

Pollen Analysis of Natural Bee Honeys from Different Regions of Sri Lanka

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ABSTRACT

Pollen analysis plays an important role when identifying the botanical and geographical origins of bee honey. The aim of the present study was to identify the major plant sources that contribute to the increase of yield of honey which was obtained from selected regions of Sri Lanka such as Ella, Elpitiya, Welimada, Minipe, Loggaloya, Anuradhapura, Kothmale, Haputhale and Nuwara Eliya. The morphology of pollen was observed with light microscope and total pollen counts were expressed in pollen percentage frequency. Based on the analysis, Welimada, Haputhale, Nuwara Eliya and Loggaloya samples were categorized as unifloral honey and remaining honeys were multifloral. Nuwara Eliya, Elpitiya, and Kothmale bee honey samples were categorized as good quality honey because they had absolute pollen count >1,000,000/10g. There were 82 pollen types belonging to 29 families identified through the study.

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Among different plant families, Myrtaceae, Fabaceae, Asteraceae, Poaceae, and Malvaceae families highly contributed for nectar and pollen sources of honey bee in the areas examined in this study. Through identifying the major plant sources for honey bees, this study demonstrated the potentiality for expanding and sustainable bee keeping practices in Sri Lanka.

Keywords: Morphology, Multifloral honey, Pollen analysis, Unifloral honey

INTRODUCTION

Bee honey is defined as the natural sweet substance produced by honey bees, from the nectar of flowers. Its composition and characteristics depend on floral origin, season of collecting honey, storage condition, and treatments of bee keepers (Da Costa Leite *et al.*, 2000; El-Metwally, 2015). Accordingly, therapeutic value and commercial value of bee honey varied. Therefore, knowing the floral origin of bee honey is extremely important.

Each flower species has a unique pollen grain which shows distinctive appearance (Petersen and Bryant, 2011). The pollen of the flower sticks to the bee's leg at the time that honey bees suck the nectar of flower (Hamid *et al.*, 2015). Some of those pollens remain in the honey after the nectar is converted into honey in the hive (Bell, 1986; Morse and Calderone, 2000). Melissopalynology is the study of pollen in

honey. Unifloral honey contains dominant pollen while if none is dominant, it is classified as multifloral or mixed floral honey (Adekanmbi and Ogundipe, 2009). Pollen analysis plays an important role when identifying the botanical and geographical origins of honey (Aronne and De Micco, 2010). Moreover, melissopalynology has been extensively used to determine the purity of honey (Ebenezer and Olugbenga, 2010).

Sri Lanka has a well described floral biodiversity and the highest species diversity is recorded among the flowering plants. Among Sri Lanka's flowering plants, 927 or 28% are endemic to Sri Lanka (Gunatilleka *et al.*, 2008). Sri Lanka has a rich ecosystem diversity due to its climatic and topographic heterogeneity and due to the coastal influence. Different ecosystems contain different plant species. For example, 60% endemic flowering plants are found in the low land wet zones and 34% in the mountain ecosystem of the island (Gunatilleka *et al.*, 2008). Therefore according to the different regions of Sri Lanka available pollen types should be different.

Qualitative and quantitative melissopalynological analyses of Sri Lankan bee honey are less available. Most of the beekeepers do not know all the important nectar plants contributing to honey production. If bee keepers get to know about the major nectar plants which contribute to the honey production in their region they can increase the yield of honey through sustainable bee keeping practices.

Based on pollen analysis, this paper aims to determine the botanical origin of honeys from different regions of Sri Lanka and to provide a useful guide for beekeeping in these regions.

MATERIALS AND METHODS

Honey sampling

Bee honey samples were purchased from the agricultural development centers and the organization of "Bingu Sampath Surakima", Kandy, during November, 2017 to March 2018. These included bee honey samples from different areas in Sri Lanka which mainly contributed to the bee honey industry such as Ella, Elpitiya, Welimada, Minipe, Loggaloya, Anura-dhapura, Kothmale, Haputhale and Nuwara Eliya. All the samples were stored in sterilized glass bottles and kept in refrigerated conditions (4 °C) until further analysis.

Pollen analysis

Pollen analysis was done according to the method described by Louveaux *et al.*, (1978) with slight modifications as described by Jayasinghe *et al.*, (2012). Sample of honey was thoroughly mixed by vortex mixture. Then 10 g of honey sample was taken and it was dissolved in 20 mL of warm distilled water (40 °C). This mixture was then centrifuged at 2500 rpm for 10 minutes. Then the supernatant liquid was removed using a dropper and the sediment was added again with warm distilled water (40 °C) and centrifuged at the same rate. Supernatant liquid was removed and the sediment was obtained. Then a drop of

sediment was spread over the glass slide and it was allowed to dry. Next, it was microscopically observed in 400× magnification under the compound light microscope and pollen images were obtained. The pollens were identified comparing to the reference pollen images using Manual for identification of pollen of Sri Lankan flora (Perera and Mudannayake, 2014). Different morphotypes of pollen grains of Sri Lankan species incorporated in this manual were used as references for identification. The photographs of plant species and their basic ecological characteristics and photographs of pollen grains taken under the light microscope are given in the manual, and those details were used for identification of plant species and families. Also published studies (Shubharani *et al.*, 2012; Sniderman *et al.*, 2018; Sivaram *et al.*, 2012; Adekanmbi 2009) were used as references to identify pollen grains.

Pollen quantification was done according to the method described by Song *et al.*, (2012). For quantification of the pollen types available in each sample, at least 500 pollen grains were counted. Then the percentage frequencies of the pollen taxa in all samples were calculated and the types of pollen were allocated to one of four frequency classes: (1) predominant pollen types (>45% of the total pollen grains counted); (2) secondary pollen types (16% - 45%); (3) important minor pollen types (3% - 15%); and (4) minor pollen types (<3%). If honey sample contained a predominant pollen type it was characterized as unifloral. Otherwise, it was considered as multifloral.

Absolute pollen count (APC) was obtained according to the method described by Jayasinghe *et al.*, (2012). An empty centrifuge tube was weighed (W1 g). The sediment was obtained as described previously and then the sediment with centrifuge tube was weighed (W2 g). The empty slide was weighed (X1 g) and after applying a drop of sediment, this slide was reweighed (X2 g). Then the pollen number was counted in the slide (N).

Weight of sediment in slide = X2 - X1

Weight of whole sediment = W2 - W1

Total count in the whole sediment (n) = $N \times \frac{(W2 - W1)}{X2 - X1}$

Absolute pollen count (APC) = n/10 g of honey

An APC <1,000 grains/10 g honey was considered as an indication of syrup adulteration or pressure filtering during processing. Analyzed samples were classified as follows. Group 1 (<20,000 grains/10 g); Group 2 (20,000–100,000 grains/10 g); Group 3 (100,000–500,000 grains/10 g); Group 4 (500,000–1,000,000 grains/10 g); Group 5 (>1,000,000 grains/10 g), which indicated extremely poor, poor, rich, very rich, and extremely rich amount of pollen respectively, in honey (Louveaux *et al.*, 1978).

RESULTS AND DISCUSSION

Variation of Pollen Content of Honey and Their Respective Families

Pollen is very important for honeybee nutrition because it provides protein for their survival and reproduction (Yao *et al.*,

2006; Dietz, 1975). Even though, bees normally collect a wide variety of pollen types, they generally concentrate on few plant species (Dimou, 2007; Bauma, 2011). Majority of bees so far recorded from Sri Lanka are polylectic. Those polylectic bees do not strictly adhere to one type of pollen source (Karunaratne *et al.*, 2005). Therefore, Sri Lankan bee honey usually contain different pollen types and present study is evident that > 4 types of are present in varying amounts (Table 1).

There were 82 pollen types belonging to 29 plant families identified in the honey samples which were obtained from different regions in Sri Lanka. According to the pollen composition, honey samples collected from Welimada, Haputhale, Nuwara Eliya and Loggaloya can be classified as unifloral honey due to containing pollens predominantly from the nectar of one plant species with the availability frequency >45%. In addition, honey samples from Welimada, Haputhale and Nuwara Eliya contained pollen of Myrtaceae family (frequency >45%) while honey samples from Loggaloya contained pollen of Sapindaceae family predominantly (frequency >45%). The third dominant pollen frequency was reported by Euphorbiaceae family (frequency 41.8%) and these pollen grains were highly available in honey samples collected from Elpitiya area, however, these honey samples were classified as multifloral honey due to frequency being <45%. All other honey samples studied can be classified as multifloral honey because they did not contain a predominant pollen type,

however, pollen of Fabaceae, Asteraceae, Poaceae, Malvaceae families were present in more than 50% of honey samples. According to the mellisopalynological survey conducted by Jayasinghe *et al.*, (2012) pollen of Asteraceae and Myrtaceae families were common in Sri Lankan bee honey. Family Asteraceae included the greatest number of floral hosts species visited by bees (Karunaratne *et al.*, 2005). Therefore, results of this study were concordance with the previously reported studies.

Pollen density can be used as an indicator for adulteration of bee honey (Jayasinghe *et al.*, 2012). According to the results the absolute pollen content per gram of honey was in the range of 34,607 - 1,323,654 (Table 2). Minimum pollen count (34,607) was found in Haputhale honey sample, the reasons may be the adulteration of the honey or bees being fed with sugar syrup. Nuwara Eliya, Elpitiya, and Kothmale bee honey samples had pollen density >1,000,000/10g. Therefore, those honey samples were categorized into group 5 and they were in good quality. Jayasinghe *et al.*, (2012) reported that among the collected Sri Lankan bee honey samples there were 34% bee honey samples with pollen density >1,000,000/10g. The present study observed that 33% bee honey samples were with pollen density >1,000,000/10g.

Pollen Morphology and Their Respective Families Identified in Honey Samples

The pollen morphologies of each honey are shown in figure 1 to figure 9. The pollen

Table 1. Pollen content (%) of honey in different regions in Sri Lanka

Plant family	A%	B%	C%	D%	E%	F%	G%	H%	I%
Myrtaceae	64.3	73.2	3.6	-	-	80.9	-	-	-
Fabaceae	1.3	2.5	5.5	5.1	-	1.8	-	-	11.6
Rubiaceae	8.4	-	-	-	4.3	1.3	-	-	-
Asteraceae	9.0	5.0	18	15.3	8.5	2.7	-	-	16.2
Acanthaceae	0.6	2.5	-	-	-	-	-	-	-
Poaceae	2.0	2.5	-	-	-	0.4	-	6.2	4.6
Rosaceae	-	-	-	0.3	-	0.9	-	-	-
Combretaceae	-	-	-	-	-	-	0.3	6.2	-
Melastomataceae	1.3	-	-	-	-	-	-	-	-
Euphorbiaceae	-	-	41.8	28.2	-	-	20	-	-
Anacardiaceae	-	2.5	-	-	25.5	-	-	16.6	6.9
Pandanaceae	-	-	-	-	-	-	0.3	-	-
Cyperaceae	-	-	-	-	-	-	-	27.7	9.3
Malvaceae	1.9	4.8	3.6	-	2.1	0.9	-	6.2	2.3
Tiliaceae	-	-	-	-	-	-	-	-	11.6
Solanaceae	-	-	-	-	-	-	-	16.6	-
Verbenaceae	-	-	-	-	-	-	-	-	6.9
Taxodiaceae-Cupressaceae-Taxaceae (TCT)	-	-	3.6	-	2.1	-	-	-	-
Typhaceae	-	-	-	-	-	-	-	11.1	-
Lobeliaceae	-	-	-	-	-	2.7	-	-	-
Lamiaceae	-	-	-	-	-	-	-	-	-
Plumbaginaceae	0.6	-	-	-	-	-	-	-	-
Oleaceae	-	-	-	-	8.5	-	-	-	-
Sapindaceae	-	-	-	16.8	-	-	63.6	-	2.3
Ochnaceae	-	-	1.8	-	14.9	-	-	-	-
Convolvulaceae	-	2.5	-	-	17.0	-	-	-	-
Proteaceae	1.3	-	-	-	-	-	-	-	-
Total no. of pollen observed	509	417	537	442	518	473	463	427	432

A - Nuwara Eliya, B – Haputhale, C- Elpitiya, D– Ella, E- Kothmale F- Welimada, G –Loggaloya, H – Minipe, I- Anuradhapura

Table 2. Absolute pollen count of each honey sample

Honey sample	Absolute pollen count (Number of grains/10 g)
Nuwara Eliya	1 060 562
Haputhale	34 607
Welimada	54 569
Elpitiya	1 237 220
Loggaloya	234 845
Kothmale	1 323 654
Anuradhapura	988 456
Minipe	968 674
Ella	984 675

characteristics are important in identifying pollen grains which belong to different families. Fabaceae family shows polyad, tetrad pollen grains and monad pollen grains which have spheroidal or prolate shape (Perera and Mudannayake, 2014). In this study polyad pollen grains belonging to Fabaceae family were found in samples from Nuwara Eliya, Haputhale, Welimada, Ella, and Anuradapura. Therefore, through the observed results, it can be suggested that bees collect nectar from Fabaceae family plants species such as *Mimosa pudica* “Nidi Kumba”, *Acacia leucophloea* “Katu andara”, *Tephrosia purpurea* “Kathurupila”, *Gliricidia sepium* “Giriseeniya”, *Acacia auriculiformis*, *Cassia fistula* “Ehala”. In Acanthaceae family, characteristic prolate-spheroidal shaped ribbed pollen are predominant in *Strobilanthes spp.* (Wood *et al.*, 2003;

Perera and Mudannayake, 2014). In Sri Lanka *Strobilanthes lupulina* “Nelu” is commonly found only in moist hill forest (Perera and Mudannayake, 2014) and is a native floral species. In this study, *Strobilanthes lupulina* type of pollen grains were found in Nuwara Eliya and Haputhale samples which revealed that bees are attracted to nectar of Nelu flowers. Asteraceae family pollen grains are unique because of the characteristic echinate and wild sunflower belongs to this family which is commonly seen in upcountry of Sri Lanka. They can be differentiated from similar pollen in other families such as Malvaceae and Convolvulaceae by the relatively small size of the pollen and the irregular arrangement of the spines (Adekanmbi *et al.*, 2009). *Tithonia diversifolia* “Wild sunflower”, *Wedelia biflora* “Moodu gampalu”, *Wedelia trilobata* “Kaha Karabu” may be the plants species in Asteraceae family which contribute to the bee honey production.

Myrtaceae family most commonly shows parasyncolpate pollen grains (Thornhill *et al.*, 2012). According to the results, honey samples obtained from Walimada and Haputhale area predominantly had pollen grains of Myrtaceae family and *Eucalyptus sp.* “Red gum” plant belongs to the same family. Punchihewa (1994) reported that growing areas of *Eucalyptus sp.* “Red gum” such as Welimada, Haputhale may have a good potential for honey production. Results of the present study suggested that *Eucalyptus sp.* may be the plant species which highly contributes to the bee honey production in

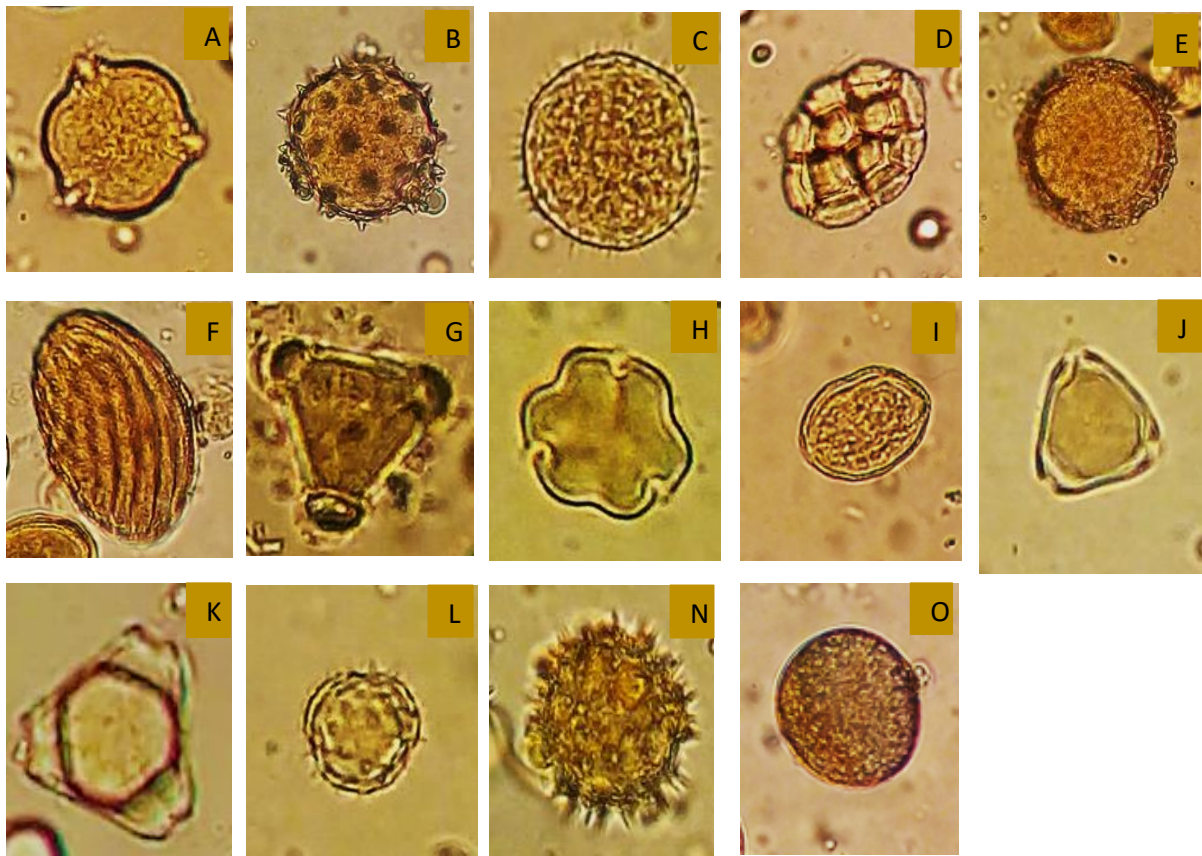


Figure 1. Morphology of different pollen identified in the Nuwara Eliya honey sample under the compound light microscope (400×)

A) Rubiaceae family; B, C) Malvaceae family; D) Fabaceae family; E) Plumbaginaceae family; F) Acanthaceae family; G) Proteaceae family; H) Melastomataceae family; I) Unidentified; J, K) Myrtaceae family; L, N) Asteraceae family; O) Poaceae family.

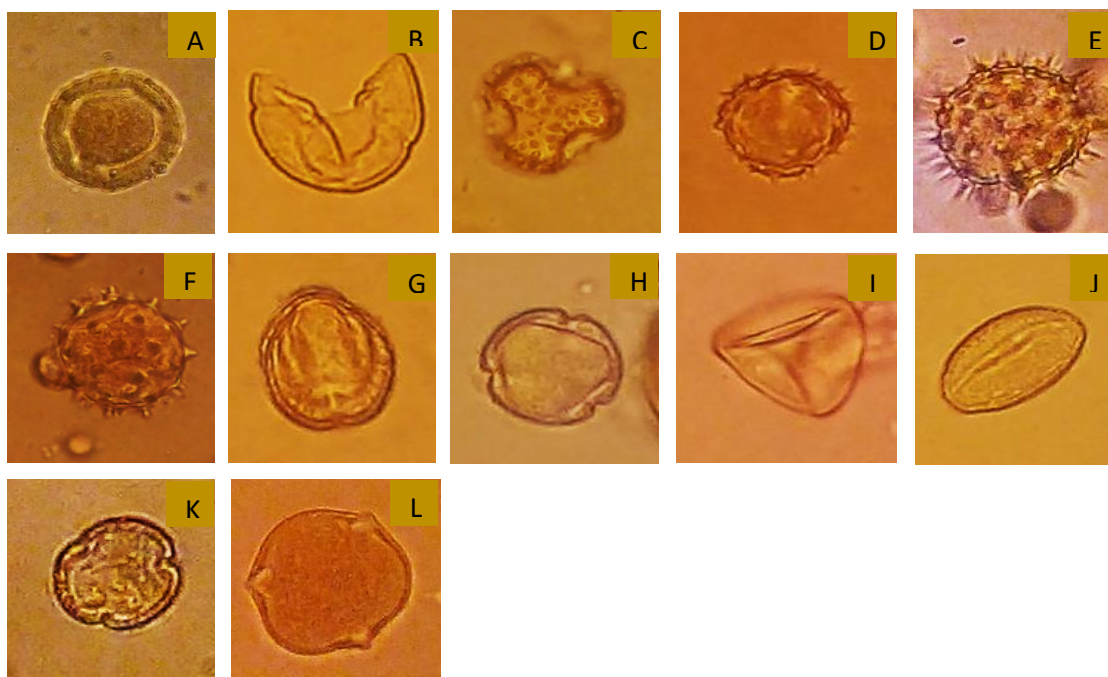


Figure 2. Morphology of different pollen identified in the Kothmale honey sample under the compound light microscope (400×)

A) Convolvulaceae family; B) TCT family; C) Unidentified; D, E) Asteraceae family; F) Malvaceae family; G) Anacardiaceae family; H) Ochnaceae family; I) Unidentified; J) Amaryllidaceae family; K) Oleaceae family; L) Rubiaceae family.

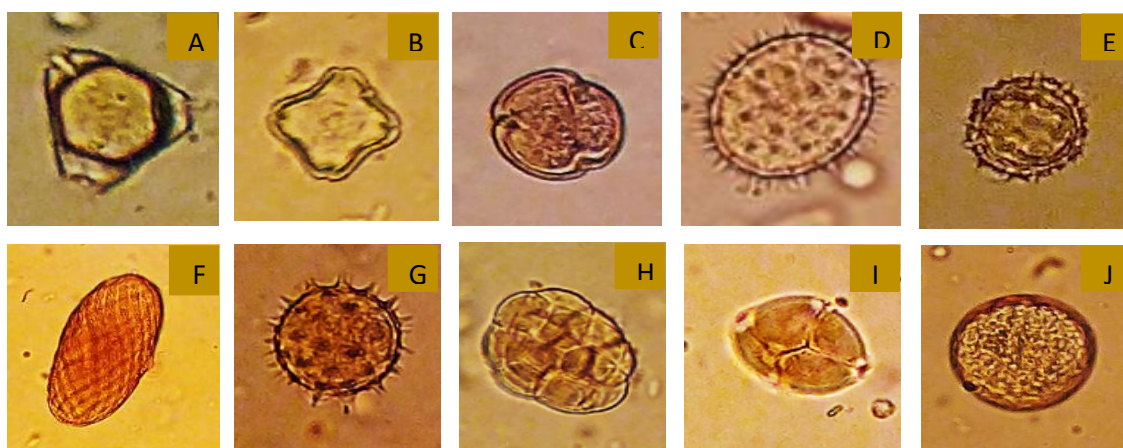


Figure 3. Morphology of different pollen identified in the Haputhale honey sample under the compound light microscope (400×)

A, B) Myrtaceae family; C) Anacardiaceae family; D) Convolvulaceae family; E) Asteraceae family; F) Acanthaceae family; G) Malvaceae family; H-I) Fabaceae family; J) Poaceae family

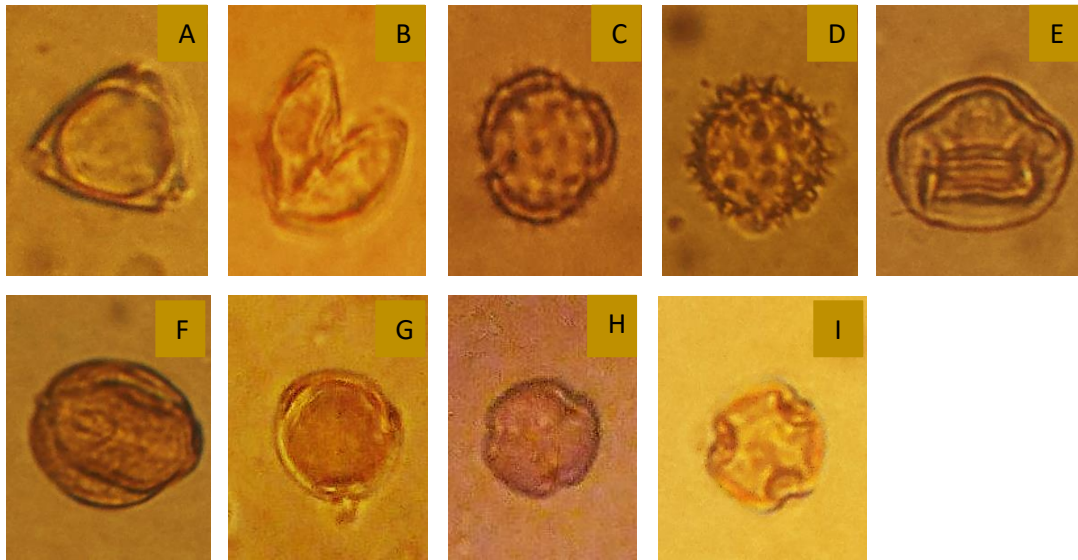


Figure 4. Morphology of different pollen identified in the Elpitiya honey sample under the compound light microscope (400×)

A) Myrtaceae family; B) TCT family; C) Asteraceae family; D) Malvaceae family; E, F) Unidentified; G) Euphorbiaceae family; H) Ochnaceae family; I) Fabaceae family.

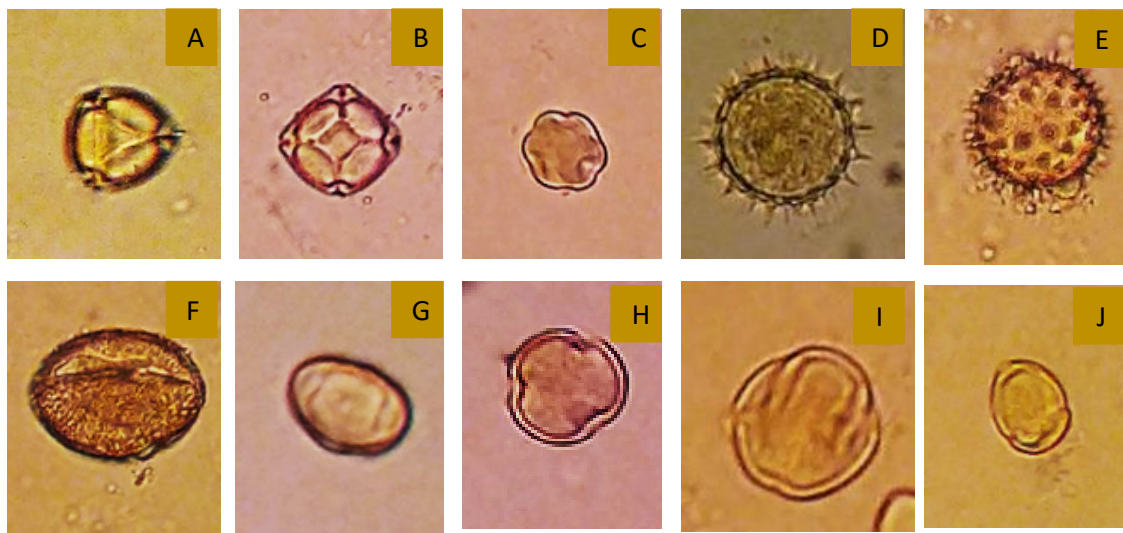


Figure 5. Morphology of different pollen identified in the Loggaloya honey sample under the compound light microscope (400×)

A, B) Sapindaceae family; C) Combretaceae family; D, E) Malvaceae family; F) Unidentified; G) Pandanaceae family; H, I) Unidentified; J) Euphorbiaceae family.

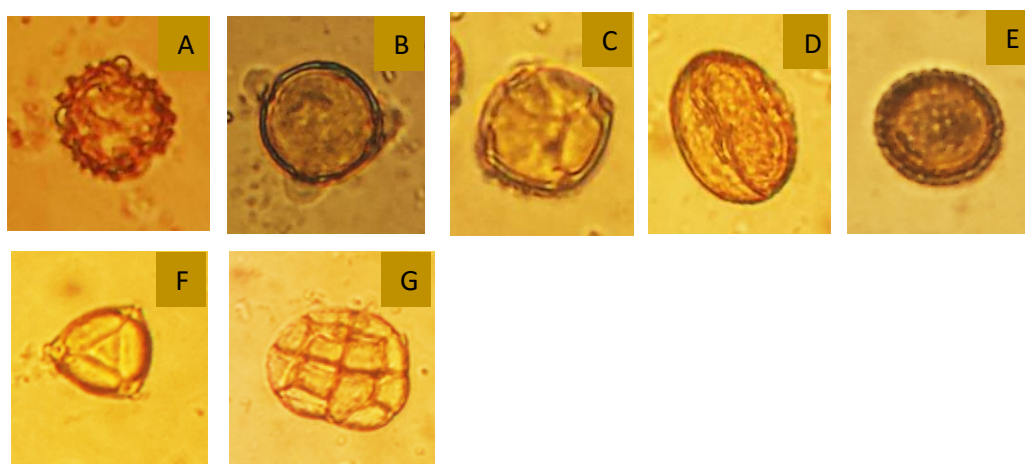


Figure 6. Morphology of different pollen identified in the Ella honey sample under the compound light microscope (400×)

A) *Asteraceae* family; B) *Euphorbiaceae* family; C) *Rosaciae*; D) *Unidentified*; E) *Euphorbiaceae* family; F) *Sapindaceae* family; G) *Fabaceae* family.

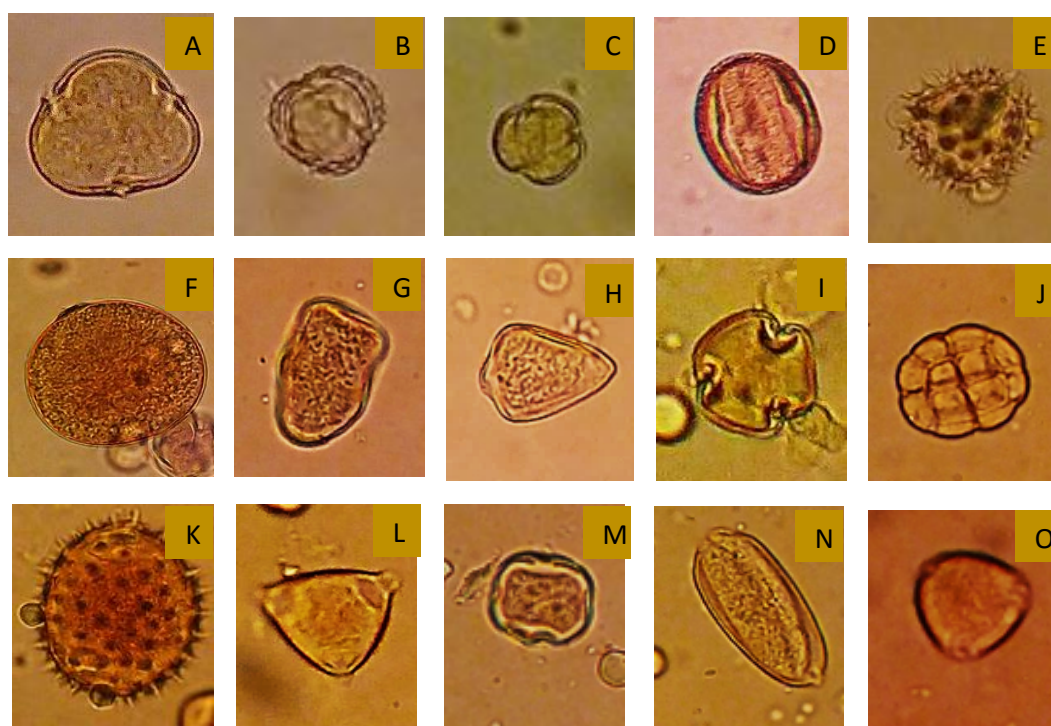


Figure 7. Morphology of different pollen identified in the Anuradhapura honey sample under the compound light microscope (400×)

A) *Tiliaceae* family; B) *Asteraceae* family; C) *Anacardiaceae* family; D) *Unidentified*; E) *Asteraceae* family; F) *Poaceae* family; G, H) *Cyperaceae* family; I, J) *Fabaceae* family; K) *Malvaceae* family; L) *Sapindaceae* family; M, N) *Unidentified*; O) *Verbenaceae* family.

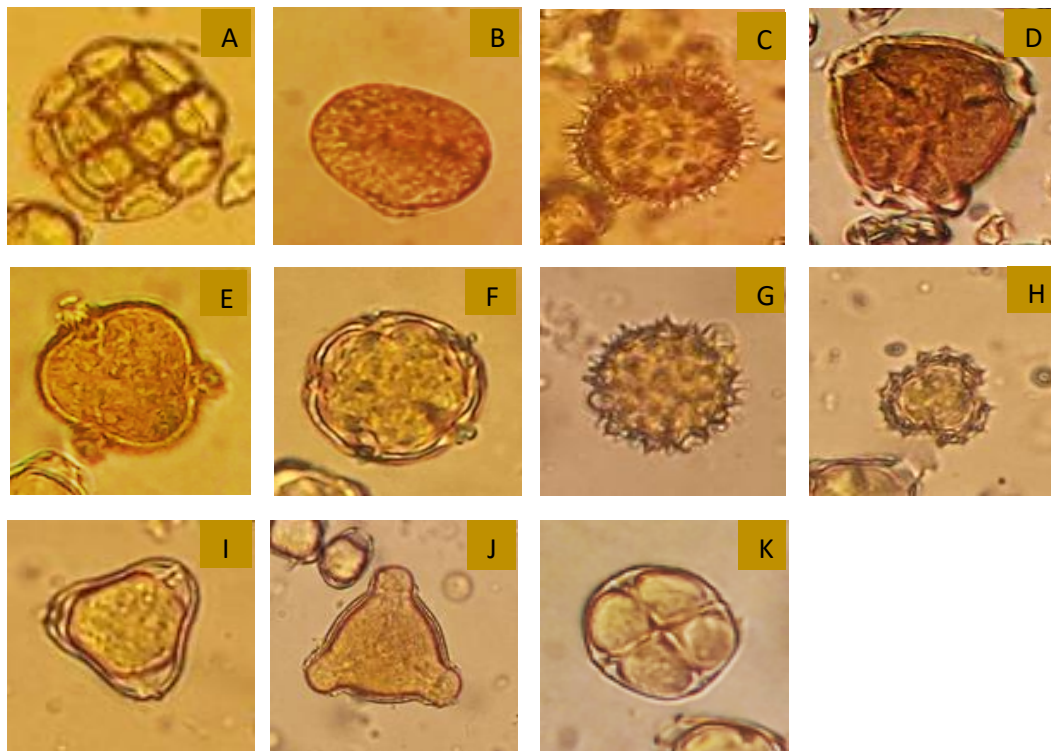


Figure 8. Morphology of different pollen identified in the Welimada honey sample under the compound light microscope (400×)

A) Fabaceae family; B) Poaceae family; C) Malvaceae family; D) Lobeliaceae family; E, F) Rubiaceae family; G, H) Asteraceae family; I) Myrtaceae family; J) Rosaceae family; K) Fabaceae family.

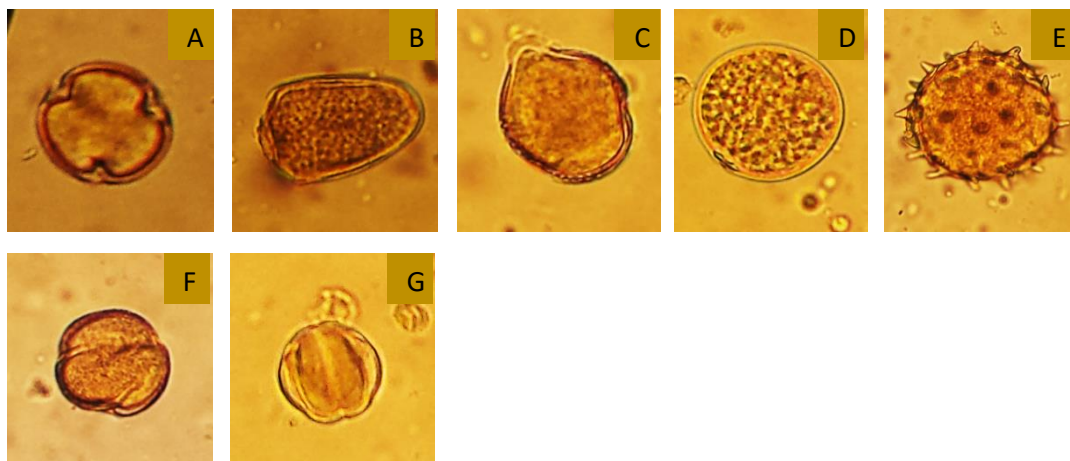


Figure 9. Morphology of different pollen identified in the Minipe honey sample under the compound light microscope (400×)

A) Solanaceae family; B) Cyperaceae family; C) Typhaceae family; D) Poaceae family; E) Malvaceae family; F) Anacardiaceae family; G) Combretaceae family.

the above areas.

Jayasinghe *et al.*, (2012) observed that there are differences in pollen types according to the provinces. In addition, in the present study, variations in pollen types were detected according to the regions within the same province. This may be due to the ecosystem diversity or agricultural crops.

CONCLUSION

The study concludes that honey samples obtained from Welimada, Haputhale, Nuwara Eliya and Loggaloya were categorized as unifloral honey based on the predominant pollen frequency that were more than 45%. Other honey samples were classified as multifloral honey because none of the pollen species were more than 45%. 82 pollen types belonging to 29 families were identified and among those different plant families, Myrtaceae, Fabaceae, Asteraceae, Poaceae, and Malvaceae families were commonly found in the analyzed bee honey samples where those forages mainly contributed to the honey production in Sri Lanka. Nuwara Eliya, Elpitiya, and Kothmale bee honey samples were categorized as good quality honey because they had absolute pollen count >1,000,000/10g. This study provides new insights into the pollen composition of honey samples from different regions of Sri Lanka and will be beneficial in increasing the commercial value of the Sri Lankan bee honey.

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