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# Pollen analysis of *Apis cerana* honey from Java, Indonesia

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**Abstract**
Honey is a natural product from honeybees which has many health benefits. Examination on pollen diversity in honey can be used as an indicator for the authenticity and quality of honey. The present study reported pollen diversity on 12 honey samples produced by *Apis cerana* Fab. The samples were collected from local farmers in various areas of Java Island, Indonesia. The objective of this study was to reveal pollen diversity in honey as a scientific evidence of the botanical origin and the authenticity of honey samples. Results indicated that all honey samples have diverse pollen grains with no particular pollen type dominating, and thus all honey samples were classified as multifloral honey. Pollen diversity in honey was strongly reflected different vegetation condition of the farming sites, and thus confirming the botanical origin and geographical origin of the samples.

**Keywords:** Melissopalynology, pollen, honey quality, *Apis cerana*

## Introduction
Honey as a natural product of honey bees has been known as a food supplement with high nutritional value. The main raw material in honey production is nectar collected by honey bees from flowers. The nectar, which typically collected from flora by honeybees, is an important compound for bees to survive due to its high concentration of vitamins, proteins, carbohydrates and minerals [1]. Java Island is a potential place for honey production as it has considerable area of forest, particularly along the mountainous regions. The diversity of plants make up the lowland and highland flora in Javanese forest provide important source of nectar for honeybees. Thus, investigation on the interaction between flora and bees is crucial given the status of flora considerably influence the life cycle of honeybees [2].
Melissopalynological analysis is considered as a suitable approach to identify the interaction between flora and bees [3] in a form of diveristy and quantity of pollen grains. In this regard, the shape, sizes, and surface texture of pollen collected by bees and found in honeys are measured [4]. Using this method, the species of producing nectar plants around the beehive can be identified. In addition, the vegetation of the region based on the geographical structure can be determined [5]. The results of melissopalynological analysis enable beekeepers to conserve the high producing nectar flora in the surrounding area to maintain sustainable honey production.
Melissopalynological analysis can be used to determine the quality of honey, and complementary to other physicochemical parameters including amino acids, protein, sugar composition, etc. [6, 7]. The diversity of pollen in the honey is a simple indicator of pure honey. Since the types of pollen in honey can be related to the source of nectar, then pollen diversity analysis indicates botanical origin and geographical origin of honey. For instance, a rape honey consists the minimum of 45% rape pollen, and classified as the unifloral honey, while multifloral honey is characterized by high pollen diversity without any type of pollen has a percentage of 45% [6].
The present study focuses on the identification of pollen content in the honey produced by *Apis cerana* from Java Island, Indonesia. This type of honey was chosen in this study given the economic value of *A. cerana* honey, which is relatively higher for the beekeepers as compared to the honey produced by *A. mellifera* [8].

## Materials and Methods
### Sample collection
Honey samples were collected from the farmers to ensure their originality. A traditional honey harvesting method was performed to extract pure honey from the honeycomb. During harvesting for sample collection, which was on June to August 2018, interview with the farmers was conducted to explore relevant information related to honey production.

The collected samples were stored in a refrigerator at 4 ºC before being processed for palynological analysis.

## Pollen identification

Preparation of palynological microscopic slides for pollen analysis was done using the following procedure: 5 mL of each honey were taken using a clean dropper pipette and placed in the centrifuge tube with the addition of 10 mL of distilled water. The centrifuge tube was shaken to obtain a homogeneous mixture of honey and distilled water. Then, the samples were centrifuged at 4000 rpm for 10 minutes with two repetitions. The precipitated pellet was colored by adding 0.25 mL of 0.25% safranin (in water), and kept at room temperature for 24 hours to ensure the adsorption of the safranin into the pollen. After 24 hours, the distilled water was added into the centrifuge tube before another centrifugation at 4000 rpm for 5 minutes. Then, the distilled water was removed, and 3 mL of glycerin was added to the pellet. A drop of pellet was placed at the microscope slide for observation using Olympus Boeco BM–180 under magnification of 4×10, 10×10 and 40×10, equipped with a Charge-Coupled Device (CCD) camera (OptiLab) to capture the images. The identification of pollen grains was carried out by comparing pollen morphology to the literatures [9, 10, 11].

## Results and Discussion

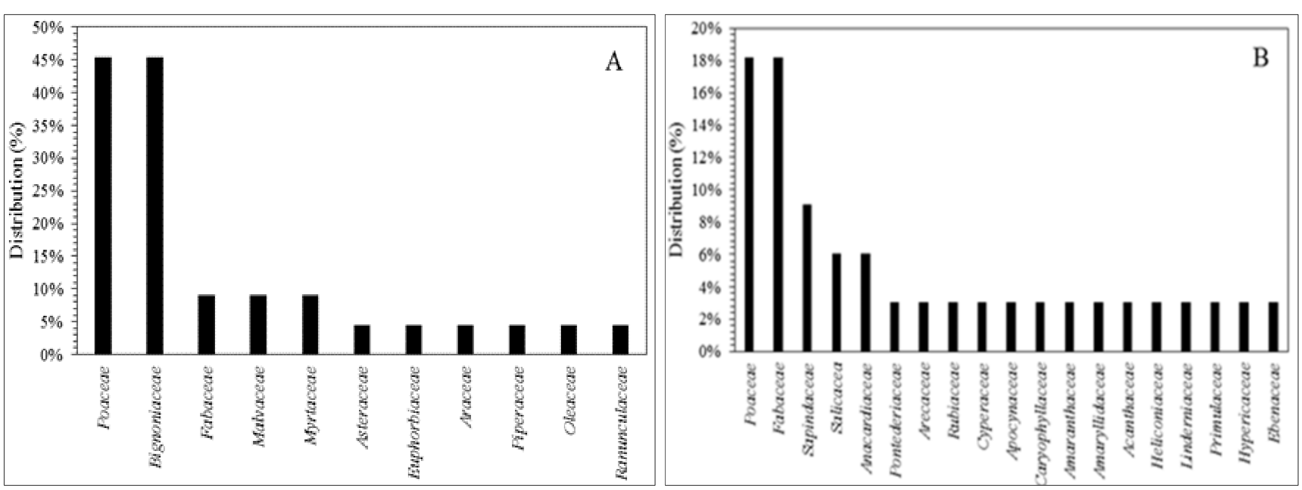
Honey samples in this study were collected during the dry season. It is worth mentioning that the harvesting frequency of the honey from *A. cerana* bees strongly depends on the seasonal change. Based on the information from the bee farmers, the dry season in 2018 was quite long, resulted in an adverse effect on the quality and production of the honey. The long dry season affected honey production in terms of limited number of blooming flowers due to minimum availability of water. Furthermore, in unfavorable climatic condition *A. cerana* tends to migrate to the locations with suitable condition and they do not store large amount of honey in their comb [12].

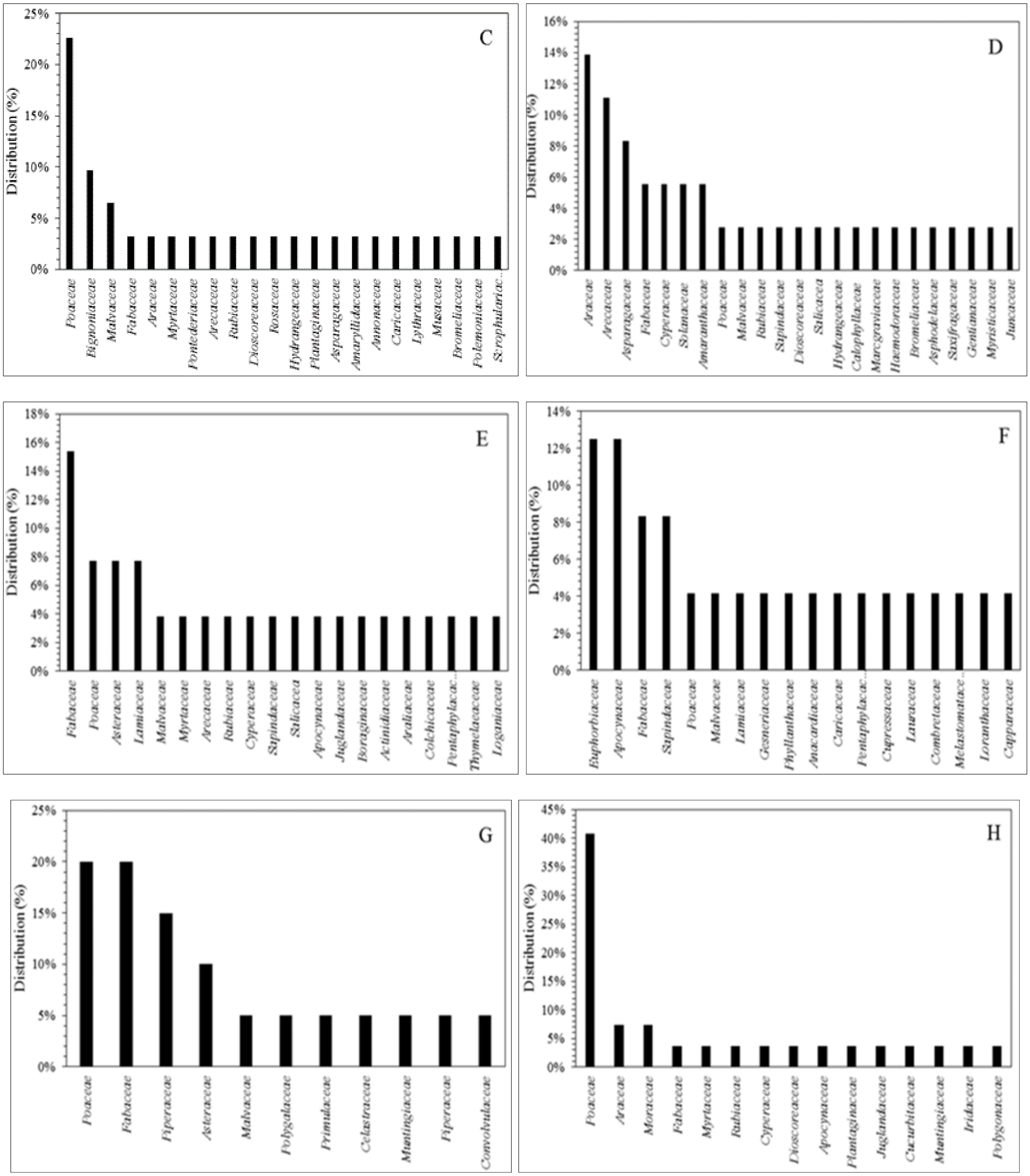
It is worth noting that the major product of *A. cerana* bees is a multifloral honey due to its ability to rove within wide foraging range in the area with high vegetation diversity [13, 14]. Thus, the pollen analysis focused on the distribution of the pollen type in the collected honeys. Twelve samples of *A. cerana* honey collected from different locations in the Java Island Indonesia varied in their color and their pollen diversity (Table 1).

**Table 1:** Variation on pollen diversity of honey samples from Java Island, Indonesia

| Sample code | Color | Number of pollen types | Origin of sample |
|---|---|---|---|
| AH-01 | Yellow | 21 | Pati, Central Java |
| AH-02 | Orange-Brown | 37 | Boyolali, Central Java |
| AH-03 | Orange | 37 | Karanganyar, Central Java |
| AH-04 | Orange | 43 | Kulonprogo, Special Region of Yogyakarta |
| AH-05 | Yellow | 31 | Karanganyar, Central Java |
| AH-06 | Yellow | 37 | Gunungkidul, Special Region of Yogyakarta |
| AH-07 | Yellow | 24 | Magelang, Central Java |
| AH-08 | Brown-Red | 32 | Solo Central Java |
| AH-09 | Dark Brown | 31 | Cilacap, Central Java |
| AH-10 | Light Orange | 69 | Solo, Central Java |
| AH-11 | Yellow | 65 | Magelang, Central Java |
| AH-12 | Orange-Brown | 41 | Depok, West Java |

Variation in honey color and other physical characteristics is attributed to several factors, including the vegetation condition [15], the season change [16], and the harvesting and processing methods [17]. The number of pollen types varied in diverse honey samples, ranging from 21 to 69. Pollen diversity in each honey samples as presented by composition of plant family were showed in Figure 1.




C
Distribution (%)
0%
5%
10%
15%
20%
25%
Poaceae
Bignoniaceae
Malvaceae
Fabaceae
Araceae
Myrtaceae
Pontederiaceae
Arecaceae
Rubiaceae
Dioscoreaceae
Rosaceae
Hydrangeaceae
Plantaginaceae
Asparagaceae
Amaryllidaceae
Annonaceae
Caricaceae
Lythraceae
Musaceae
Bromeliaceae
Polemoniaceae
Scrophulariac...
D
Distribution (%)
0%
2%
4%
6%
8%
10%
12%
14%
16%
Araceae
Arecaceae
Asparagaceae
Fabaceae
Cyperaceae
Solanaceae
Amaranthaceae
Poaceae
Malvaceae
Rubiaceae
Sapindaceae
Dioscoreaceae
Salicacea
Hydrangeaceae
Calophyllaceae
Marcgraviaceae
Haemodoraceae
Bromeliaceae
Asphodelaceae
Saxifragaceae
Gentianaceae
Myristicaceae
Juncaceae
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Distribution (%)
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2%
4%
6%
8%
10%
12%
14%
16%
18%
Fabaceae
Poaceae
Asteraceae
Lamiaceae
Malvaceae
Myrtaceae
Arecaceae
Rubiaceae
Cyperaceae
Sapindaceae
Salicacea
Apocynaceae
Juglandaceae
Boraginaceae
Actinidiaceae
Araliaceae
Colchicaceae
Pentaphylacac...
Thymelaeaceae
Loganiaceae
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Distribution (%)
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6%
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10%
12%
14%
Euphorbiaceae
Apocynaceae
Fabaceae
Sapindaceae
Poaceae
Malvaceae
Lamiaceae
Gesneriaceae
Phyllanthaceae
Anacardiaceae
Caricaceae
Pentaphylac...
Cupressaceae
Lauraceae
Combretaceae
Melastomatace...
Loranthaceae
Capparaceae
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Distribution (%)
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5%
10%
15%
20%
25%
Poaceae
Fabaceae
Piperaceae
Asteraceae
Malvaceae
Polygalaceae
Primulaceae
Celastraceae
Muntingiaceae
Piperaceae
Convolvulaceae
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Distribution (%)
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10%
15%
20%
25%
30%
35%
40%
45%
Poaceae
Araceae
Moraceae
Fabaceae
Myrtaceae
Rubiaceae
Cyperaceae
Dioscoreaceae
Apocynaceae
Plantaginaceae
Juglandaceae
Cucurbitaceae
Muntingiaceae
Iridaceae
Polygonaceae

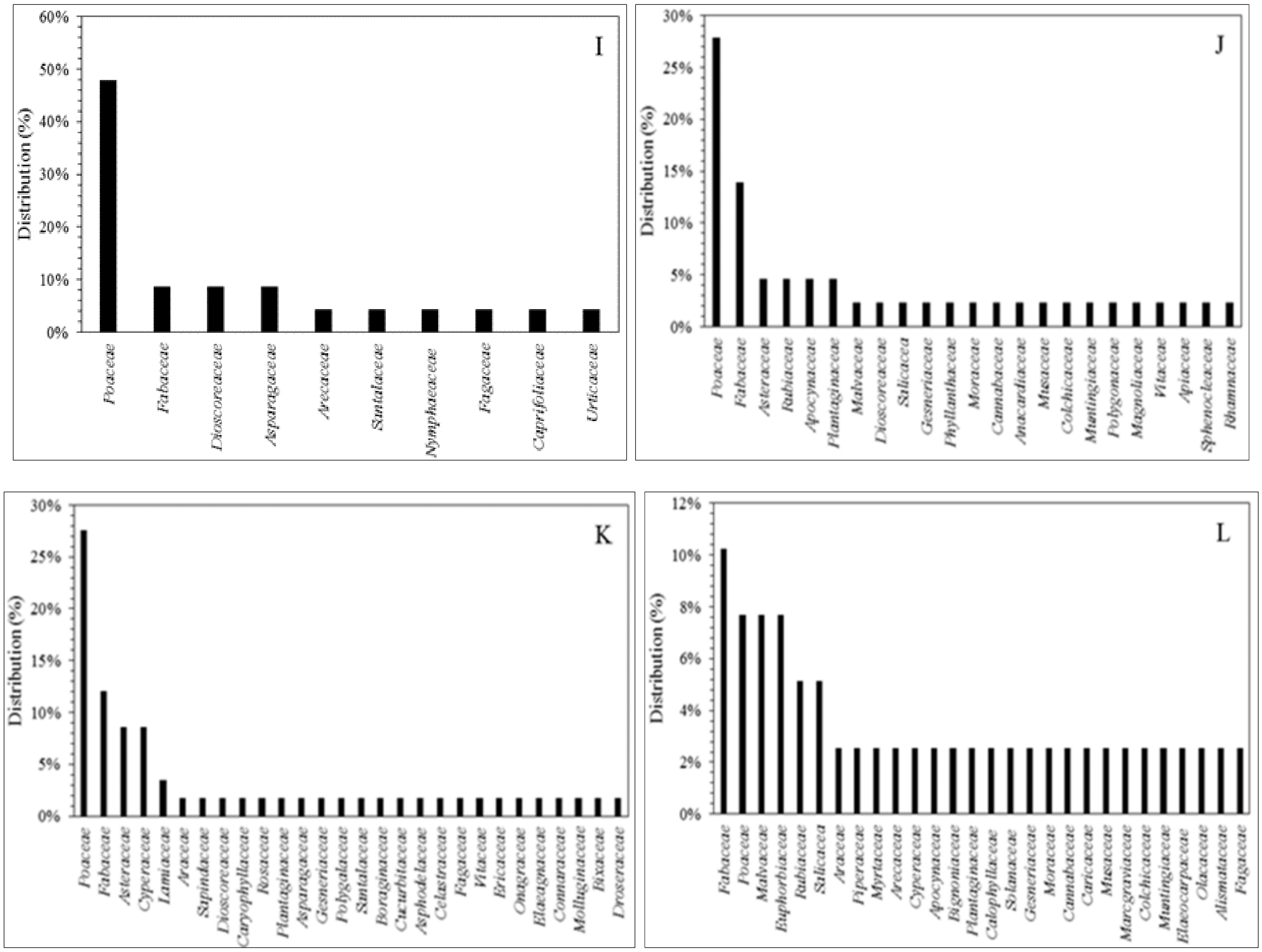


**Fig 1:** Pollen diversity based on the plant family as pollen source

This result is in accordance with the finding by [18], which investigated the pollen diversity in the honey from *A. dorsata* bee in West Bengal. They reported that the ecological factor (i.e., pollen plants, humidity, and light intensity) due the seasonal change significantly influenced the honey farming sites, and directly affected the pollen diversity in honey. Similarly, [19] also concluded that different ecological factors were found on different honey production from farm sites across Sichuan province.

The lowest diversity of pollen was found on AH-01 sample. Based on field observation during honey collection, the honeycomb was placed on the areas which were dominated by *Ceiba pentandra*, and the honey harvesting was only carried out during the blooming season of this plant species. However, the pollen in the AH-01 was dominated not only by *Ceiba pentandra* pollen, but also by the pollen of plants from Poaceae family including *Oryza sativa* and *Zea mays* (Figure 1A). These Poaceae plants grow in the surrounding area of the honey farm. This finding can be attributed to the nature of *A. cerana* which prefers to hove at low altitude. This is in accordance with [14], who reported the relatively low height nesting zone (average height of 1 – 2 m) of *A. cerana*. As the diversity of pollen grains is strongly dependent to the diversity of the surrounding area vegetation [20], the narrow pollen diversity of the sample indicates the low diversity of vegetation in the respective sites.

The highest pollen diversity was observed on the AH-10 sample, which was collected from the residential area in Solo. This is surprising when comparing the respective honey sample with the honey sample collected from non-residential area. In this regard, the honeycomb was set around the house yard with various vegetations of trees and herbs including *Muntingia calabura*, *Mangifera indica*, and *Antigonon leptopus*. The bee farming site was also close to the public araea, which is dominated by *Plumeria alba*. Pollen grains of these pant species were notably present in AH-10 honey sample. Pollen from other plants such as *Disporum kawakamii*, *Ceiba pentandra*, and *Mimosa* sp. were also observed, indicating that the *A. cerana* collect the nectar outside the bee farming sites.

This result clearly indicated that the nectar sources strongly determines the diversity of pollen in honey [1]. This is also in line with those reported by [13] and [14], who reported the ability of *A. cerana* to hover within long-distance foraging area. This finding is confirmed by the observation on vegetation outside bee farming sites, where various flowering plants were found on the road and city parks adjacent to the farming sites. The existence of plants in city park explains the domination of Poaceae family in the honey sample of AH-10, as shown in Figure 1J.

Sample AH-02, AH-05, AH-07, and AH-11 have identical vegetation condition, in which the sites were close to the rice field, vegetable gardens, and residence. It is worth noted that the beekeepers planted various type of plants close to the bee nesting place. The honeycomb of AH-02 was placed in the house yard with dense vegetation such as *Dimocarpus longan*, *Mangifera indica*, and *Parkia speciosa*. Pollen analysis on these honey samples revealed that the honey contains the pollen grains of those plants species. However, the pollen of *Oryza sativa* and *Zea mays* (both are Poaceae family) dominates the sample AH-02, as shown in Figure 1B. Similar result was found on the sample AH-07 and AH-11, showing the highest percentage of pollen from Poaceae family in the honey samples, as shown in Figure 1G and 1K, respectively. This result can be attributed to the preference of *A. cerana* to forage at low altitude [14]. In the case of sample AH-05, the presence of Fabaceae family lays over the Poaceae family (Figure 1E). Considering the nature of *A. cerana* during foraging activity, the result indicated that Fabaceae plants was sufficiently available and on flowering time around the honey farm of AH-05.

The sample AH-03 was collected from a beekeeper in Metesih, Karangayar, Central Java. The honeycomb was placed in the yard, near to the residential areas and industrial plantation of *Albizzia chinensis* and *Tectona grandis*. Pollen analysis of this honey sample showed that the number of pollen grains from woody plants such as *Albizzia* sp., *Fissistigma oldhamii*, *Lagerstroemia speciosa*, and *Ceiba pentandra* were lower than that of non-woody plants, particularly the pollen from Poaceae family, as shown in Figure 1C. Surprisingly, the pollen of *Tectona grandis* was unable to be identified, suggesting that this plant species was not at blooming period during honey production. It is obvious that the blooming period of some plants strongly depends on the corresponding season due to differences of ecological factors [16]. Sample AH-03 was also classified as multifloral honey due to the presence of pollen from various plants, such as *Hydrangea angustipetala* and *Tabebuia chrysantha*, and other plant species in low percentage.

The honeycomb of sample AH-04 was placed in the yard and close to the community forest, which lays along the Menoreh Hills of Kulonprogo, Special Region of Yogyakarta to Muntilan, Central Java. Pollen analysis showed that the pollen of garden plants (i.e., *Hibiscus rosa-sinensis*, *Carica papaya*, and *Musa paradisiaca*) were present at lower number as compared to those of forest plants (such as *Albizia procera, Fissistigma oldhamii*, and *Rubus shinkoensis*). This expected result can be attributed to the close distance between the honeycomb and the forest. In Figure 1D, it is obvious that pollen of Araceae family existed at high percentage in the honey, indicating the presence of Araceae family plants in the forest. The identical vegetation condition was found on farm site of AH-06 from Kedung Poh, Gunungkidul, Special Region of Yogyakarta. In addition of community forest, the beekeepers in that area planted various type of fruit plants such as *Nephelium lappaceum*, *Mangifera indica*, *Persea americana*, and *Carica papaya,* around the honeycomb to provide source of nectar for the honeybees. Pollen analysis showed that pollen grains of these plants were found in the honey sample. However, the major pollen diversity of the honey was belong to Euphorbiaceae family, as shown in Figure 1F. This finding lays on the fact that the surrounding area of the farming site is dominated by plants from Euphorbiaceae family such as *Ricinus communis*, *Mercurialis leiocarpa* and *Claoxylon brachyandrum.*

Sample AH-08, AH-09, and AH-12 were collected from the locations with similar vegetation, in which the honeycomb was placed in the yard of the beekeeper's house, close to the city park and public facilities. The sample AH-08 was collected from the farm surrounded by garden plants including *Plumeria alba, Mangifera indica,* and *Psidium guajava*. Accordingly, pollen analysis showed that pollen grains from these plants grow in the yard and city parks, such as *Plumeria alba*, *Psidium guajava*, *Acacia glomerosa*, and *Muntingia calabura* were found in the honey. Honey sample AH-09 was produced by a farm which was surrounded by *Smilacina formosana*, *Rhipidocladum racemiflorum*, and *Nymphaea blanda*. Pollen of *Desmodium paniculatum*, *Panicum grande*, and *Cassia obtusifolia* were observed in the honey. Sample AH-12 was collected from an area neighbored by *Ceiba pentandra*, *Musa paradisiaca*, and woody plants such as *Acacia* sp., *Macaranga tanarius*, and *Spathodea campanulata*. Pollen from these plants were found in the honey sample, but interestingly pollen from Poaceae family were found in high percentage, as shown in Figure 1L. This finding might be attributed to the limitation of *A. cerana* to collect nectar from plants with high crown or canopy [14].

In geneal, plant families which their pollen was found in high percentage across all honey samples were Poaceae (22%) and Fabaceae (11%), as shown on Figure 2. Poaceae is a family of grasses found in the tropics in closed canopy areas, open canopies, and near waters. The presence of Poaceae plants which is more commonly found around honeybees indicated open areas, such as yards and fields. Poaceae is group of vascular plants commonly found as understory vegetation and they need high light intensity [21]. High representation of pollen from Poaceae plants is also due to the presence of paddy fields (for *Oryza sativa*), community gardens (for *Zea mays*), yards (for *Pennisetum purpureum*), and city parks (for *Cenchrus brownii*).

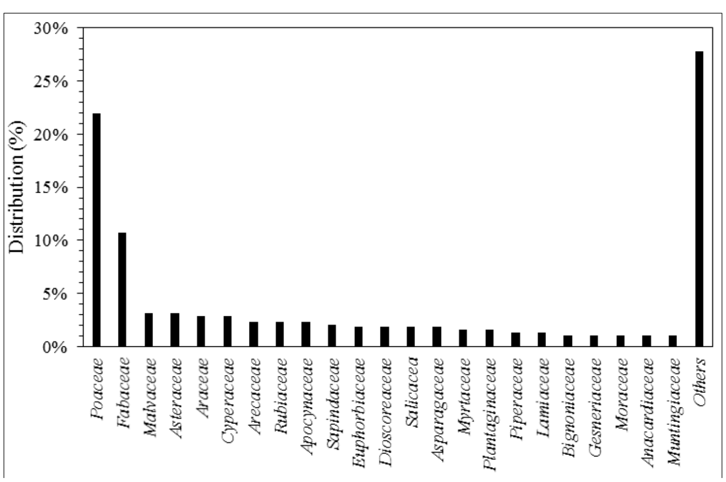


**Fig 2:** Average distribution of plant families represented by their pollen in all honey samples

Fabaceae is a group of plants known as legumes with various habitats for herbs, shrub, to trees. Fabaceae was found in quite high number during field observation and sample collection, either planted as garden plants or ornamental plants such as *Delonix regia* and *Calliandra calothyrsus*, and some were found in manufacture plantation such as *Acacia* sp. And *Albizzia* sp.. In addition, several locations of *A. cerana* beekeeping were close to community forests where Fabaceae was found such as *Inga hayesii*. *Mimosa pudica* from Fabacae was also found in open spaces such as rice fields and gardens. Some representation of pollen grains in honey samples were presented in Figure 3.

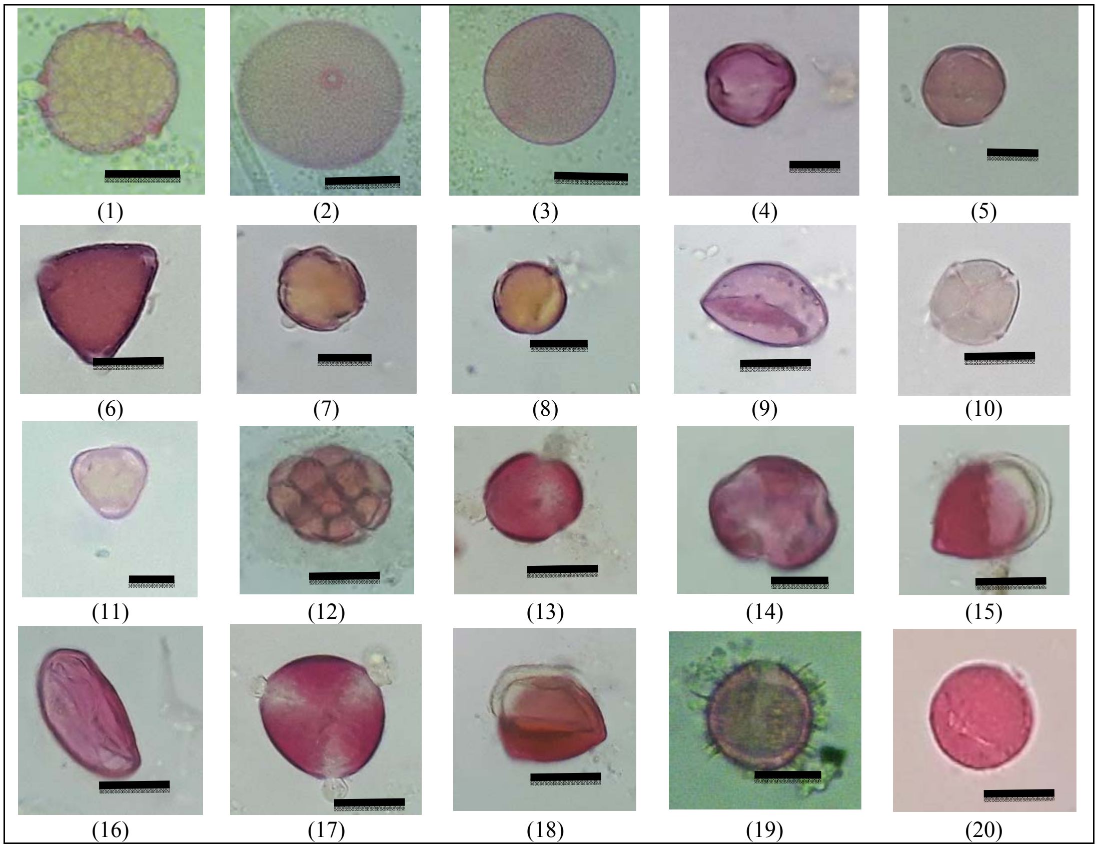


**Fig 3:** Pollen diversity in honey samples: (1) *Ceiba pentandra;* (2) *Zea mays*; (3) *Oryza sativa*; (4) *Muntingia calabura*; (5) *Antigonon leptopus*; (6) *Psidium guajava*; (7) *Mangifera indica*; (8) *Plumeria alba*; (9) *Disporum kawakamii*; (10) *Mimosa* sp.; (11) *Dimocarpus longan*; (12) *Albizzia* sp.; (13) *Hydrangea angustipetala*; (14) *Tabebuia chrysantha*; (15) *Fissistigma oldhamii*; (16) *Dioscorea urophylla*; (17) *Carica papaya*; (18) *Musa paradisiaca*; (19) *Hibiscus rosa-sinensis*; (20) *Persea Americana.* Magnification 400x. Scale 10 µm.

Other plant families such as Malvaceae, Rubiaceae, Araceae, Asteraceae, Euphorbiaceae, Lamiaceae, and Myrtaceae were found as ornamental plants, fruit plants, garden plants, and as wild plants. These plants were intentionally planted by farmers due to their beneficial flower or edible fruits, and at the same time these plants could serve as nectar source for honeybees.

Results of pollen diversity analysis clearly showed that all honey samples are categorized as multifloral honey because that is particular pollen type dominantly in the samples and read the percentage of 45% [6]. Differences of pollen grains diversity found in honey are due to differences in vegetation and corresponding micro-climate in the area of honey production sites. Vegetation has an important influence in honey production which different seasonal nearby of in particular area [22]. Microclimate affects the flowering process of plants, and thus honey harvested at different session will have different pollen compositions.

The activity of *A. cerana* honeybee is considerably affected by environment condition. The activity of *A. cerana* honeybee consists of flying to look for nectar, collecting pollen, and roaming out of the nest [23]. These activities influenced by differences in environment, especially the physical conditions of the environment such as temperature, humidity and light intensity, and the attitude of the place.

The foraging pattern of honeybees is also an important factor in determining the quality of honey in term of quantity and diversity of pollen and nectar found in honey [24]. The fact that some farmers intentionally grow certain plants near their honeycomb sites for the purpose of providing source of nectar indicated that they are aware of the important role of plants as source of nectar and pollen for their honeybees. By growing various plant species, the farmers hope that honeybees will do foraging activity close to their nest and preventing the bee escaping from the nesting area. Some farmers also intentionally look for area with high diversity of plants, as location to place their honeybee hives, especially area with plants of flower.

Results of pollen analysis proved the importance of biological indicators as one of the honey quality criteria, in the form of pollen diversity [2]. The pollen diversity indicator may serve as standard reference for honey quality. Knowledge of biological indicators may have a great impact on the maintenance of vegetation around honeybee hives as a source of nectar and pollen for honeybee feed and a source for producing honey. The adoption of pollen diversity criteria as biological indicator is certainly expected to increase the selling value of Indonesian local honey products in order to be able to compete in the international market. Scientific information of pollen diversity in honey will certainly increase public confidence in consuming local Indonesian honey. This will give a positive impact in the recognition of local honey as food supplement having high health benefits.

## Conclusion

All honey samples produced by *A. cerana* from Java are classified as multifloral honey. Pollen diversity varies among honey samples from different bee farming. Variations in pollen diversity was attributed to differences in vegetation condition of the surrounding area of bee farming sites.

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