International Journal of Botany Studies
ISSN: 2455-541X; Impact Factor: RJIF 5.12
Received: 04-02-2019; Accepted: 06-02-2019
www.botanyjournals.com
Volume 4; Issue 3; May 2019; Page No. 20-22

Melissopalynological analysis of honey samples collected from Nilambur Taluk in Malappuram district, Kerala

Amrutha N Divakaran[1], Sincy Joseph[2]
[1, 2] Department of Botany, Nirmala College for Women, Coimbatore, Tamil Nadu, India

Abstract
Melissopalynological analysis of honey samples was done in five regions of Nilambur taluk during the period of June to September in 2018. In this study 14 pollen grains was identified to species level, 2 in generic level, 2 in family level and 1 as type. The identified pollen grains belongs to 11family. Of these plants, 9 are tree, 4 are shrubs, 1 is herb and 2 are climbers. Due to the variation of bee visits in plants, the pollen density varies in each sample.

Keywords: Melissopalynology, honey, pollen grains, Nilambur taluk

Introduction
Honey bees are used in the agriculture field for the harvest of honey and pollen grains. Pollen grains is the major food source for honey bees. It is also called bee bread. During plant visit, Forager bees collect pollen grains. These pollen grains are never eaten by themselves and are stored in the pollen basket. Foragers unload the pollen directly into open cells of honey comb. These grains are mixed with honey during collection and unloading. Palynology is the study of pollen grains, spores and of other biological materials that can be studied by means of palynological techniques (Hyde and Williams, 1944). Melissopalynology is one of the applied branch of palynology that deals with the microscopic analysis of the pollen contents of honey and pollen loads from a locality. It will provide reliable information regarding the floral types which serves as major or minor nectar and pollen sources for the honey bees (Lieux, 1975). Analysis of honey samples from a particular area provide reliable information about the floral situation of that area and geographical origin of honey. Calculation of pollen density indicate the frequency of bee visit, it is useful for apiculture farmers.

Materials and Methods
Study Area
Nilambur taluk is located at Malappuram district in Kerala with a latitude of 11º 16´ 29 N, longitude of 76º 13´ 30 E and altitude of 55 meter. The main economy of this Taluk is agriculture. The details of the locations from which the apiaries were collected are:

Table 1

Sample	Location	Latitude	Longitude
Sample-1	Karulai forest	11.232º N	76.0723º E
Sample-2	Nilambur forest	11.2748 º N	76.2250 º E
Sample-3	Chungathara	11.3344º N	76.2775º E
Sample-4	Vazhikkadavu	11.3861ºN	76.3449º E
Sample-5	Munderi	11.4414º N	76.2537º E

Preparation of slides by acetolysis (Erdtman, 1960)
Dissolve 30 g of honey with 50 ml of hot distilled water not above 40°C. Centrifuge the solution in equal parts in two tubes (50 ml capacity) for 10 min at 2500 r/min. Draw off the supernatant liquid with a fine pipette. Not more than 1 ml should be left in the tubes. Disperse the sediment again and transfer it with several portions of water into two smaller centrifuge tubes of 20 ml capacity. Centrifuge for 5 minutes and draw off the supernatant. Place one of the tubes upside down on filter paper. The sediment should be as dry as possible. Set aside the second tube. Prepare 10 ml acetolysis mixture by adding 1 ml of sulphuric acid to 9 ml of acetic anhydride. All glassware must be absolutely dry. Add a drop of the acetolysis mixture to the sediment. If there is little water left, the reaction will not be too violent. Redisperse the sediment with a thin glass rod and add the rest of the mixture. Place the tube in a 70°C water- bath for 10 minute. Take care to prevent contact between the water and the acetolysis mixture. Centrifuge the tube after incubation. The acetolysis sediment settles less tightly than an untreated one. Fill the centrifuge tube with distilled water and shake vigorously and centrifuge again for 5 min. Draw off the supernatant liquid. The adhered pollen grains is shake again with distilled water and centrifuge at high speed (3500 r/min) for 5 min. Put the entire sediment on a slide and spread it out over an area about 20 X 20 mm. The addition of a drop of glycerine water mixture (1:1) to the sediment prevents the formation of air bubbles. A drop of glycerine is added to the sediment. A very small drop of glycerine in the centre of the cover glass prevents the enclosure of air bubbles when the cover glass is placed on the sediment-glycerine mixture.

Microscopical examination
The prepared pollen grains were observed under compound microscope, then the grains are photographed.

Pollen density
By selecting three regions of 2mm diameter field view in each slides of five samples under compound microscope, the average pollen density were calculated.

The formula used for the calculation of field view is;

$$\text{Field view} = \frac{\text{field number (F N)}}{\text{Objective magnification}}$$

$$= \frac{20}{10x} = 2 \text{ mm}$$

Results

The present investigation was carried to classify the honey samples on the basis of unifloral and polyfloral origin, found the geographical origin of honey, average pollen density and honey sample that process more types of pollen grains.

Table 1: Pollen grains present in different samples indicates the geographical origin of honeys

PLANTS	SAM-1	SAM-2	SAM-3	SAM-4	SAM-5
Acacia auriculiformis, A. Cunn.; Mimosaceae		✓	✓		
Bombax ceiba, Linn.; Malvaceae	✓	✓			
Cocos nucifera, Linn;. Arecaceae	✓			✓	
Croton sp. Euphorbiaceae		✓			
Cucurbita sp. Cucurbitaceae			✓		
Delonix regia, Raf.; Fabaceae	✓				
Hibiscus rosa-sinensis, Linn.; Malvaceae	✓	✓			✓
Mangifera indica, Linn.; Anacardiaceae		✓		✓	
Mimosa invisia, Linn.; Mimosaceae		✓	✓		
Mimosa pudica, Linn.; Mimosaceae		✓		✓	
Moringa oleifera, Linn.; Moringaceae		✓			
Passiflora foetida, Linn.: Passifloraceae	✓		✓		
Peltophorum pterocarpum, (DC.) K. Heyne.; Fabaceae	✓				
Schleichera pololeosa, Willd.; Sapindaceae	✓				✓
Tecoma stans, L(Juss) Kunth.; Bignoniaceae	✓				
Vicia faba, L.; Fabaceae		✓			

✓ - presence, SAM - Sample

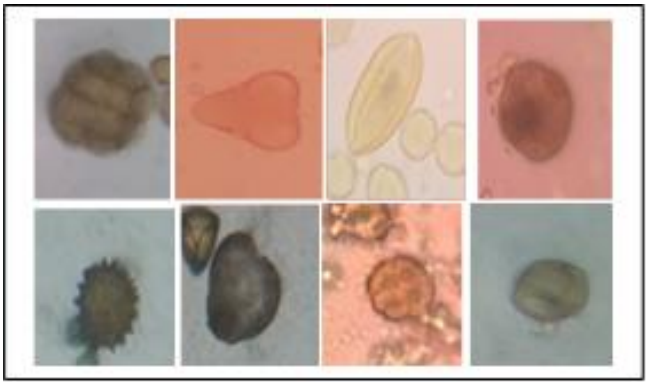

Fig 1: Images of pollen grains: **a**-*Acacia auriculiformis*, **b**-*Bombax ceiba*, **c**-*Cocos nucifera*, **d**-*Delonix regia*, **e**-*Hibiscus rosa*-sinensis, **f**- *Mangifera indica*, **g**-*Mimosa invisia*, **h**-*Mimosa puica*

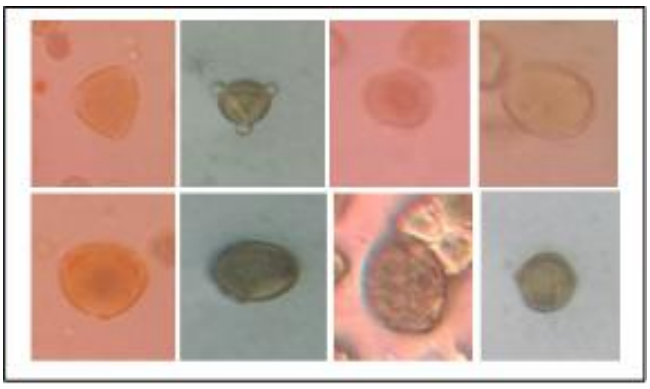

Fig 2: Images of pollen grains: **a**-*Tecoma stans*, **b**-*Moringa oleifera*, **c**-*Passiflora foetida*, **d** –*Peltophorum pterocarpum*, **e** -*Schleichera pololeosa*, **f**-*Croton sp.*, **g**-*Cucurbita sp.*, **h**-*Vicia faba*

Samples collected from Karulai Forest, Nilambur Forest and Chungathara are polyfloral. Sample from Vazhikkadavu is bifloral. Unifloral honey is obtained from Munderi.

Table 2: Classification of honey on the basis of floral origin.

Honey	Unifloral	Bifloral	Polyfloral
SAM 1			✓
SAM 2			✓
SAM 3			✓
SAM 4		✓	
SAM 5	✓		

Height and habit of the plant is not a factor of collecting honey in the case of honey bees. They visit plants invariably the habit.

Table 4: Distribution of plant species according to their habit in each sample.

Samples	Habit			
	Herb	Shrub	Tree	Climber
Sample 1	0	2	6	1
Sample 2	1	4	4	0
Sample 3	1	0	1	2
Sample 4	1	0	2	0
Sample 5	0	1	1	0

Of these 16 identified plants 9 are tree, 4 are shrubs, 1 is herb and 2 are climber. Considering the habit, there is a chance of variation in the pollen content and amount of honey collected by bees. That indicates that, the contribution of pollen and honey are depend on the characters and the availability of plants.

Table 5: Habit of individual plants

S. No.	Plants	Habit
1	*Acacia auriculiformis*	Tree
2	*Bombax ceiba*	Tree
3	*Cocos nucifera*	Tree
4	*Croton sp.*	Shrub
5	*Cucurbita sp.*	Climber
6	*Delonix regia*	Tree
7	*Hibiscus rosa-sinensis*	Shrub
8	*Mangifera indica*	Tree
9	*Mimosa invisia*	Herb
10	*Mimosa pudica*	Shrub
11	*Moringa oleifera*	Tree
12	*Passiflora foetida*	Climber
13	*Peltophorum pterocarpum*	Tree
14	*Schleichera pololeosa*	Tree
15	*Tecoma stans*	Tree
16	*Vicia faba*	Shrub

Pollen densityof individual plat indicates the frequency of bee visit in that particular plant. It provides information about the major and minor sources of honey. Sample based analysis of honey shows that, sample contain high pollen density have variations in pollen types. The pollen density is high in sample- 1 collected from Karulai Forest and least pollen density showed by sample- 4, collected from Vazhikadavu.

Table 6: Pollen density of each sample

Apiaries	Average pollen density (field view- 2mm)
Sample 1	607.66
Sample 2	421.75
Sample 3	149.33
Sample 4	140
Sample 5	156.33

Conclusion

Melissopalynology is one of the applied branch of palynology that deals with the microscopic analysis of the pollen contents of honey and pollen loads from a locality. It will provide reliable information regarding the floral types which serves as major or minor nectar and pollen sources for the honey bees. The slides were prepared by acetolysis (Erdman, 1960) and without acetolysis (Louveaux, 1970). SAM-1 contain pollens of *Delonix regia* Raf.; *Bombax ceiba* Linn.; *Hibiscus rosa-sinensis* Linn.; *Passiflora foetida* Linn.; *Cocos nucifera* Linn.; *Schleichera pololeosa* (Lour.)Oken.; *Tecoma stans* L(Juss)Kunth.; and *Peltophorum pterocarpum* (DC.) K.Heyne.; In SAM-2 pollen grains of *Croton sp.*, *Acacia auriculiformis* A. Cunn.; *Moringa oleifera* Lamk.; *Bombax ceiba* Linn.; *Hibiscus rosa-sinensis* Linn.; *Mangifera indica* Linn.; *Mimosa invisia, Linn*.; and *Mimosa pudica* Linn.; are present Pollen grains of *Acacia auriculiformis* A. Cunn.; *Cucurbita sp*., *Mimosa invisia Linn*.; and *Passiflora foetida* Linn.; are obtained from SAM-3 and *Mimosa pudica* Linn.; *Mangifera indica* Linn.; and *Cocos nucifera* Linn.; from SAM-4. SAM-5 contain pollen grains of *Schleichera pololeosa*, Willd.; and *Hibiscus rosa-sinensis* Linn.; Individual analysis of honey samples shows the major and minor sources of honey. Height and habit of the plant is not a barrier to bees for collecting pollen and nectar, they visit both the tall trees (*Cocos nucifera*) and small plants (*Mimosa pudica).* The increased pollen density of honey indicates the frequent visit of plants by honey bees and the more number of plant species in that area. The pollen density is high in SAM-1 and low in SAM-4.

References

1. Aswini S, Amritha VS, Devanesan S. Melissopalynology studies on the Indian honey bee (*Apis cerana indica*, Fab.;) in southern Kerala, Thesis, 2015; vol. 40(1).
2. Dinkov S. Quantitative melissopalynological analysis of bee honey using a Burker chamber. International food research journal. 2015; vol. 22(4).
3. Evelin Kirsa, Raili Palla, Kaie Martverka, Katrin Laosa. Physicochemical and melissopalynological characterization of Estonian summer honeys, 2011; pp: 616-624.
4. Gilliam, Martha. Microbiology of pollen and bee bread: the yeasts, *Apidologie*, 1979; vol. 10.
5. Katarzyna Komosinska-Vassev, Pawel Olczyk, Justyna Kaźmierczak, Lukasz Mencner, Krystyna Olczyk. Bee Pollen: Chemical Composition and Therapeutic Application, *Review article*, 2015.
6. Laxmikant Borkar, Devendra Mate. Melittopalynological study of squeezed summer honey collected from forest area of Sindewahi tahasil of Chandrapur district, (Maharashtra state). International journal of science and research. 2016; 5(6), ISSN: 2319-7046.
7. Lea Maria Medeiros Carreira, Ricardo De Souza Secco. Pollen morphology of the lianescent species of the genus *croton* (Euphorbiaceae). Journal. 1996; 35(2), ISSN: 0017-3134.
8. Louveaux J, Anna Maurizio, Vorwohl G. Methods of melissopalynology, 1970; vol. 51(3).
9. Manju Sahney, Snehalata Rahi, Ajay Kumar, Roma Jaiswal. Melissopalynological on winter honeys from Allahabad, Uttar Pradesh, India, 2018; vol. 42(4).
10. Michael S Engel. The taxonomy of recent and fossil honey bees (Hymenoptera: Apidae: Apis). Journal of Hymenoptera Research, 1999; vol. 8.
11. Nair MC. Palynological identification of resources for development of apiculture in Kerala- A case study. Journal of Palynology. 2005; 41(1-2).
12. Saharia D, Sarma GC. Melissopalynological studies in the Darrang district of Assam (India), 2016; 10(1):116-122. ISSN: 0973-9467.
13. Nawaz A, Khan MA, Naz R, Zeb S. Extraction of venom from honey bee in district swat, Khyber Pakhtunkhwa, Pakistan. Extraction. 2019 Nov;4(6).