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# MELISSOPALYNOLOGY CHARACTERIZATION OF PAKISTANI HONEY

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SQ & MS conceived the idea; BA, ZH, SK honey samples collection and data analysis; HU, UN & S conducted analysis for pollen; KK, ZA & M identification of pollens.

***Key words:***
Pakistani honey, Pollens, *A. mellifera*, *Ziziphus* spp.

## ABSTRACT

The aim of the study was to add latest scientific information regarding bee flora through pollen analysis available for honeybees in different districts of Punjab province, Pakistan. A total of 50 honey samples, natural (n=32) and branded (n=18) were collected and examined as per the method recommended by International Honey Commission. Natural honey samples were collected from beekeepers. Pollens of families *Poacea* (26.5%), *Azadirachta* spp. (22%), *Citrus* spp. (17.45%), *Pisum* spp. (16.41%), *Ziziphus* spp. (13.99%), *Prosopis* spp. (13.13%), *Brassica* spp. (8.57%), Malvacea (8.08%), *Syzygium* spp. (7.29%), *Cassia* spp. (6.2%), *Acacia* spp. (5.17%) and *Eucalyptus* spp. (4.35%) were common in both branded and fresh honeys. Whereas *Morus* spp. (8.5%), *Moringa* spp. (4.46%), *Psidium* spp. (4.23%), *Bombax* spp. (1.9%), *Mangifera* spp. (1.9%) were found in fresh honeys only. Similarly, four different types of Pollens (*Melilotus* spp. (8.6%), *Alfa* spp. (6.4%), *Benincasa* spp. (6.4%) and *Halianthus* spp. (4.3%)) were detected exclusively in various branded honeys Muqeet (n=4), Sary (n=2), Swat honey, Marhaba (n=3), Youngs (Beehive), Ubqari, Salman (Pak honey) Al-Shifa, Ponam, Langanase and Aftab Qarshi. There was a correlation (r=0.24) between pollens of same taxa and families in branded and fresh honeys. High quality pictures were taken by camera fitted on light microscope.

## 1. INTRODUCTION

Pollen grains are fine powdery material formed by the anthers of seed plants. During nectar collection from flowers, bees visited number of flowers and get some quantity of pollen with them. Pollen either adheres to the "hairy" legs and body of bees while crawling over flowers or removed from an anther by them using tongue and mandibles. The bees' combs pollen from her body, head, and forward appendages, collected and mixed with salivary glands secretion or nectar before placing in specific baskets known as "corbicula" that is located at their tibia (hind legs), and finally transferred to the beehive (Von Der Ohe *et al.*2004; Paray *et al.*, 2020). As pollen s are packed into the comb, they are supplemented with phytocidal acid to prevent bacterial growth and delay pollen germination. To prevent anaerobic metabolism and fermentation other enzymes produced by worker bees are also added for enhancing longevity of the stored pollen (Sajwani, *et al.*, 2014) . The pollen comb is referred to as "bee bread" when it is completely processed for storage, (Pospiech *et al.*, 2019 & 2021). After the nectar has converted into honey some of the pollen

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remains in honey and served as blueprint of botanical origin of the honey. Airborne pollen is also a source of pollen in honey. When ripened honey is being removed from a hive by beekeeper sometimes airborne pollen is deposited into it (Jones & Bryant, 2014).

## 2. MATERIALS AND METHODS

***Sample collection***

A total of 50 samples (fresh and branded) were collected from the different districts of Punjab, Pakistan, including: Jhang, Faisalabad, Kahnewal, Gujranwala, Bahwalpur, Toba tek singh and Muzaffargarh district. Branded honeys were collected from the local markets of Punjab, Pakistan. Honey brands were including: Marahaba, Salman Pak honey, Al-Shifa, Ubqari, Muqeet, Sary, Langanase, Youngs honey, Ponam, Swat honey and Aftab Qarshi. All honeys were subjected to the pollen analysis.

***Method***

Melissopalynological studies in present research was carried out according to the method that was recommended by International Commission for Bee Botany of IUBS and (Louveaux *et al.*, 1978; Ullah *et al.*, 2019).

***Procedure***

Five (5) g of honey was weighed out and dissolved in 10ml hot (not above 40°C) water (distilled water or clean tap water) in a beaker. This honey solution was transferred to the falcon tubes of 15ml capacity. Honey solution was centrifuged for 10 min (2500 r/min) and the supernatant liquid was decanted with the help of pipette. Honey sediment was distributed again with 10 ml of distilled water to eliminate sugars and centrifuged for 5 min. This step was repeated until a clear supernatant appeared; the honeys rich in colloidal matter were initially centrifuged with distilled water thrice. Then the pellets were centrifuged and washed with dilute Sulphuric acid followed by potassium hydroxide (5 g $H_2SO_4$ or 100 KOH to 1 liter of water). The sediment was washed with distilled water twice to remove remaining chemicals.

***Preparation of slides***

After washing the honey sediment was put over a slide and spread over it with the help of a micropipette of 10-100 µl capacity. After drying, the sediment was mounted with stained glycerin gelatin. For the preparation of glycerin jelly 0.48g of gelatin was dissolved with 25.5 ml of distilled water, 30ml pure glycerin was added to the mixture to prevent excess dehydration, followed by 0.6ml of phenol to prevent microbial decomposition. Safranin stain was added to this mixture to make the pollens more visible. About 2-3 drops of this glycerin-gelatin mixture were placed in the center of slide and a cover slip was placed over the slide in a way to prevent air bubble formation.

***Performance of microscopic analysis***

The determination of identification and counting of pollen grains in honey is a base to determine the geographical origin. Identification was made by comparing pollen grains with the reference to the literature. Microscopic examination was carried out at 40X and 100X magnification. An imaging software named "TCapture" was used to capture the micrographs from microscope.

**Pollen counting**

For pollen counting, heamocytometer was used. About 10 µl of the sediment was transferred to the haemocytometer, and it was allowed to charge. After these pollens were counted in the squares at 10X magnification of microscope. And for the identification of pollens magnification was increased to 40X. Formula of cell counting was applied to the calculated No. of pollens to calculate the No. of pollens per µl of the sample.

The formula is:

$$\textbf{Particles per µl volume} = \frac{(counted\ particles)}{(counted\ surface \times Chamber\ depth)}$$

To calculate the No. of pollens per ml of the sample following formula was applied:

$$\textbf{Particles per ml volume} = \frac{(counted\ particles)}{(No.of\ squares\ counted)} \times 10000$$

## 3. RESULTS AND DISCUSSION

***Poacea***

In branded honeys average pollens of Poacea was higher (15.28%) than fresh honeys that showed 11.17% of this pollen.

***Azadirachta* spp.**
Average %age of *Azadirachta* spp. pollen was greater 12.23% in fresh honeys and a lower value of 9.84% was there in branded honeys.

***Citrus* spp.**
Fresh honeys presented a higher average percentage (12.11%) of this pollen than that of branded honeys having lower (5.35%) percentage of this pollen.

***Pisum* spp.**
*Pisum* spp. had pollens in higher percentage (12.11%) in fresh samples as compared to the branded honeys those consisted of a lower percentage (4.28%) of this pollen type.

***Ziziphus* spp.**
Slightly greater (8.46%) average percentage of this spp. was calculated in fresh honeys as compared to branded honeys which showed 7.84% pollen of this taxa.

***Prosopis* spp.**
An average of 7.13% of *Prosopis* spp. pollens were counted in branded honeys and fresh honeys were having lower (3.95%) percentage than honey brands.

***Brassica* spp.**
An average percentage of the *Brassica* spp. pollens was recorded as 5.7% of branded honeys that was higher than that of fresh honeys which showed 2.8% of this pollen.

**Malvacea**
Pollens belonging to family Malvacea in branded honeys were in higher (4.28%) average percentage as compared to the pollens in fresh honeys those were having 3.76% of total pollens.

***Syzygium* spp.**
Pollens of this taxa showed a moderately higher percentage (4.28%) in branded samples and lower (3.01%) in fresh honeys.

***Cassia* spp.**
*Cassia* spp. showed a higher (4.28%) percentage in branded honeys as compared to fresh honeys those contained 1.88% of this pollen.

***Acacia* spp.**
*Acacia* pollens were having a higher (3.17%) average percentage in fresh honeys and branded honeys were consisting of 2.03% pollens of this taxa.

***Eucalyptus* spp.**
Fresh honeys contained greater (4.3%) average percentage of this pollen than that of branded honeys those were having 3.56% of this pollen.

*Melilotus* spp., *Alfa* spp., *Benincasa* spp. and *Halianthus* spp. were recorded only in branded honeys having 8.56%, 6.4%, 6.4%, and 4.28%, respectively. *Morus* spp., *Moringa* spp., *Psidium* spp., *Bombax* spp. and *Mangifera* spp. were found only in fresh honey samples having a percentage of 8.46%, 4.46%, 4.23%, 1.88% and 1.88%, respectively.

Pollen analysis provides very valuable information and knowledge about the botanical and ecological sources of honey. It presents an idea about the vegetation of the specific area from where the honey is collected. Bahadur *et al.*, (2019); Gul *et al.*, (2021) conducted Palynological characteristics of selected.

Lamioideae taxa and its taxonomic significance. Greater or lesser frequencies of *Citrus* spp. (14.41%), *Eucalyptus* spp. (9%), *Psidium* spp. (15%) and Poacea pollens (<3%) were reported by Sahney *et al.* (2018) in the honeys from Bankura and Paschim Medinipur districts of West Bengal. Mangi *et al.* (2018) studied the shapes, size and number of pollens in honey such as *Ziziphus* spp., *Azdirachta* spp., *Psidium* spp., *Brassica* spp. and *Acacia* spp. in natural honeys of district Dadu, Sindh Pakistan.

Adekanmbi and Alebiosu (2018) identified pollens of native flora from Nigeria only. Pollens of *Syzigium* spp. (3.01%) and (4.28%), *Brassica* spp. (2.87%) and (5.7%), *Eucalyptus* spp. (4.35%) and (3.56%), *Prosopis* spp. (4%) and (7.13%), *Acacia* spp. (3.17%) and (2%), *Mangifera* spp. (1.9%), *Bombax* spp. (1.9%) and *Moringa* spp. (4.46%) in fresh and branded honeys, respectively were found in the study of honey pollens of Punjab, Pakistan closer to the findings of Chauhan *et al.* (2017) in his work with Indian honeys, frequencies of *Syzigium* spp. pollens (3-15%) in the New Hyderabad (Lucknow ) honeys, *Bombax* spp. pollens (<3%) in the honey of Jhansi, pollens of

*Brassica* spp. (<3%) in the New Hyderabad (Lucknow), Ashakhera and Jhansi honeys, *Eucalyptus* spp. pollens (3-15%) in the honeys of Girar, Bahraich, Ashakhera, New Hyderabad (Lucknow) and Malihabadand districts, pollens of *Prosopis* spp. (3-15%) in the honeys of Ashakhera district, *Bombax* spp. pollens (<3%) in the Jhansi and Trilokpur honeys, pollens of *Acacia* spp. (3-15%) in the Trilokpur and Mallawan district's honeys, *Mangifera* spp. pollens (<3%) in the honeys of Jahnsi, *Moringa* spp. pollens (3-15%) in the honeys of New Hyderabad (Lucknow) made this study closer to the current finding.

Pollen types in the current study were having following frequencies: *Syzygium* spp. 3.01% and 4.28%, *Brassica* spp. 2.87% and 5.7%, *Ziziphus* spp. 8.46% and 5.53%, *Cassia* spp. 1.9% and 4.3%, *Acacia* spp. 3.17% and 2% in fresh and branded honeys, respectively. These frequencies were closer to the (*Syzygium* spp. 2.26% in Kakragaon 3.78% in Chapaguri, 3.64% in Bidyapur district 3.18% in Chatibargaon, 3.78% in Chapaguri and 4.60% in Sidli, *Ziziphus* spp. 4.78% in Bidyapur, 6.57% in Basugaon, 6.78% in Bidyapur, 4.50% in Chapaguri and 10.43% in Dalogaon *Cassia* spp. 1.78% in Chalekati and 4.50% in Sidli, *Acacia* spp. 1.87% in Basugaon and 1.67% in Chapaguri and 1.32% in Sidli honeys) frequencies of pollens calculated by Tripathi *et al.* (2017) in the honeys collected from different regions of northeast India (Bongaigaon district of Assam).

## 4. CONCLUSION

This study contributes valuable insights into the diverse bee flora present in Punjab, Pakistan, as evidenced by the pollen analysis of honey samples from different regions. The distinct pollen profiles in natural and branded honeys highlight the potential regional variations in floral sources, providing a foundation for future research and quality monitoring in the apicultural industry.

## 5. CONFLICT OF INTEREST

All authors have declared that there is no conflict of interests regarding the publication of this article.

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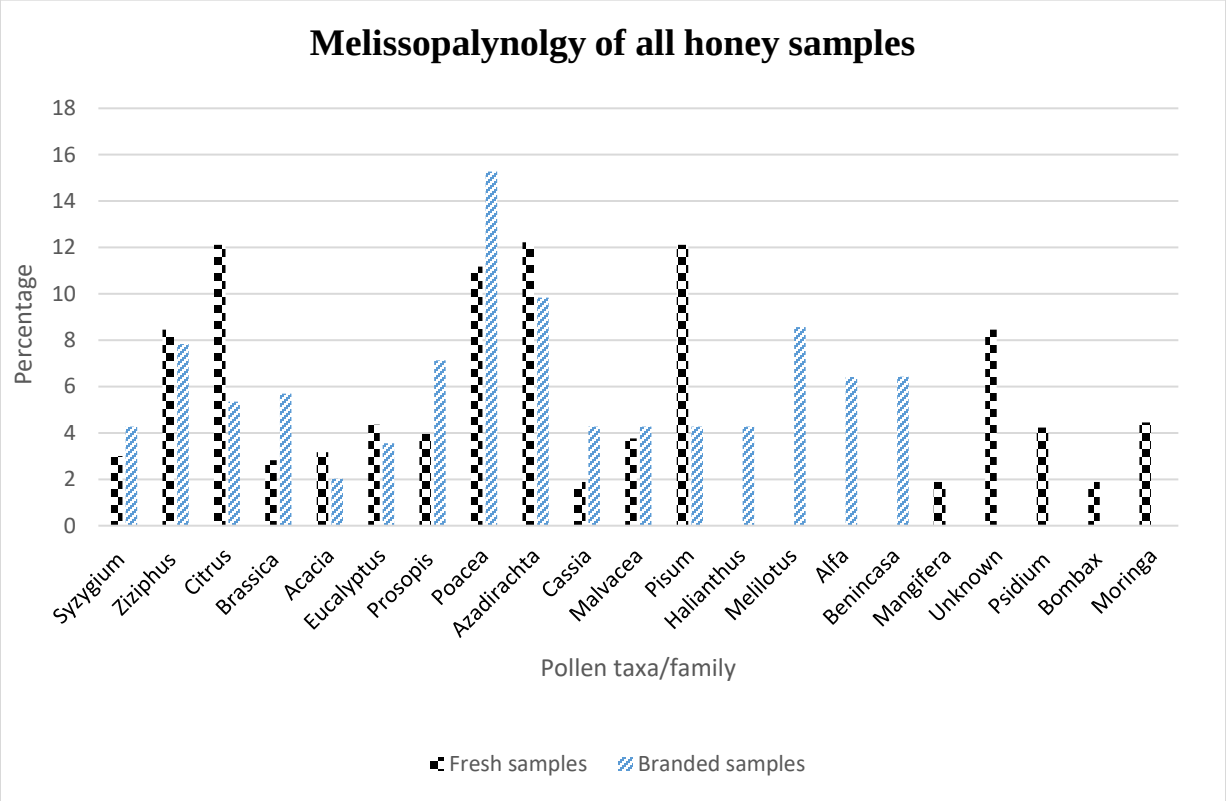
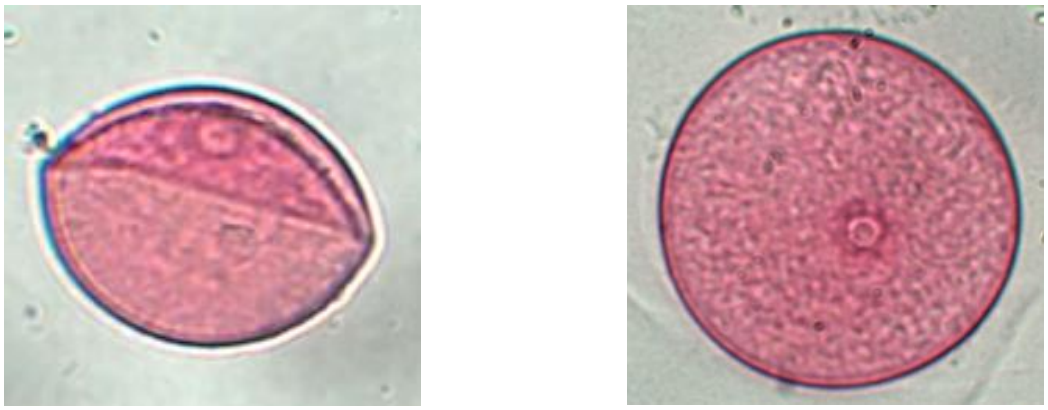
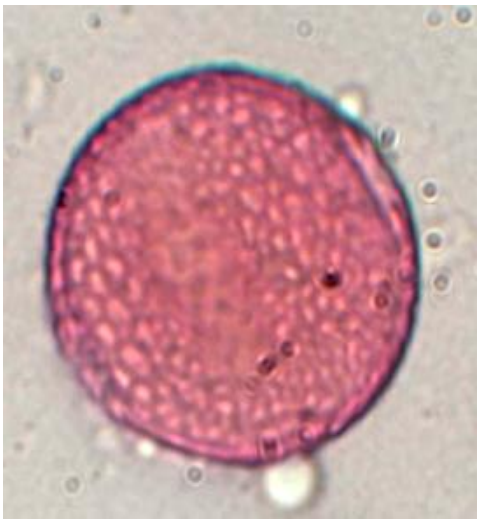


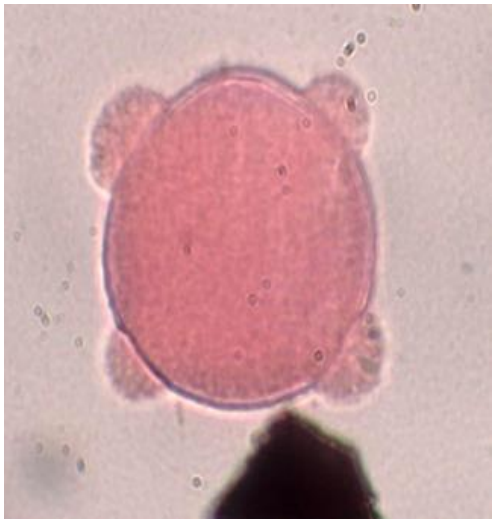

Fig. 1: Percentage of pollen taxa/family in fresh and branded honeys



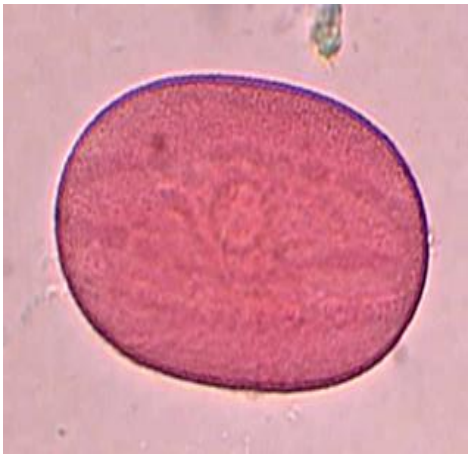
*Pollens of Poacea*



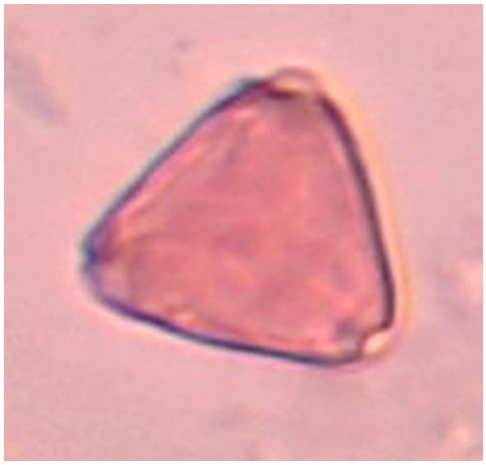
*Azadirachta spp.*



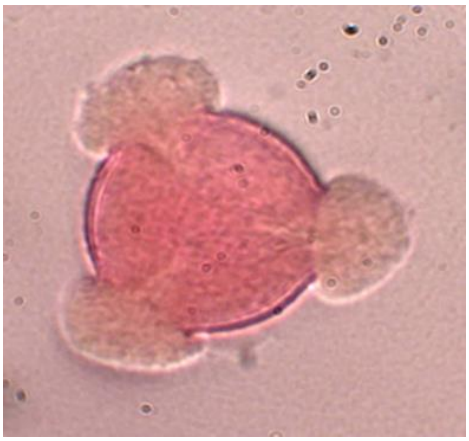
*Citrus spp.*



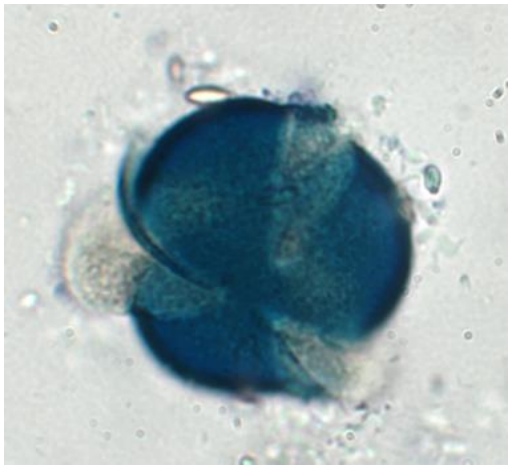
*Pisum spp.*



*Ziziphus spp.*

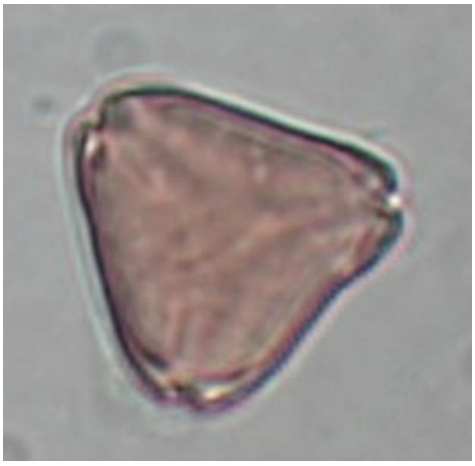


*Prosopis spp.*

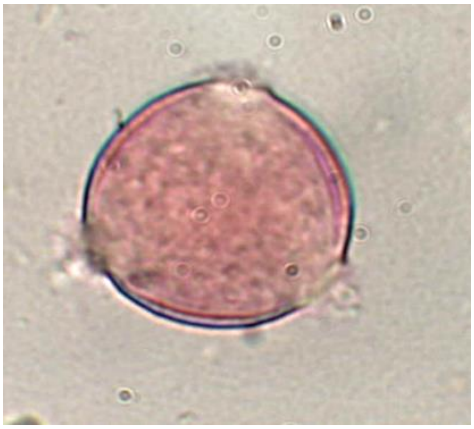


*Brassica spp.*

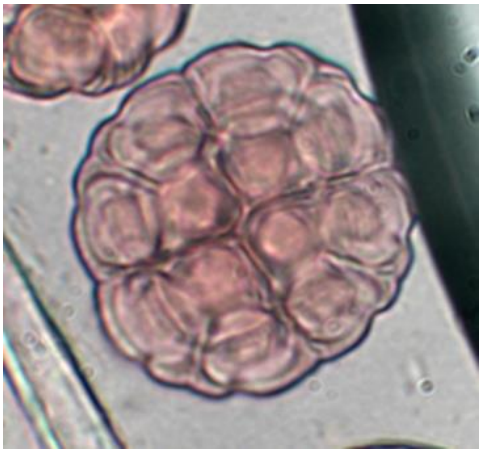
*Malvacea*



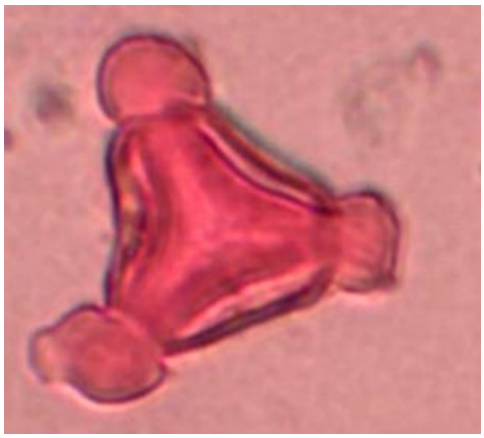
*Syzygium spp.*



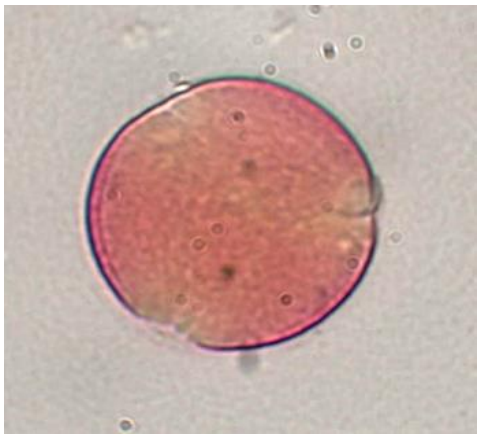
*Cassia spp.*



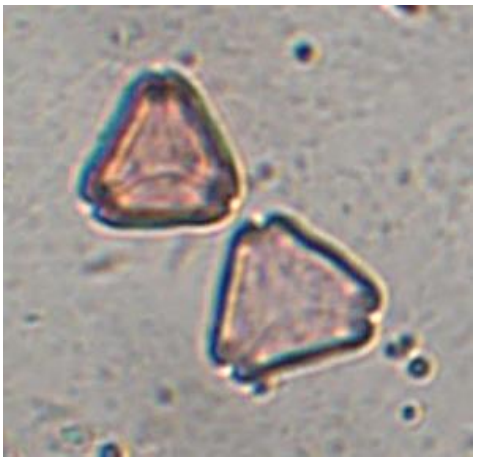
*Accacia spp.*



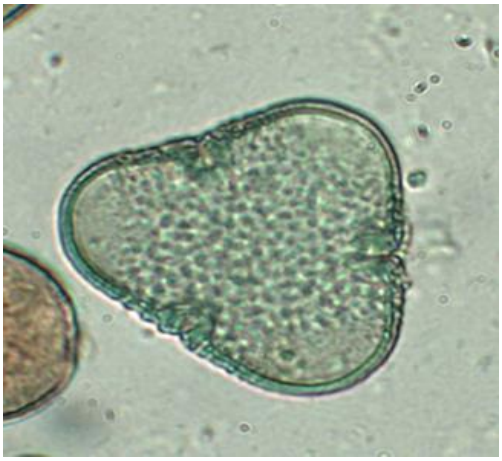
*Eucalyptus spp.*



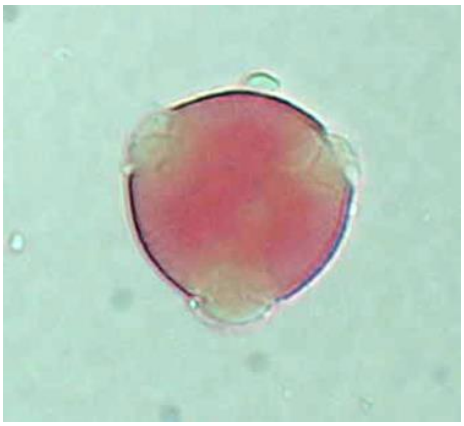
*Moringa spp.*



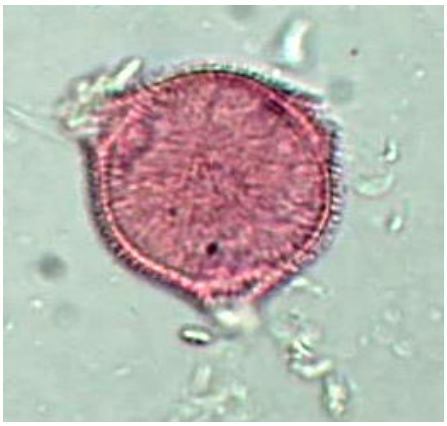
*Psidium spp.*



*Bombax spp.*



*Mangifera spp.*



*Alfa spp.*