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Melissopalynological Analysis of Forest Honey from North Malaysia

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Abstract

Honey is a natural product widely used by humans due to its sweet taste and health benefits produced by bees from nectar and honey dew of various plants. To establish and increase the production of honey one must know the plants that take part in the production of honey. In this study pollen analysis of forest honey samples from northern part of Malaysia was carried out to determine the botanical sources playing role in the production of honey in that region. The pollen samples were acetolyzed and identified microscopically. Out of the three samples studied Baling sample was unifloral having *Mimosa scabrella* as predominant pollen while Jeli and Gerik samples are multifloral containing *Tipo myrcia* and *Elais guineensis* as major secondary pollen. Fabaceae family represented four pollen types and accounted 80% of pollen in Baling sample and 34 % in Gerik sample while completely absent in Jeli sample. These results showed the dominance of plants from Fabaceae family in honey production. All the samples analysed have *Albizia falcataria*, *Eupatorium* sp., *Sparganium typha*, *Tilia* sp. and *Tipo myrcia* in common indicating that these plants are present in all the three places and these results also can be used as a tool in geographical identification of North Malaysian honey from others.

1. Introduction

Honey is a natural sweetening agent produced by bees from nectar of plants, secretions of living parts of the plants or excretions of plant-sucking insects of the living part of plants. Honey is the most ancient sweetener, and it was noted to have been in use throughout the world several million years ago [1]. Honey contains about 200 substances, essentially composed of a complex mixture of carbohydrates and other minor substances, such as phenols, organic acids, amino acids, proteins, minerals, vitamins and lipids along with minor amounts of pollen [2]. The composition of honey is affected by contributions of the nectar sources, location, climate, environmental conditions and ability of the beekeeper [3] [4]. The usage of honey and other honey related products like royal jelly and honeybee pollen are increasing these days due to their acclaimed nutraceutical and therapeutic properties.

Honey bees while collecting nectar from flowers also collect pollen which is a crucial part of diet for bee larvae [5]. Pollen is a fine powder produced by anther-the male reproductive organs of flowering plants. Pollen is rich in carbohydrates, amino acids, proteins, lipids, vitamins, minerals, phenolic compounds, flavonoids, and phytosterols [6]. The pollen collected by the honey bees pass into the honey and remains in the honey. This pollen present in the honey is used in determination of the botanical origin of honey [7]. The identification and quantification of pollen in honey is known as melissopalynogical studies. These studies are quantitative and qualitative microscopic determination of pollen present in honey which helps in determining floral or botanical origin which is essential for standardization of honey and geographical origin of the honey and foraging ecology of honeybees [8] [9]. Pollen analysis also helps in determining whether the honey has been contaminated with poisonous pollen or adulterated [10]. These studies help to discriminate multifloral honeys from unifloral or specific type of honeys which are of high commercial value. Additionally the pollen spectra identified will provide information about the flowering plants utilized by the bees in the study area. Knowledge regarding botanical source of honey is a must for beekeepers for increasing honey production [11].

Malaysia, a tropical country rich with a variety of flora and fauna and harbors many different types of honey like Tualang honey, Gelam honey and many others. But the Malaysian apiculture was far undeveloped compared to neighbor countries; Thailand and Vietnam, although many attempts had been carried out by the government to promote the apiculture industry since the 1980s [12]. Since then few advancements have been made toward strengthening bee farming infrastructure and the industry in Malaysia. Most of the Malaysian honey is imported and a study of Mardan and Osman [13], showed

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adulteration of honey was found common in Malaysia market. The inclusion of honey as a new Agro-resource and service in RMK10 indicates the importance of beekeeping and its related activities in Malaysia's national agenda [14]. Honey is largely produced in Malaysia in the states Johor, Perak and Selangor in Selangor [15] [12]. Since the areas of North Malaysia are not playing big role in honey production, these areas are chosen for the analysis of pollen in the honey to develop relation between the plant sources and honey so that it will be helpful in developing the honey production in this area and also serve as reference for honey quality analysis.

2. Materials and Methods

2.1. Honey Sample Collection

Squeezed forest honey samples (each three) were collected from Jeli, Gerik and Baling of north Malaysia (Figure 1) and examined for different pollen types and their percentages.

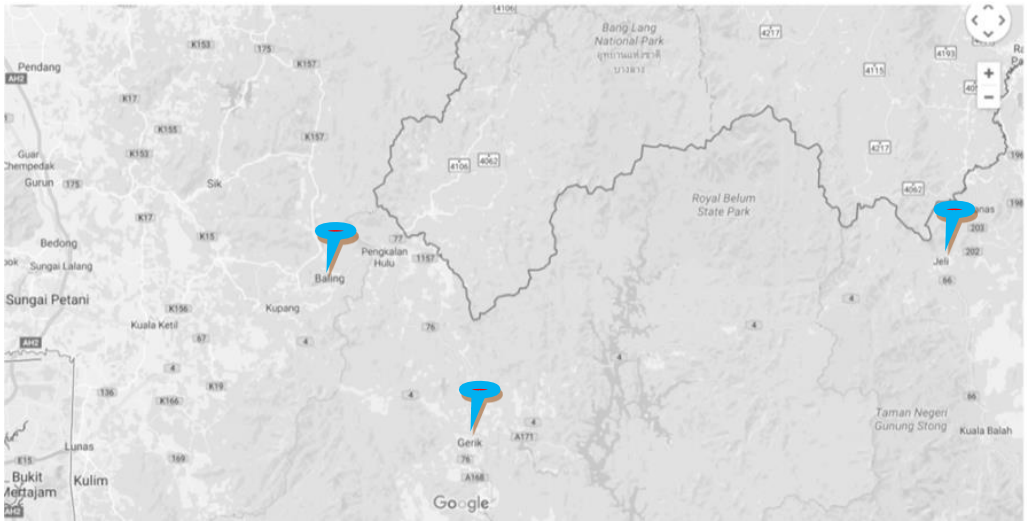

Figure 1: Map showing the honey sample collection places. (Source: Google maps).

2.2. Pollen Characterization and Identification

Pollen characterization and identification was done according to the guidelines given by International Commission of Bee Botany [16]. Pollen samples for the analysis were prepared using acetolysis method as per Louveaux *et al.* [17]. 10 ml of honey mixed with 20 ml of distilled water and centrifuged at 5000 rpm for 10 minutes. The supernatant was removed and glacial acetic acid was added to the residue and allowed to stand for five minutes before centrifuging and decanting. Then 1ml of 10% potassium hydroxide (KOH) was added to the sediment and boiled for 5 minutes on a water bath at 70°C. This process turns the pollen into light to golden brown in colour. The mixture is centrifuged and KOH was removed, the residue containing pollen was mounted on glycerine jelly and observed under compound microscope with 400X magnification. Pollen identification was done by comparing with references of flora and pollen description published [18], [19], [20].

2.3. Pollen Count

Total number of pollen present in the honey samples were counted according to Adeonipekun [21]. Pollen is counted in groups of 100, following parallel equidistant lines uniformly distributed from one edge of cover slip to other until 500 grains are counted. The total number of pollen is calculated by using dilution factor and number of pollen in the sample. The characterization of pollen was based on percentages of each pollen type: Predominant Pollen -PP (>45%), Secondary Pollen -SP (16-45%), Important Minor Pollen -IP (3-15%), Minor Pollen -MP (1-2%), and "Present" (<1%) [16].

$$\text{Total pollen count per slide} = \frac{N \times 2500}{8}$$

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

3. Results and Discussion

The results of melissopalynogical analysis of the three honey samples are shown in Table 1. The total pollen count showed that honey sample from Baling (272,812) has the most abundance of pollen compared to the sample of honey from Jeli (133,748) and Gerik (243,600). According to Ige and Apo [22] the more pollen type or pollen content in honey, the more the source of nectar collection and the more richness of the honey. A total of 19 pollen types were identified, 12 were identified up to species level, 6 were identified upto Gnus level and one left identified as wild grass pollen (Figure 2). Baling sample has 14 types of pollen followed by Gerik with 12 types and Jeli had 9 types of pollen. Two of the three honey samples viz Jeli and Gerik were multifloral in origin while Baling sample is unifloral in origin containing pollen of *Mimosa scabrella* more than 45%. Unifloral honey is mostly produced from one plant species which represents more than 45% of the total pollen content [23].

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Table 1: Pollen abundance in different samples collected from forest environs of east coast and north Malaysia.

Sample name	Predominant pollen (PP >45% of pollen grains counted)	Secondary pollen (SP, 15-45%)	Important minor pollen (IMP, 3-15%)	Minor pollen (MP, 1-3%)
Jeli		*Tipo myrcia* (30.14) *Sparganium typha* (23.83)	*Cocos nucifera* (11.92) *Elais guineensis* (9.58) *Albizia falcataria* (7.01) *Tilia* sp. (9.07) *Eupatorium* sp. (6.67)	*Osmunda sp.* (0.58) *Polygala laureola* (0.7)
Baling	*Mimosa scabrella* (45.41)	*Sophora japonica* (20.91)	*Mimosa pudica* (11.45) *Psidium sp.* (3.21) *Sparganium typha* (5.04) *Syzygium caryophyllatum* (4.24) *Tipo myrcia* (5.01)	*Albizia falcataria* (0.34) *Eupatorium* sp. (0.57) *Juniperus* type (0.23) *Polygala laureola* (0.69) *Tilia* sp.(0.46) *Tribulus terrestris* (0.54) Wild grass type (0.8)
Gerik		*Elais guineensis* (16.36) *Sparganium typha* (15.52)	*Mimosa scabrella* (14.0) *Mimosa caesalpinifolia* (12.5) *Tipo myrcia* (12.5) Wild grass type (10.92) *Tilia* sp. (3.78) *Sophora japonica* (7.9)	*Albizia falcataria* (0.66) *Eupatorium* sp (2.96) *Polygala laureola* (1.29) *Psidium* sp. (1.11)

The honey samples contained pollen from 18 species belonging to 12 families along with umbelliferae and wild grass types and the identified species belong to varying genera of native herbs, grass and trees (Figure 3). Fabaceae family represented five species *Albizia falcataria, Mimosa pudica, Mimosa caesalpinifolia, Mimosa scabrella, Sophora japonica* and is the most prominent in the samples forming about 80% of pollen in Baling sample and 34% in Gerik sample. The frequency of pollen from Fabaceae in pollen analysis undertaken in Brazil demonstrated the importance of this family to bees in honey production [24] [25]. Genus *Mimosa* showed prominent presence in two samples as predominant pollen and secondary pollen indicating the dominant role of *Mimosa* of Fabaceae in honey production.

Next to Fabaceae, Myrtaceae family played good role in honey production with its three species *Tipo myrcia, Psidium* sp. and *Syzygium caryophyllatum* is common in all samples and present as secondary pollen in sample from Jeli and important minor pollen in samples from Baling and Gerik. Areacace represented by two species *Cocos nucifera* and *Elais guineensis* is present as important minor pollen in sample from Jeli and secondary pollen in Gerik sample. Though *Elais guineensis* is present in honey it does not play any role in honey production because both male and female flower of this tree do not produce nectar and bees mostly visit them for their pollen only [26]. *Sparganium typha* is present in all the three samples, as secondary pollen in sample from Jeli and Gerik and important minor pollen in samples from Baling. Predominant and Secondary pollen groups play important role in the formation of honey by acting as source of nectar [27].

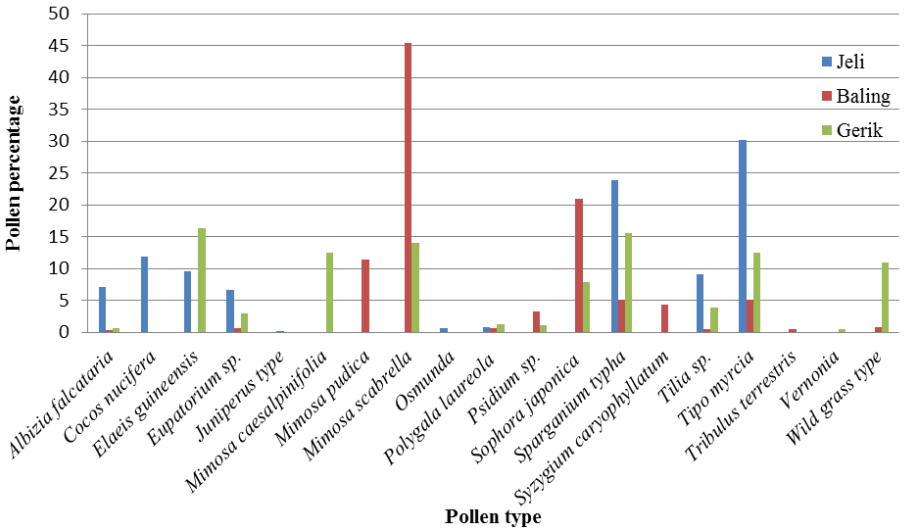

Figure 2: Pollen type and abundance in Baling, Gerik and Jeli samples.

Pollen analysis is currently used to determine the geographical origin of honey as the pollen in honey reflects the vegetation type where the nectar has been collected by the bees. Each location of honey production produces a unique pollen print which is mostly so specific that it can be used to identify the geographical origin of

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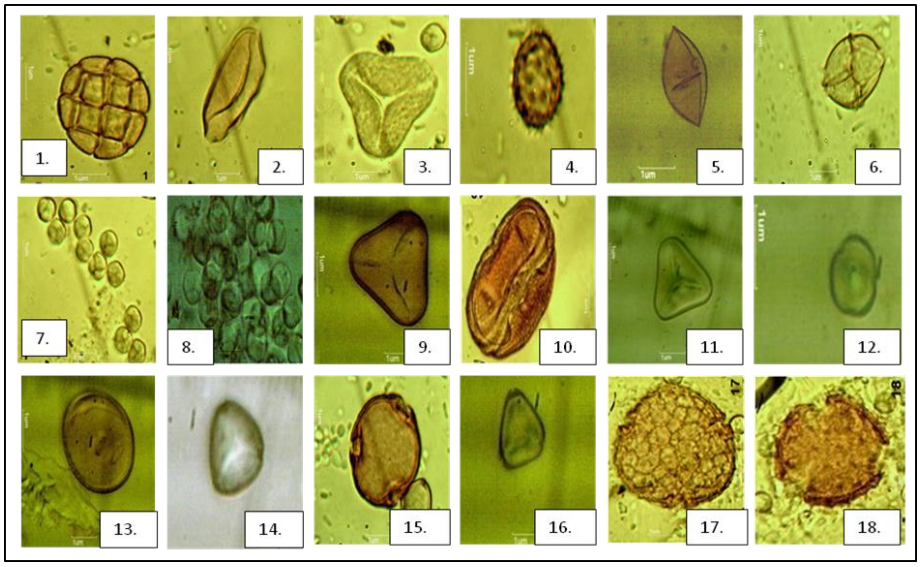
the analysed honey. In the samples analysed all of the three have *Albizia falcataria*, *Eupatorium* sp., *Sparganium typha*, *Tilia* sp. and *Tipo myrcia* in common indicating that these plants are present in all the three places and these results also can be used as a tool in geographical identification of north Malaysian honey from others. Previous researches showed that geographical origin of honey can be established through pollen content [28] and the determination is based on the entire pollen spectrum being consistent with the flora of a particular region or with any reference pollen spectra [16].



1. *Albizia falcataria* (Fabaceae) 2. *Cocos nucifera* (Arecaceae) 3. *Elais guineensis* (Arecaceae)
4. *Eupatorium* sp. (Asteraceae) 5. *Juniperus* type (Cupressaceae) 6. *Mimosa caesalpinifolia* (Fabaceae)
7. *Mimosa pudica* (Fabaceae) 8. *Mimosa scabrella* (Fabaceae) 9. *Osmunda* sp. (Osmundaceae)
10. *Polygala laureola* (Polygalaceae) 11. *Psidium* sp. (Myrtaceae) 12. *Sophora japonica* (Fabaceae)
13. *Sparganium typha* (Poaceae) 14. *Syzygium caryophyllatum* (Myrtaceae) 15. *Tilia* sp. (Tiliaceae)
16. *Tipo myrcia* (Myrtaceae) 17. *Tribulus terrestris* (Zygophyllaceae) 18. *Vernonia* sp. (Asteraceae).

Figure 3: Morphology of different pollen identified in the three forest honey samples.

4. Conclusion

Honey from the Baling forest contains *Mimosa scabrella* as predominant pollen while the sample from Jeli forest contains mostly *Tipo myrcia* as dominant pollen and Gerik forest rich in *Elais guineensis* as the dominant pollen. But all the samples analysed have *Albizia falcataria*, *Eupatorium* sp., *Sparganium typha*, *Tilia* sp. and *Tipo myrcia* in common indicating that these plants are present in all the three places and these results also can be used as a tool in geographical identification of North Malaysian honey from others based on the presence of pollen types that resembles the pollen spectra of the particular region. The presence of the large number of pollen types also indicated that the honeys were pure and not adulterated. This study has led to identification of major plants visited by honeybees in North Malaysia and provides possibility of utilizing this rich bee flora of the region for the development of apiculture and increased honey production of Malaysia.

References

[1] Crane E. In: Honey, A Comprehensive Survey. Crane E, editor. William Heinemann, London; 1975. History of honey; pp. 439–488.

[2] White, J. W. (1975). Composition of honey. In E. Crane (Ed.). Honey, a comprehensive survey. London, UK: Heinemann, Vol. 5, pp. 157–206.

[3] Nair P. K. K. (1964). A pollen analytical study of Indian honeys. Journal of the Indian Botanical Society, 43, 179-191.

[4] Persano Oddo, L. and Piro, R. Main European unifloral honeys: descriptive sheets. Apidologie 2004, 35 (special issue), 38-81.

[5] Wiese, H. (1985). Nova Apicultura, sixth ed. Agropecuária, Porto Alegre.

[6] Carpes, T. (2008). Estudo das Caracteristicas Fisico-Quimicas e Biológicas do Polén Apícola de Apis mellifera da região Sul do Brasil. Tese apresentada ao Programa de Pós-Graduação em Tecnologia de Alimentos, Sector de Tecnologia da Universidade Federal do Paraná.

[7] Jones, G.D. and Bryant, Jr. V. M. (2004). The use of ETOH for the dilution of honey. Grana, 43, 174-182.

[8] Santos, F. A. R. S., Oliveira, J. M., Oliveira, P. P., Leite, K. R. B., and Carneiro, C. E. (2006). Plantas do semi-a´rido importantes para as abelhas. In, Santos, F.A.R. (Ed.), Apium Plantae. IMSEAR, MCT, APNE, Recife, pp. 61–86.

[9] Oliveira, P.P., van den Berg, C., Santos, F.A.R., 2010. Pollen analysis of honeys of *Apis mellifera* L. from Caatinga vegetation of Bahia, Brazil. Grana, 49, 66-75.

[10] Bryant, V. M., Jones, J. G. and Mildenhall, D. C. (1990). Forensic palynology in the United States of America. Palynology 14, 193-208.

[11] Shubharani, R., Sivaram, V., and Roopa, P. (2012). Assessment of Honey Plant Resources through Pollen Analysis in Coorg Honeys of Karnataka State. The International Journal of Plant Reproductive Biology, 41, 31-39.

[12] Wah, L. Y., and Baharun, R. (2008). Apiculture strategies in Malaysia: Planning Implementation. In, R. Baharun, A. B. A. Hamid, H. H. Tat, (Eds.) Contemporary Issues in Marketing. Penerbit UTM, ISBN: 978-983-52-0717-4, pp. 33-53.

[13] Mardan, M., and Osman, M. S. (1983). Beekeeping in Coconut Small Holding in Pontian, Johor, West Malaysia. In Proceeding of

eISSN Number: 2462-2389

the Second International Conference on Apiculture in Tropical Climate, New Delhi, pp.179-186.

[14] Resnick, J. A., Mann, J. M. (2014). A Snapshot of Meliponiculture in Malaysia: An Industry in Infancy. http://www.journals-ofscience.com/uploads/6/8/9/3/6893524 /meliponiculture_in_malaysiafinal-2.pdf

[15] Mardan, M. (1985). Current status, problems, prospects and research needs of A. mellifera in Malaysia. Proc. Expert consultation on beekeeping with A. mellifera in tropical and sub-tropical Asia. Bangkok, 191-197.

[16] Louveaux, J., Maurizio, A., and Vorwohl, G. (1978). International Commission for Bee Botany of IUBS. Methods of Melissopalynology. Bee World, 59, 139–157.

[17] Louveaux, J., Maurizio, A., and Vorwohl, G. (1970). Methods of Mellissopalynology. Bee World, 51, 125-138.

[18] Ibrahim, I. F, Balasundram, S. K, Abdullah, N.A.P, Alias, M.S, Marden, M. (2012). Morphological Characterization of Pollen Collected by Apis dorsata from a Tropical Rainforest. *International Journal of Botany*, 96-106.

[19] Anthonysamy, S., and Abdullah, M, S. (1991). Beekeeping in Malaysia: Pollen Atlas. Malaysian Beekeeping Research and Development Team.Retrieved from http://www.sciencedaily.com.

[20] Maishihah, A & Kiew R. (1988). The Pollen Spectrum as a means of Characterising Malaysian Honeys. *Proceedings of the Fourth International Conference on Apiculture in Tropical Climates, Cairo, Egypt*.

[21] Adeonipekun P. A. (1989). A palynological study of an apiary in Ibadan, Nigeria. Report for B.Sc. (Hons). Department of Botany and Microbiology, University of Ibadan, Nigeria, pp: 57.

[22] Ige, O. E. and Apo, K. A. (2007). Pollen analysis of honey samples from two vegetation zones in Nigeria. Advanced Science Focus, 13, 36-43.

[23] Maurizio, A. (1975). Microscopy of honey. In, Crane, E., (Ed.) A comprehensive survey of honey. London, Heinemann. pp. 240-257.

[24] Novais, J. S., Lima, L. C. L., & Santos, F. A. R. (2009). Botanical affinity of pollen harvested by *Apis mellifera* L. in a semi-arid area from Bahia, Brazil. Grana 48, 224-234.

[25] Sodré, G. S., Marchini, L. C., Carvalho, C. A. L., and Moreti, A. C. (2007). Pollen analysis in honey samples from the two main producing regions in the Brazilian northeast. Anais da Academia Brasileira de Ciências 79, 381-388.

[26] Agwu, C.O.C and Akanbi, T.O. (1985). A palynological study of honey from four vegetation Zones of Nigeria. Pollen et spores, 27, 335-348.

[27] Kaya, Z., Binzet, R., and Orcan, N. (2005). Pollen analyses of honeys from some regions in Turkey. *Apiacta* 40:10- 15.

[28] Maurizio, A. (1951). Pollen analysis of honey. Bee World, 32,1-5.

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