BioEksakta: Jurnal Ilmiah Biologi Unsoed
Volume 2, Nomor 1 (2020) : 42 - 48
E-ISSN : 2714-8564

Identification of Pollen Characteristics as *Apis cerana* Feed Sources in Honeycomb, in Serang Purbalingga

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Article History:

Received : 28/08/2019
Accepted : 19/02/2020

Abstract

Serang Purbalingga village was a fertile area and has the potential for the development of *Apis cerana* honeybee business. Honeybee products are known to have high economic value. The development of honeybee business will be better if supported by the availability of pollen from flowering plants as feed sources. Pollen that uses to be *A. cerana* feed sources are taken from plants flower around the beehive and matched with pollen inside the honeycomb. The purpose of this research is to determine the diversity and character of pollen from plants found around the beehive and inside *A. cerana* honeycomb. This research conducted by descriptive survey method where the data obtained from the field used as material for analysis and describing the characteristics of pollen found. The variable in this research is pollen characters include pollen units, size, shape, apertures and ornamentation. Based on results, there are 23 species of plants included in 17 families found around the beehive with varying of pollen shape, namely spheroidal, prolate-spheroidal, sub-prolate, and prolate. The smallest to largest pollen sizes are minutae, mediae, and magnae. Types of pollen ornamentations are rugulate, reticulate, echinate, psilate, scabrate, to baculate. Pollen apertures are varied: monosulcate, monoporate, tricolporate, tricolpate, tetracolpate, hexacolpate to syncolpate. Pollen characters inside honeycomb are identical to 12 pollen of plant species found around the beehive where the pollen shapes are spheroidal, prolate-spheroidal, sub-prolate and prolate. There are several types of ornamentation, namely reticulate, rugulate, echinate, psilate and sacbratte. Apertures are varied from monosulcate, monoporate, tricolporate, tricolpate to syncolpate..

Keywords: : Apis cerana, characters, diversity, pollen, Purbalingga

INTRODUCTION

Insects were one of the fauna that has high adaptability in Indonesia's climate. Some insect species not only can interfere with agricultural production due to being a pest for cultivated plants but also can give benefit to humans and the environment. Honeybees are one of the insects which have beneficial products for humans and from an ecological perspective, it may help the plant's pollination process (Wibowo *et al*., 2016). Honeybee's production can be influenced by the availability of feed. Feed is a very important requirement for the survival of honeybees. Pollen and nectar are honeybee feed sources which obtained from flowering plants around their beehive to fulfill their daily needs (Keller *et al*., 2015).

Plants that are used as honeybee feed sources can be wild or cultivation plants. Flowers from those plants contain nectar and pollen which can affect the production of honeybees (Mulyono *et al*., 2014). Plants that have bright flower colors and attractive scents are preferred honeybees to visit the flowers to pick the nectar and pollen up that they will use as a feed source (Pratama *et al*., 2015).

This research conducted in Serang Purbalingga which is a highland area with an average temperature about 20°C. This village has greatly fertile land, it's known by many agricultural and plantation lands. Besides plantation, there are also beekeepers of *Apis cerana* honeybee, that produced products utilized as a source of livelihood. Honeybee products used by beekeepers are honey, royal jelly, pollen, propolis, and bee venom that have high economic value because if sold it may help to increase the income of the villagers, especially beekeepers (Saepudin, 2013). The problem is still little information about flowering plants that potential as a source of feed for *A. cerana* honeybees. Honeybee feed sources can be identified by identifying pollen characters inside honeycomb compared to pollen characters found around *A. cerana* honeycomb.

Pollen can be used as an identification material because each plant species has a specific pollen characteristics. The aims of this research are to know the diversity and characteristics of pollen from plants around the *A. cerana* honeycomb and to know the diversity and characteristics of pollen inside honeycomb which used as *A. cerana* feed sources. The benefits of this research can be used

as a reference to find out which plant species that *A. cerana* use as feed sources.

MATERIAL AND METHOD

The pollen sample inside the honeycomb of A. cerana and fresh flowers of different plant species around the honeycomb are collected in Serang Village, Karangreja Subdistrict, Purbalingga Regency (Figure 1). The pollen characteristics identified in Pengajaran 1 Laboratory and Struktur Perkembangan Tumbuhan Laboratory Faculty of Biology, Jenderal Soedirman University.

The research used descriptive survey method, samples observed and taken directly in the field. The results obtained then described systematically according to the variables data of pollen morphological characters include pollen unit, pollen shape, diameter size of pollen from the longest axis, pollen ornamentation and pollen aperture.

Figure 1. Map of sampling location. Source: View ranger.

To observe the pollen characteristics using acetolysis method by Erdtman (1943) with modification. Pollen samples were collected into labeled test tubes and 3 mililiters of glacial acetic acid was added. After 24 hours transferred the pollen samples into centrifugation tube and concentrated sulfuric acid was added 1 – 2 drops or with a ratio of glacial acetic acid and concentrated sulfuric acid 9 : 1. Heated in temperature 600C using waterbath into slightly brownish. Let stand the tube containing the sample for 2-3 minutes and centrifuged on 1000 rpm for 10 minutes. Removed the liquid and replaced with 1 ml of aquadest and centrifuged again on 1000 rpm for 10 minutes. Stain the pollen sample with safranin and glycerin gell then stirred with the stirring rod. Pollen sample placed on object glass and covered by cover glass. Labelled the samples and observed under the microscope.

This research used Plant net application to identified the name of plant species. The characteristic and morphology of pollen samples identified by using several books by Hesse *et al*., (2009), Erdtman (1952), Faegri & Iversen (1989), Kapp (1969), and Moore & Webb (1978). Pollen samples inside A. cerana honeycomb were compared and matched with pollen morphological characters found around the beehive to find out which plant species were carried by honeybees to be used as their feed source.

RESULT AND DISCUSSION

The diversity of pollen characteristics in plants around the honeycomb

Based on the identification of diversity flowering plant that have potential to be Apis cerana honeybee feed sources were found 23 species of plants, including in 17 families namely:

1. Acanthaceae family

   Pachystachys lutea Nees. has open flower stamen located inside white flower, with anther in green colour. The white flowers that arise between bracts can make easier pollinators to take pollen. Based on the characteristics of pollen this species has a monad unit, with prolate shape, magnae in size, reticulate ornamentation and tricolporate aperture.
2. Apiaceae family

   Daucus carota L. or known as carrots has a white compound flower which is umbrella-shaped, and there are several flower petals that are larger in size located at the edge to attract the pollinators visit. In one stalk there are several flowers in which consist of stamens. Based on the results of pollen identification, this species has pollen with monad units, spheroidal shape, magnae size, rugulate ornamentation and tricolporate apertures.
3. Asteraceae family
   a. *Tagetes erecta* L. has bright red petals with stamen located in the middle of flower, this can make easier for pollinators to take pollen. Two types of *T. erecta* were found, type A had more tube flowers than type B. Based on the identification of pollen characters *T. erecta* type A and B have similarities in units of pollen namely monad, mediae in size and echinate ornamentation. There are differences in the shape and aperture of pollen. *T. erecta* type A has spheroidal pollen shape and tricolpate aperture, while *T. erecta* type B has a prolate-spheroidal shape and syncolapate ornamentation.
   b. *Cosmos sulphureus* Cav. has a single flower and bright yellow petals. Stamen and pistil are located in the middle of flower. Bright flower colors can attract the pollinator to visit. The results showed pollen of *C. sulphureus* has monad units, prolate-spheroidal shape, mediae size,

echinate ornamentation and monoporate aperture.

c. *Praxelis clematidea* (Griseb.) R.M.King & H.Rob. has purple, small and clustered at the end of a branch. On each flower head, there are small flowers surrounded by involucre. Small flowers are purplish or purple in colored. Flowers appear almost during the summer months of January to May (Kementrian Pertanian, 2017). Based on the observations of *P. clematidea*, has pollen with monad unit, prolate-spheroidal shape, size minutae with the tricolporate aperture.

d. *Emilia sonchifolia* (L.) DC. ex DC has a small corymbus terminal flower surrounded by bractea. The stamen is located in a purple tube flower. Pollen *E. sonchifolia* in the identification results is known to have prolate-spheroidal shape, minutae size and tricolporate aperture.

e. *Zinnia elegans* L. has pink compound flower with two types of flowers namely ribbon flowers and tube flowers. Stamen of *Z. elegans* located in the tube flower. Some pollinators such as honeybee and dronefly are known often visit *Z. elegans* to collect nectar and pollen in tube flowers (Miyajima, 1995). The result of the identification pollen shows that this pollen has a sub-prolate shape, size mediae with tricolpate aperture.

f. *Dahlia pinnata* Cav. has bright purple petal colored flowers and in the middle, there are disc flowers consisting of several flower tubes containing stamen. In the study conducted by Priya and Raichal (2018) in India *D. pinnata* included is included in agricultural crops that visited by pollinators such as honeybees. The results of pollen identification *D. pinnata* showed it has prolate spheroidal shape, media in size, and monoporate aperture.

4. Balsaminaceae

Impatiens balsamina L has single axillary flowers and purple petals. The petal at the lower part has a larger size than the petal at the upper, based on this structure it may facilitate the pollinator to alight. The identification results of *I. balsamina* pollen have a monad unit with prolate shape, mediae in size, reticulate ornamentation and a tetracolpate aperture

5. Begoniaceae

Begonia cucullata Willd. it has pink flower petals. In the middle of the flower, there are a number of stamen that is free to each other. Some pollinators that often visit B. cucullata are honeybees, meliponinae bees, flies and beetles (Avila *et al*., 2017). The results of the identification showed that pollen characters of this species had units with monad type, prolate shape, minutae size, psilate ornamentation and tricolpate aperture.

6. Brassicaceae

Brassica oleracea L. has bright yellow flower petals and in the middle there are open stamens that make pollinators easy to take pollen. According to Stanley *et al*. (2017), there are several order of pollinating insects that often visit *B. oleracea*, namely Hymenoptera, Dyptera and Lepidoptera. Among the three orders Hymenoptera is the most dominant order to visit this plant. A. cerana is also known to be a good pollinator for *B. oleracea* because it can affect agricultural production. The results of the observations show the character of pollen *B. oleracea* has a monad unit with prolate-spheroidal shape, minutae size, ornamentation scabrate and tricolpate aperture.

7. Capparidaceae

Cleome houtteana Schltdl. has purple flowers and is known as a spider flower because it has a long stamen which lay below the flower that blooms. This plant blooms at the beginning of summer until the beginning of winter. Some *C. houttena* pollinators are hummingbirds, moths, bees and butterflies (Mahr, 2017). The results of pollen identification of this species have monad units with prolate-spheroidal shape, mediae size, baculate ornamentation and tricolpate aperture.

8. Gesneriaceae

Chrysothemis pulhella (Donn ex Sims) Decne. flower consist of corolla shaped like a yellow bell surrounded by orange sepals. In the middle of flower, there are stamen containing pollen. The pollen character of *C. pulchella* has been observed to have monad units, prolate shape, mediae size, with psilate ornamentation and tricolporate apertures.

9. Hidrangeaceae

Hydrangea *macrophylla* (Thunb.) Ser. have a compound flower with two types of flowers, green flowers consist of stamen in the middle, while purplish-blue flowers do not exist. The results of pollen identification of H. macrophylla are known to have a monad unit with the sub-prolate shape, minutae size, psilate ornamentation and tricolpate aperture.

10. Iridaceae

Neomarica longifolia (Link & Otto) Sprague. has the characteristics of a bright yellow flower where in the middle there is an open stamen with anther which leads out, it makes easier for the polinator to reach the pollen. Based on the identified pollen character known that this species has units with monad

type, prolate shape, mediae size, ornamentation of reticulate and monosulcate apertures.

11. Lamiaceae
    a. *Salvia splendens* Sellow ex Schult. has a bright red compound flower in which there is a stamen. Identification results showed *S. splendens* has pollen with monad units, sub-prolate shape, magnae size, ornamentation of reticulate and aperture hexacolpate. According to Cantino & Senders (1986), based on the type apertures *S. splendens* included in the subfamily Nepetoidae.
    b. *Cleodendrum thomsoniae* Balf.f. has a compound flower with white flower sepals and red flower petals which in the middle there are stamen. Based on the results of the identification it is known that this species has pollen with monad units, sub-prolate form, magnae size, reticulate ornamentation and tricolpate apertures. According to Cantino & Senders (1986), *C. thomsoniae* belongs to the Lamioideae subfamily based on the type of aperture.
12. Lythraceae

    Cuphea hyssopifolia Kunth. has a single flower with purple petals and forms like a tube in which there is stamen. Insects such as the order Hymenoptera, Lepidoptera, Coleoptera, Diptera and Hemiptera are known to frequently visit these plants because of the availability of pollen and nectar (Safriya & Karunaratne, 2011). The results of pollen identification *C. hyssophyfolia* are known to have pollen with monad units, prolate-spheroidal shape, minutae size, psylate ornamentation and tricolporate apertures.
13. Melastomataceae

    Tibouchina *urvilleana* (DC.) Cogn. has a single terminal inflorescence with purple petals. Based on the results of T. urvilleana pollen identification, it has monad unit, sub-prolate shape, minutae size, psilate ornamentation and monosulcate aperture.
14. Poaceae

    Zea mays L. is a species of Poaceae which is widely planted by local farmers. On each flower stem *Z. mays* can produce pollen. Honeybee pollinators such as *A. cerana, A. mellifera* and *Trigona* sp. collect pollen from *Z. mays* and at the same time it may help the pollination process (Agussalim, *et al*., 2017). Pollen identification shows that *Z. mays* has units with monad type, prolate-spheroidal shape with magnae size, ornamentation of scabbrate and monoporate aperture.
15. Poligonaceae

    Persicaria capitata (Buch. Ham. Ex. Don) H. Cross is a Polygonaceae species that has pink compound flowers arranged in a circle. Based on the results of identification, these plants have pollen with monad units, spheroidal shapes, minutae sizes, echinate ornamentations and tricolporate apertures.
16. Rosaceae

    Rosa sp. has a single *flower* with bright red petals and stamens located in the middle of the flower. The results of the identification of pollen are known that *Rosa* sp. have monad unit types, prolate shapes, mediae size, psilate ornamentation and tricolporate apertures.
17. Tropaeolaceae

    Tropaeolum majus L. is a species of Tropaeolaceae *family*. This plant grows in single flowers and has a bright red color. Flowers form a trumpet-like structure in which there is a stamen with yellow anther. The bright colors of *T. majus* can attract pollinators to visit. Based on the results of identification *T. majus* pollen had monad units, spheroidal prolate in shape, mediae size, psylate ornamentation and tricolporate apertures.

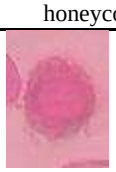
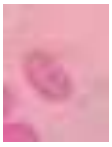
    Based on the identification flowering plants around the beehive of A. cerana, Asteraceae is the family with the most species found in up to 6 species. The majority of the plants identified are ornamental plants such as *P. lutea, D. pinnata, T. erecta, C. sulphureus, Z. elegans, B. cucullata, C. hassleriana, C. pulchella, H. macrophylla, N. Longifolia, S splendens, C. hyssopifolia, and T. urvilleana*. Serang Village, Purbalingga is a fertile area that is widely used as agricultural land for growing crops. Based on the results in the field at the time of the research found 3 cultivated plant species, namely *D. carota, B. oleracea* and *Z. mays*, and there were also weeds such as *E. sonchifolia, P. clematidea* and *P. capitata.*

The diversity of pollen characteristics inside the honeycomb

There are 17 pollen characters inside the honeycomb, were identical with 23 species of pollen characters found from plants around the honeycomb and there are 5 unidentified pollen characters.

Species that were successfully identified based on table 1 are *P. clematidea, E. sonchifolia, T. erecta* (Type A), *T. Majus, B. cucullata, S. splendens, C. hyssophyfolia, H. Macrophylla, T. urvilleana, D. carota, Z. mays* and *N. longifolia*. According to Sarwono (2001), Apis cerana has a flightrange about ± 700 m, but the identified plant species are located close to honeycomb ranging from 10 to 160 m. Farland (1985) states that bees tend to collect feed that is close to the honeycomb when the amount is available.

Table 1. Pollen characteristics inside and around the honeycomb of Apis cerana

Species name	Inside honeycomb	Around honeycomb
Praxelis clematidea		
Emilia sonchifolia		
Tagetes erecta (Type A)		
Tropaeolum majus		
Begonia cucullata		
Salvia splendens		
Cuphea hyssophyfolia		
Hydrangea macrophyla		
Tibouchina urvilleana		
Daucus carota		
Neomarica longifolia		
Zea mays		

The pollen inside the honeycomb of *A. cerana*, the smallest equatorial diameter pollen is known as H. macrophylla which has a size about 11.5 µm and the largest is *Z. mays* which has an equatorial diameter up to 80.0 µm based on this results honeybees in collecting pollen which is used as feed sources do not depend on certain pollen diameters. The pollen shape observed also vary, which are spheroidal, prolate-spheroidal, sub-prolate and prolate. The observations found several types of ornamentation from pollen inside the honeycomb where 1 species with rugulate ornamentation namely *D. carota*, 2 species with reticulate types namely *N. longifolia* and *S. splendens*, 3 echinate type species namely *P. clematidea, T. erecta* and *E. sonchifolia*, 5 species of psilate type *B. cucullata, H. macrophylla, C. hyssopifolia, T. urvilleana* and *T. majus*, and 1 scabrate species which is *Z. mays*. The aperture observed also vary from tricolporate, tricolpate syncolpate, monoporate, monosulcate and hexacolpate.

Factors such as flowering time effect *A. cerana* choose certain pollen, which is coincides with the sampling time in the field, in February. Based on the results of the identification of inflorescence, most plants collected by *A. cerana* honeybees are flowering around the year and they tend to take pollen based on the availability of flowering plants around the honeycomb.

According to Faheem *et al*. (2004), bees will also take flowering plants based on their color and scent. The results showed that plant species as honeybee *A. cerana* feed sources consisted of various colors, such as red, yellow, purple, white to blue. These results are supported by research conducted by McCall & Primack (1992), bees tend to visit yellow and purple flowers, but when these yellow flowers are removed bees will make a greater proportion of visits to pink and red flowers, then white to blue flowers.

Flower shape are also often regarded as characters that can limit the types of pollinators that pollinate flowers. The results of the

identification show that *A.cerana* honeybees tend to visit flowers with an open form, where the stamen is located higher than the flower petals and hypanthium flower both in compound or single flowers. Tubular-shaped flowers such as *T. majus, C. hyssophyfolia,* and *S. spelendens* according to McCall & Primack (1992), are considered frequented by specialist pollinators such as long-tongued bees and butterfly and hypanthium flower often visited by general pollinators such as bees, flies and beetles.

The pollen from *Cuphea hyssophyfolia* has the highest frequency found inside *A. cerana* honeycomb. This can occur because the location of plants that are not far from the honeycomb and available in abundant quantities. The study of the characteristics of *C. hyssophyfolia* flowers carried out by Safriya & Karunarathe (2011), stated that polinators such as honey bees visiting this species related to entomophilous syndrome flower where the plants have their own mechanism to attract insects with bright colors, flower shapes and scent so its able to make pollinators to pollinate. In addition, insects from the order Hymenoptera have proboscis which are used to extract nectar located at the base of the corolla C. hyssophyfolia small tube. In addition, this plant is also able to produce flowers throughout the year, so pollen and nectar are always available for pollinators (Safriya & Karunaratne, 2011).

There were 5 types of pollen that can not be identified because they had different characters from the plants identified around the honeycomb. According to Sulistia (2016), this can be happen because the pollen is obtained from high-flowering plants or flowering outside the time of observation and may also be out of reach when sampling flowers in the field.

CONCLUSION

The pollen shape characteristics of 17 flower families found around *A. cerana* beehive are varies, from spheroidal, prolate-spheroidal, sub-prolate, and prolate. Pollen sizes vary from minutae, mediae, and magnae. Ornamentation types also varies from rugulate, reticulate, echinate, psilate, scabrate, to baculate. Aperture types from monosulcate, monoporate, tricolporate, tricolpate, tetracolpate, hexacolpate to syncolpate.

The pollen characters contained inside A. cerana honeycomb are identical with the pollen characters from 12 plant species found outside the honeycomb where the pollen forms are spheroidal, prolate-spheroidal, sub-prolate and prolate. There are 5 types of ornamentation, namely reticulate, rugulate, echinate, psilate and sacbratte. Aperture varies from monosulcate, monoporate, tricolporate, tricolpate to syncolpate. Based on observations also known the pollen from C. hyssophyfolia has the highest frequency found inside A. cerana honeycomb. This can occur because the location of plants that are not far from the honeycomb and available in abundant quantities.

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