			Number of pollen count			
No.	Family	Plant Species	Tualang honey	Gelam honey	Nenas honey	Acacia honey
1	Acanthaceae	*Asystasia gangetica*	-	-	3	-
2	Apocynaceae	*Thevetia neriifolia*	25	-	4	-
3	*Bromeliaceae*	*Ananas Comosus*	-	-	10	-
4	Clusiaceae	Garcinia *hombroniana*	42	-	-	-
5	Fabaceae	*Acacia species*	40	-	-	18
		Mimosa Pudica	38	15	-	-
		Robinia pseudoacacia	-	-	-	8
		Trifolium repens	-	-	-	-
6	Myrtaceae	*Leptospermum scoparium*	-	-	-	-
		Melaleuca cajuputi	68	28	-	-
7	*Nelumbonaceae*	*Nelumbo nucifera*	-	-	-	-
8	Palmae	*Cocos Nucifera*	32	-	-	-
		Elaeis *guineensis*	90	5	-	5
9	Rubiaceae	*Ixora species*	45	-	-	-
10	Rosaceae	*Rosa canina*		-	-	-
11	Unidentified pollen		36	4	1	2

Pollen morphology characteristics of Tualang Honey

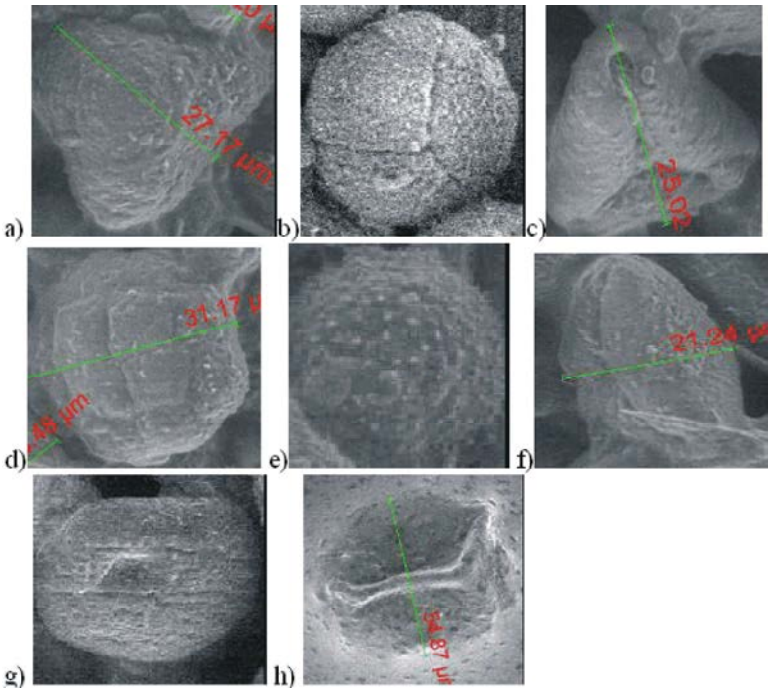

Fig. 1: Pollen source identified from Tualang honey using ESEM a) *Elaeis guineensis* b) *Mimosa Pudica* c) *Melaleuca Cajuputi* d) *Acacia sp* e) *Garcinia hombroniaca* f) *Ixora sp* g) *Cocos nucifera* h) *Thevetia nerifolia*

Pollen morphology characteristic of Gelam Honey

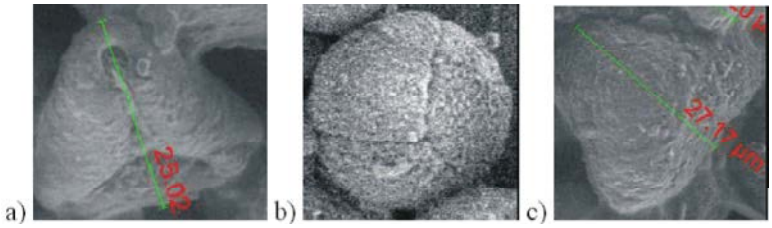

Fig. 2: Pollen source identified from Gelam honey using ESEM a) *Melaleuca Cajuputi* b) *Mimosa Pudica* c) *Elaeis guineensis*

Pollen morphology characteristic of Nenas honey

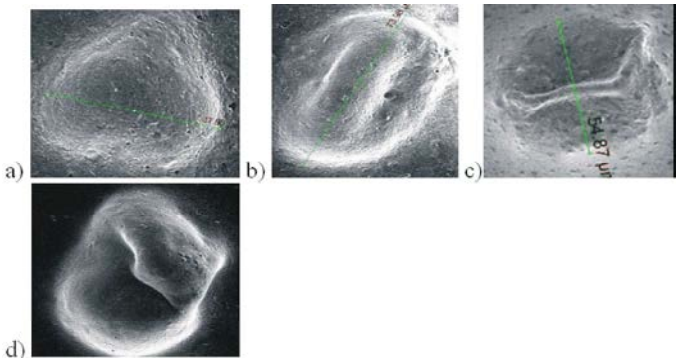

Fig. 3: Pollen source identified from Nenas honey using ESEM, a) *Ananas Comosus* b) *Asystasia gangentica* c) *Thevetia nerifolia* d)unidentified species

Pollen morphology characteristic of Acacia honey

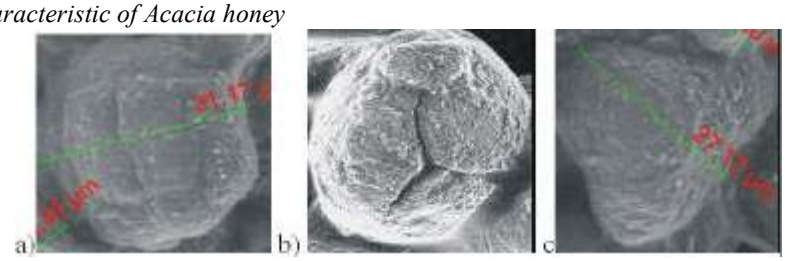

Fig. 4: Pollen source identified from Acacia honey using environmental scanning electron microscopy, a) *Acacia species* b) *Robinia pseudoacacia* c) *Elaeis guineenis*

From the results, number of pollen count was highest in Tualang honey followed by Gelam honey, Acacia honey and Nenas Honey. The pollen characteristics and morphology of each honey was shown in Figure 1 to Figure 4. Number of pollen count was calculated as pollen percentage frequency to categorize as unifloral or multifloral honey.

There were eleven different pollen sources identified in the local honeys. Based on the results, Tualang honey was classified as multifloral honey because of the pollen species involved was not more than 45%. The secondary predominant frequency was found in *Elaeis guineenis* (Palmae 21.6%) and *Melaleleuca cajuputi* species (Myrtaceae 16.3%). Tualang honey also contain important minor pollen including *Ixora species* (Rubiaceae 10.9%), *Garcinia hombroniaca* (Clusiaceae 10.5 %) *Mimosa Puidica* (Fabaceae 9.6 %) and multifloral species which the source of origin cannot be identified.

CONCLUSION

Form the analysis we can conclude that Gelam honey, Acacia honey and Nenas honey were categorized as unifloral honey based on primary predominant pollen frequency that were more than 45%. Pollen grain frequency of *Ananas comosus* was 55.5% in Nenas honey, 53.8% of *Melaleuca cajuputi* in Gelam honey and 54.5% of *Acacia sp* in Acacia honey that confirmed these species are unifloral honey. On the other hand, Tualang honey was classified as multifloral honey because none of the pollen species were more than 45%. These findings provide knowledge on the origin source of honey that is necessary to maintain the adequate and abundant supply sources of nectar and pollen for the bees thus maximize honey yields.

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