Melissopalynology study from Anuppur district, Madhya Pradesh

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Abstract- **Melissopalynology, the study of pollen grains in honey, can reveal valuable information about the pollen and nectar sources that bees use to produce honey. This information can be used to determine the geographical and botanical origin of the honey. the aim of the presentinvestigation was to identify the plant taxa consumed by bees during nectar collection and incorporated into winter honey samples. The honey samples were collected from wild areas in Anuppur District, Madhya Pradesh of Central India, to identify the important source plants in the region. Pollen-analytical examinations were conducted on five honey samples collected from different localities of Anuppur.**
Analysis of 05 squeezed honey samples revealed a diversity of 20 pollen morphotypes belonging to 15 different plant families were identified. The palynoassemblage of most honey samples mainly consist of pollen from summer blooming plants such as *Syzigium*, *Schleichera*, *Terminalia*, *Lannea*, *Lagerstroemia*, and Anacardiaceae, indicating the occurrence of a tropical moist deciduous forest in the region with high monsoonal conditions (core monsoon zone).

Keywords: Honey, melissopalynology, pollen, bee foraging.

INTRODUCTION

Honey, popularly known as golden liquid is a natural sweetener that is produced by honey bees by collecting nectar from flowers. It has generated employment to many families and can be used in innumerable edible products. From centuries, honey has been consumed as one of the oldest foods of mankind (Zumla and Lulat, 1990). Honey is a natural product of very high nutritive value which is made when the nectar (floral) and sweet deposits from plants (non-floral) are gathered, modified and stored in the honeycombs by honeybees of the genera *Apis* and *Meliponini* (Manyi-Loh *et al*., 2011).

Pollen grains are essential food for honey bees as they are source of proteins, vitamins, minerals and fatty substances (Gary, 1975). They are an important tool in analyzing honey. For identifying floral nectar sources utilized by honey bees to produce honey, different types of pollens are used. This helps in labelling of major and minor nectar sources of honey sample (Sadia Bibi *et al*., 2008). For understanding bee foraging preferences of honey bees, it is essentially important to have the basic understanding of the local flora. Most easy and simplest method widely followed by majority of botanists to do so is by performing melissoplaynological studies of the honey samples collected from the desired area.

Qualitative and quantitative analysis of pollen content of honey is melissopalynology (= melittopalynology). This term comes from the Greek words for “bee” and “honey” along with the word for “study of dust,” now referred to “pollen”. Such studies are beneficial in determination of the floral content and origin of honey samples present in a particular geographical area during different seasons (Parades *et al*., 2020). Today, it is recognized worldwide as being the least expensive and quickest way to determine the pollen content of honey. Concentration of representative floral pollen in honey is dependent upon several factors such as structure of the flower, size and shape of the pollen grain and how long it spends in the bee’s honey stomach. Melissopalynological studies are used to verify honey types produced from floral sources that are “under represented” or “over represented” in the relative pollen counts of the honey samples. Verification of preferred (premium) types of honey is often difficult because many of them come from plant sources that are either weak pollen producers or have pollen that is under-represented in honey (Bryant & Jones, 2001).

Apis dorsata, *A. cerana* and *A. mellifera* are the major bee species largely responsible for nectar collection in India (M.S. Chauhan *et al*. 2017), though *Apis cerena indica* is most common and utilized in bee keeping apiaries. Study of pollen grains in honey samples helps in identifying the purity of honey. Presence or absence of different pollen types helps in identifying the main plant sources for nectar which in turn can help the native people to encourage apiculture at a larger scale and thereby generating employment opportunities to locals.

During the last few decades, analysis of pollen using honey samples have been reported from districts of Lucknow (Chaturvedi, 2009, Chauhan *et al*., 2013), Western Himalayan Region of Uttar Pradesh (Chaturvedi, 2004), Adikmet area, Hyderabad (Kalpana *et al*., 1990), Prakasam, Andhra Pradesh (Jhansi *et al*.,1991,1994), South Indian Western Ghats (Balachandra, 1999), North Eastern hill region (Singh, 1999), Karnataka (Sivaram ,2001), Maharashtra (Bhusari, 2005), Upper Gangetic Region of India (Datta *et al.*, 2008), Nilgiri Biosphere (Sivaram, 2012), Nadia, West Bengal (Bhattacharya, 2014), Tropical South India (Ponnuchanney *et al.*, 2014), Chandrapur, Maharashtra (Lakshmikant *et al.*, 2014, Borkar *et al.*, 2016), Varanasi, Uttar Pradesh (Sahney*et al.*, 2016), Vindhya Pratishthan Campus, Baramati, Pune (Harugade*et al.*, 2016), Chandrapur District, Maharashtra (Borkar and Mate., 2013), Assam (Tripathi, 2017), Kangra, Himachal Pradesh (Saklani, 2017), Garhwal Himalaya, Uttarakhand (Chaudhary *et al.*, 2018), Newasa tehsil, Maharashtra (Dhawan *et al.*, 2018), Nilambar Taluk, Malappuram, Kerala (Divakaran*et al.*, 2019), Dakshina Kannada Karnataka (Krishna and Patil, 2019), Paderu Vishakhapatnam Andhra Pradesh (Devender *et al.*, 2020) and Eastern dry zone Karnataka (Kumar, 2020). In the present work an attempt has been made to sum up the melissopalynological studies by different investigators in different places of India.

From centuries, honey has been consumed as one of the oldest foods of mankind and there have been many references to it (Zumla & Lulat, 1990). Manyi-Loh et al., 2011 reported in a study that honey is a natural product of very high nutritive value which is made when the nectar (floral) and sweet deposits from plants (non floral) are gathered, modified and stored in the honeycombs by honeybees of the genera *Apis* and *Meliponini*.

In India, honey is used in Ayurvedic treatment for almost 4,000 years. Honey is used for the treatment of eyes for the improvement eyesight, weight loss, for the treatment of pathological disorders like asthma, diarrhoea, and vomiting. Honey can reach up to the smallest blood vessel of the body and so is referred as "yogavahi" in Ayurveda (Shastri, 2001). Honey carries the effects of the drugs added to it, without changing its own properties. It means it acts as a bioenhancer that increases the herbal properties and effects of the substances with which it combines (Ram et al., 2019). Also it has been emphasized that the use of honey is highly beneficial in the treatment of irritating cough. Honey is regarded by Ayurvedic experts, as valuable in keeping the teeth and gums healthy. As mentioned in Charaka Samhita, honey is of four different types: Makshika, Bhramara, Kshaudra and Paittaka. Makshika is considered to be the best type of honey. It is produced by *Apisflorea*. According to Susrutha Samhita, honey is of eight types: Pauttika, Bhramara, Ksaudra, Makshika, Chatra, Arghya, Auddalaka and Dala Madhu (Ediriweera and Premarathna, 2012).

In Islamic medical system, honey is considered a healthy drink. The holy Qur'an illustrates the potential therapeutic value of honey. Avicenna, the great Iranian scientist and physician, almost 1000 years ago, had recommended honey as one of best remedies in the treatment of tuberculosis (Asadi et al., 2003).

Honey was the most popular Egyptian drug being mentioned 500 times in 900 remedies (Al Jabri, 2005). Medicines in Egypt contained honey together with wine and milk. People in Egypt offered honey to their deities as a sacrifice (Eteraf-Oskouei T et al., 2013). They also used honey for embalming the dead. Honey was utilized for its antibacterial properties that helped heal infected wounds. Moreover, honey was used as a topical ointment.

Oenomel is an ancient Greek beverage consisting of honey and unfermented grape juice. It is sometimes used as a folk remedy for gout and certain nervous disorders (Eteraf-Oskouei T et al, 2013). Hippocrates, the great Greek scientist, prescribed a simple diet, favouring honey given as oxymel (vinegar and honey) for pain, hydromel (water and honey) for thirst, and a mixture of honey, water and various medicinal substances for acute fevers (Zumla & Lulat, 1989).

The role of honey has been acknowledged in the scientific literature and there is convincing evidence in support of its antioxidant and antibacterial nature, cough prevention, fertility and wound healing properties. Several research groups, after laboratory and clinical investigations, have reported honey as an antioxidant, anti-inflammatory, anti-bacterial agent that augments the adherence of skin grafts and wound healing process. (Eteraf-Oskouei et al., 2013, S.A.Meo et al., 2017). It has been used as ointment for wounds and skin infections. It has anti-inflammatory, immune boosting property, and exhibits broad spectrum antibacterial activity, owing to factors such as acidity, osmolarity, hydrogen peroxide, volatiles, beeswax, nectar, pollen and propolis. Its antioxidant activity is attributed to glucose oxidase, catalase, ascorbic acid, flavonoids, phenolic acids, carotenoid derivatives, organic acids, Maillard reaction products, amino acids, and proteins (Abeshu MA, et al., 2016). Honey prevents and treats gastrointestinal disorders such as peptic ulcers, gastritis and gastroenteritis. It also promotes health of gastrointestinal tract because of its probiotic activity.

Use of honey for medicinal purpose has been contentiously discussed and has not been very well accepted in the modern medicine due to lack of authentic scientific reports. Therapeutic potential of uncontaminated honey is grossly underutilized. It is easy to find in most populations and some of its properties remains dubious and needs further investigation.

Methodology:

Pollen-analytical examinations were made for five squeezed honey samples procured from different localities of Anuppur district, Madhya Pradesh (Central India) to ascertain the plant taxa which were incorporated into honey during nectar consumption by the bees.Quantitative pollen evaluation was based on the method recommended by the International Commission for Bee Botany (Louveaux et al., 1978). Pollen counts were taken at random, covering the maximum mounted area to avoid repetition.After the precise identification and counting, the pollen grains were placed into one of following pollen frequency classes as predominant pollen types (> 45%), secondary pollen types (16-44%), important minor pollen types (3-15%), minor pollen types (< 3%) and pollen present (< 1%). Honey samples consisting of more than 45% of single pollen taxa were considered as 'unifloral' honey. However, the honey sample originated from more than one floral source and having no predominant pollen type is considered to be 'multifloral' honey. An elaborated list including sample number, locality, nature and type of honey, collected season and frequency of pollen types recovered areprovided in Table 1.

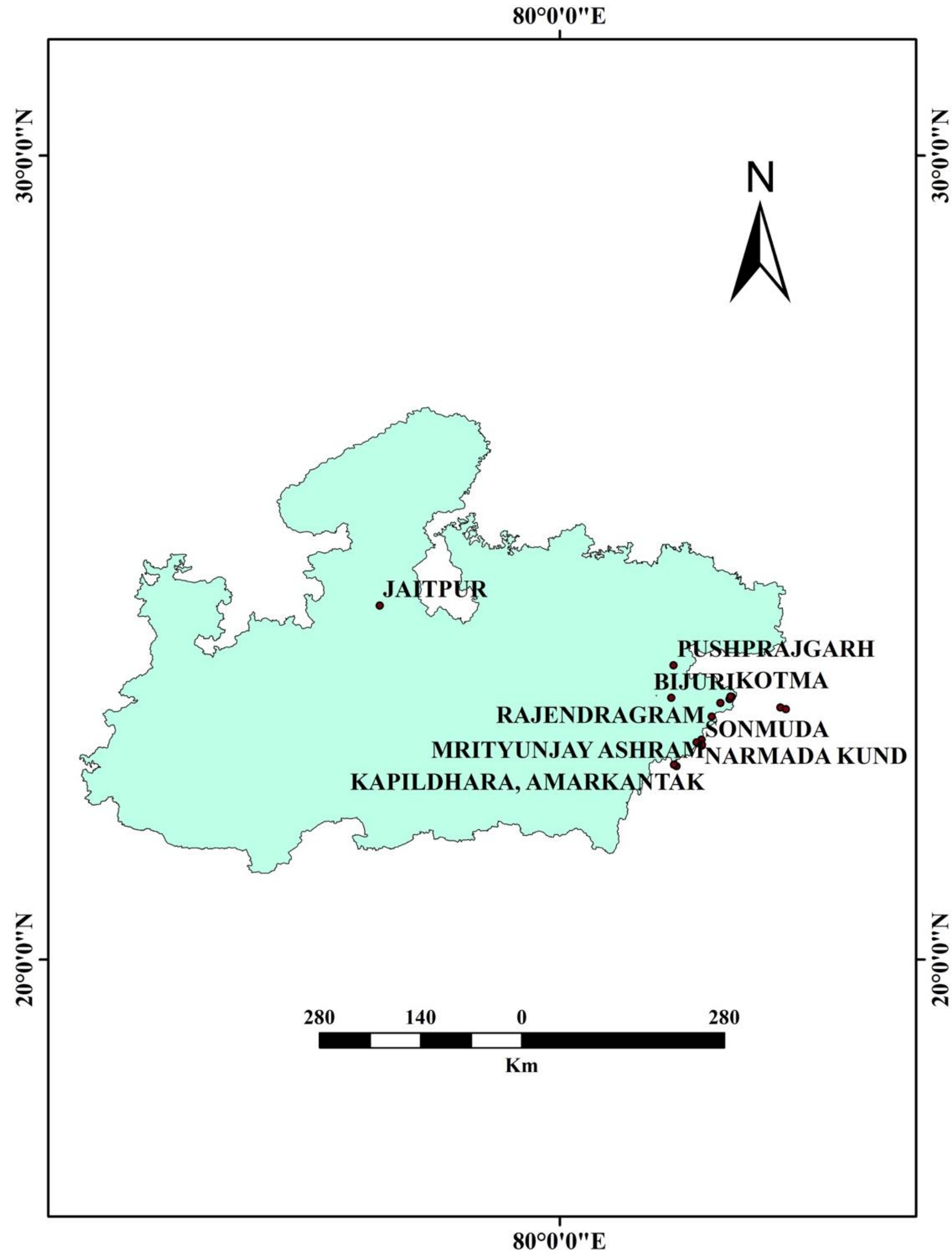
Table 1: Collection details of honey samples from selected localities from Anuppur district in Madhya Pradesh and timeline of data.

Honey samples	Date	Collection site	Latitude	Longitude
H1	22 October 2019 to 28 October 2019	Bijuri (h1b)	23.2501813 e	82.1223827 n
		Kotma (h1k)	82.1387 e	23.2687 n
		Sheetal dhara coal mines (h1sd)	23.239816 e	82.112069 n
H2	14 August 2020 to 31 August 2020	Rajnagar ro (h2r)	23.0205 e	81.8906 n
		Rajendragram (h2rg)	23.25507 e	81.38836 n
		Jaitpur (h2j)	24.40397 e	77.76065 n
		Kurja temple (h2k)	82.112069 e	23.23986 n

H3	09 November 2020 to 16 November 2020	Narmada kund (h3n)	81.7595 e	22.6724 n
		Sonmuda (h3s)	81.7650 e	22.6601 n
		Jwaleshwar temple (h3j)	81.7627 e	22.7355 n
		Mrityunjay ashram (h3m)	81.4529 e	22.4036 n
		Kapildhara, amarkantak (h3k)	81.4219 e	22.422 n
H4	25 March 2021 to 31 March 2021	Kapildhara colliery (h4kc)	82.1245700 e	23.2707100 n
		Kewai river (h4k)	23.1907 n	81.9985 e
		Ravi nagar (h4r)	23.1139 n	82.811 e
		Dola, bijuri (h4d)	82.742 e	23.136 n
H5	15 June 2021 to 25 June 2021	Amarkantak forest nursery (h5a)	22.6749 e	81.7591 n
		Dudhdhara waterfall (h5d)	81.7025 e	22.7023 n
		Pushprajgarh (h5p)	23.65922 e	81.41525732 n
		Narmada maakibagia (h5n)	81.7702 e	22.6687 n

Light Microscopy and Scanning Electron Microscopy Study of Pollen Grains from Honey:

1. Sample Preparation: Collected honey samples from different locations werestored in sterilized containers at room temperature. The honey samples were centrifuged at 3000 rpm for 10 minutes to separate the pollen grains from the honey. The sediment is washed with distilled water to remove any remaining honey and other impurities to separate the pollen grains. pollen grains dried at room temperature or desiccator were used for observation.
2. Light Microscopy: Examined the dried pollen grainsmounted in glycerine under light microscope at 400x, 1000x, and 2000x magnifications to identify and classify the pollen grains according to their morphological features using standard pollen identification keys (Louveaux et al., 1978; Erdtman, 1960).
3. Scanning Electron Microscopy (SEM): dried pollen grains were placed on a carbon adhesive tab and mounted on an SEM stub and sputter coated with gold or gold-palladium alloy. The samples were observed under a SEM at various magnifications (100x-5000x) to obtain high-resolution images of the pollen surface structure. The pollen surface features such as exine ornamentation, shape, size, and aperture was analyzedusing software such as ImageJ/SEMrush (Punt et al., 2007).
4. Data Analysis: The observations and images obtained from the light microscopy and SEM analyses were recorded.The data wereanalysedto determine the frequency and relative abundance of different pollen types present in the honey samples. Using statistical software such as SPSS or R the data wereanalysed to draw conclusions about the pollen sources and botanical origin of the honey.



Results

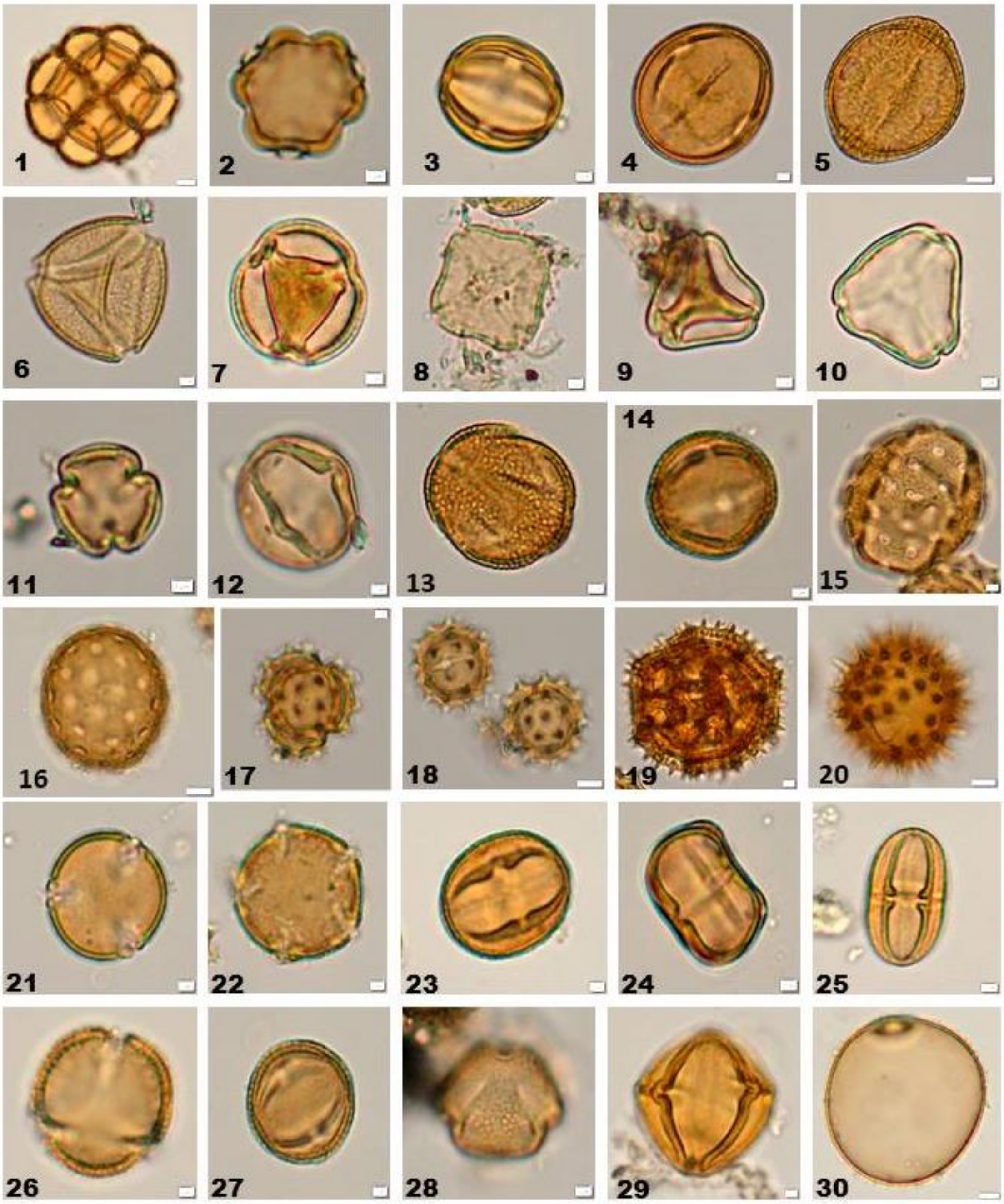
More than 20 pollen morphotypes belonging to 15 different plant families were identified and counted. In most cases identification was made down to the family and generic level and in some up to species level based on the precision in the pollen morphology(Shukla et al., 2019).

The sample number H-2 and H-5 consist of least number of pollen morphotypes, whereas; H-1 contains the maximum number of pollen morphotypes. The pollen analytical data of each honey samples(H-1 to H-5) are discussed below according to their abundance in the samples. The analytical examination of each honey sample provided detailed information on the pollen types present, and data are arranged in descending order of their frequency class with the predominant pollen type mentioned first, followed by secondary and minor pollen types.

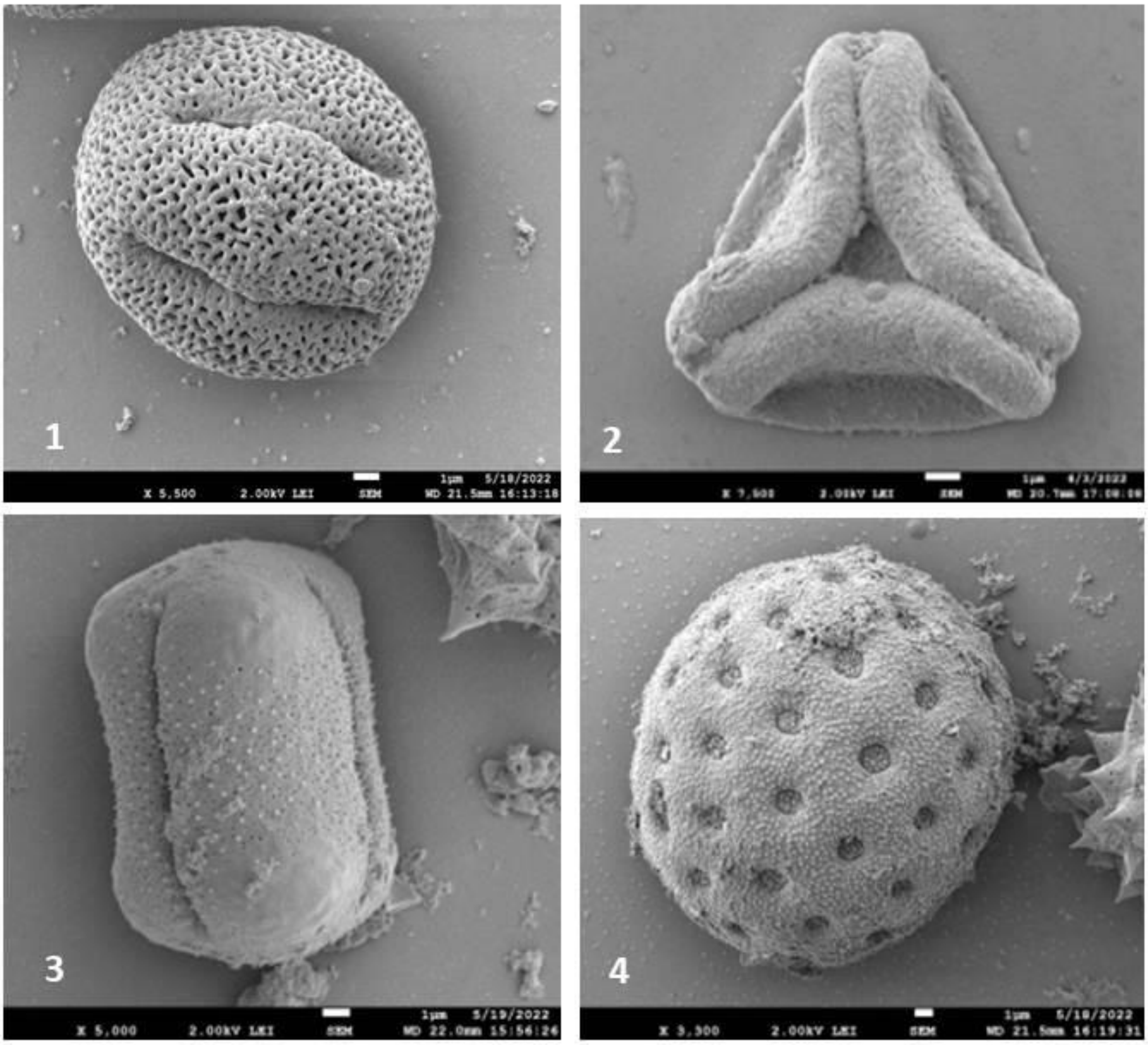
H-1: - The sample procured is palynologically productive and proved to be unifloral as evident by the presence of single predominant pollen morphotype of subfamilyAsteroideae (56.35%), followed by the occurrence of Poaceae (7.23%), Solanaceae (6.83%), *Terminalia* (6.12%),*Coriandrum*(6.05%),Euphorbiaceae(4.22%)and Chenopodiaceae(3.67%)as important minor pollen types, whereas *Brassica* (2.30%), Malvaceae (1.34%), Anacardiaceae (1.22%) and Cichorioideae (1.05%) are observed as minor pollen types. The pollen taxa like, *Acacia*, *Myriophyllum*, *Aeglemarmelos*, *Mitragyna*, *Emblica* and *Shorearobusta* are also scantly found in the palynoassemblage in the category of pollen present.

H-2:- The samples acquired is less-productive and documented to be multifloral as evident by the presence of two secondary pollen types i.e.,*Lanneacoromandelica*(40.85%), andAnacardiaceae(35.22%)and deficit of any predominant pollen type. Important minor pollen types are represented by *Schleichera*(11.22%), *and Syzygium*(9.50%). The minor pollen type is represented by only single morphotype of Bignoniaceae (3.21%).

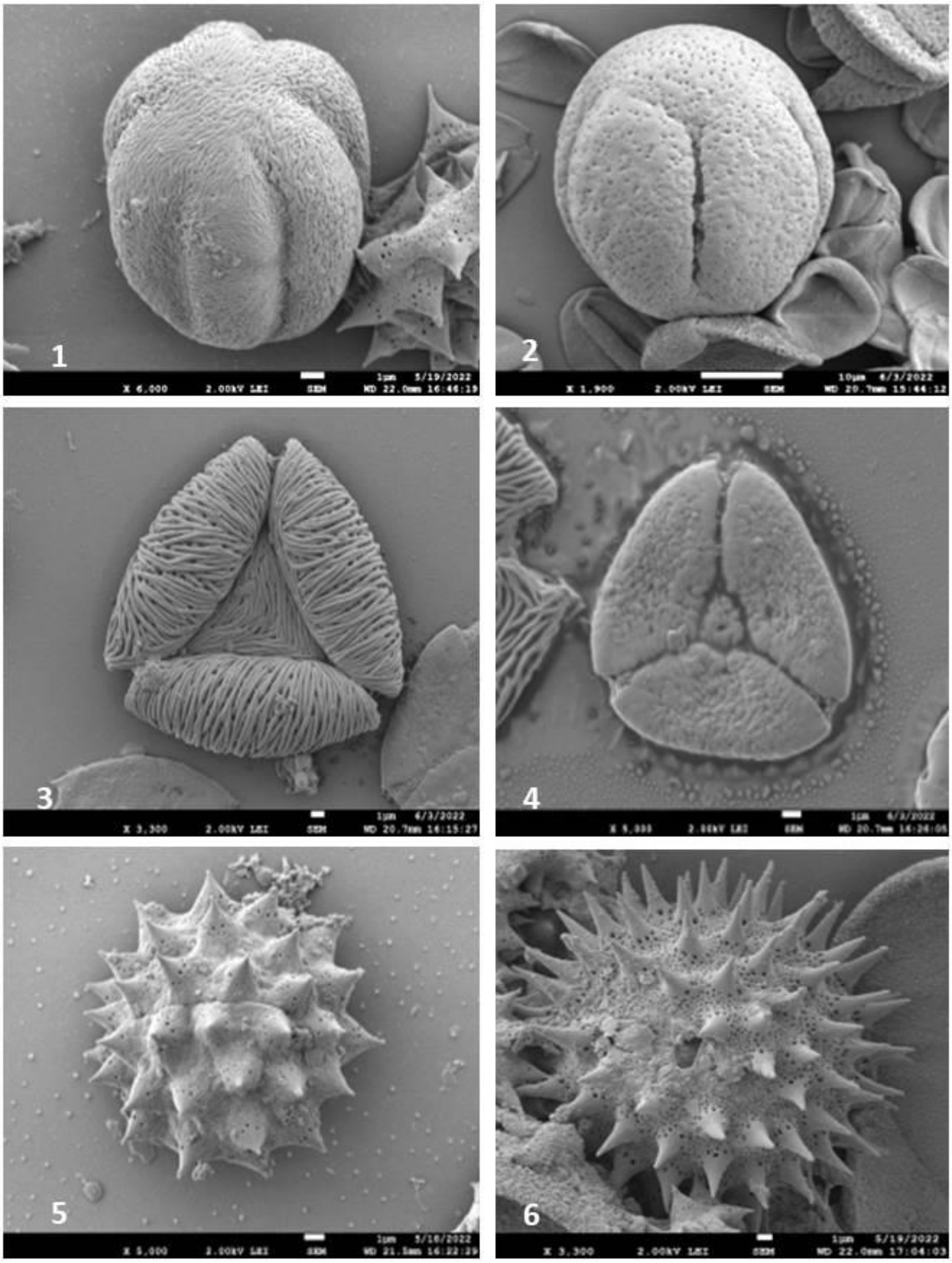
H-3: - The sample procured is productive and proved to be unifloral as evident by the presence of predominant pollen taxa *Syzygium*(49.94%),followed by the presence of *Schleichera*(20.51%) as secondary pollen type**.** The remaining taxa like Solanaceae(8.21%), *Buchanania (7.42%)*,*Terminalia* (5.55%), *and* Acanthaceae *(Justicia)* (3.40%)were markedas important minor pollen types. The minor pollen types are represented by Euphorbiaceae (2.85%), and *Bauhinia* (2.12%).
H4:- The sample acquired is palynologically productive and documented to be unifloral as evident by the presence of single predominant taxa i.e., *Syzygium*(45.42%), followed by the presence of by *Schleichera* (19.05%)and *Cassia* (16.33%) as secondary pollen types. The other tree taxa like *Lagerstroemia (*6.53%), Sapotaceae(5.18%) and *Bauhinia* (4.77%) are represented as important minor pollen types. The minor pollen types are represented by Poaceae (1.59%) and *Barringtonia* (1.13%).
H5: - The sample procured is less-productive and proved to be multifloral as evident by the presence of two secondary pollen types i.e., *Syzygium* (36.67%)andSolanaceae (25.09%) with lack of any predominant pollen type, followed by *Terminalia* (14.42%), Asteroideae (10.23%) and *Lagerstroemia* (9.21%) as important minor pollen types. The minor pollen types are represented by Anacardiaceae (2.78%), whereas,Sapotaceae has been scantly observed in the palynoassemblage.



LM micrographs: 1. *Acasia*, 2&3. *Terminalia*, 4. *Sapotaceae*, 5. *Lagerstomia*, 6. *Schlecheira*, 7. Myrtaceae, 8. Syzigium. 9. Ziziphus, 10.Myrtaceae, 11&12. Fabaceae, 13. Convolvulaceae, 14. *Aegle* sp. 15 & 16. Chenopodiaceae/Amaranthaceae 17 & 18. Asteroideae, 19. Cichorioidea, 20. Sid asp., 21. Unidentified, 22. *Microphyllum*, 23. Euphorbiaceae, 24. Pisum sp., 25. Coriandrum, 26-28. Brassica., 29. Solanaceae, 30 Poaceae (Non- cereal)



FESEM micrographs: 1. Equatorial view of Bauhinia, 2. Ppolar view of Ziziphus, 3. Equatorial view of Coriandrum, 4. Chenopodiaceae pollen.



FESEM micrographs: 1. Polar View of Terminalia, 2. Equatorial view of Terminalia. 3. Polar view of *Syzigium*, 4. Polar View of *Scleichera*, 5 & 6. *Asteroideaea* (echinate) pollen

Table 2: Category wise categorisation of pollens observed in the 5 samples

S. No.	Sample code	Nature of Honey	Type of Honey	Season collected	Predominant pollen type (>45%)	Secondary pollen type (16-44%)	Important Minor pollen type (3-15%)	Minor pollen type (1 to <3%)	Pollen present (0.5 to <1%)
1	**H1**	Unifloral	Squeezed		Asteroideae (56.35%)		Poaceae (7.23%), *Terminalia* (6.12%), *Coriandrum* (Apiaceae) (6.05%), Solanaceae (6.83%),	*Brassica* (2.30%), Cichorioideae (1.05%), Malvaceae (1.34%), Anacardiaceae (1.22%)	*Acacia* (1.02%), *Myriophyllum* (0.5%), *Aegle marmelos* (0.6%), *Mitragyna* (0.8%),

							Euphorbiaceae (4.22%), Chenopodiaceae (3.67%)		*Shorea robusta* (0.7%)
2	**H2**	Multifloral	Squeezed			*Lannea coromandelica* (40.85%), Anacardiaceae (35.22%)	*Schleichera* (11.22%), *Syzygium* (9.50%)	Bignoniaceae (3.21%)	
3	**H3**	Unifloral	Squeezed		*Syzygium* (49.94%)	*Schleichera* (20.51%)	Solanaceae (8.21%), *Terminalia* (5.55%), Acanthaceae (*Justicia*) (3.40%), Rosaceae (7.42%)	Euphorbiaceae (2.85%), *Bauhinia* (2.12%)	
4	**H4**	Unifloral	Squeezed		*Syzygium* (45.42%)	*Schleichera* (19.05%), *Cassia* (16.33%)	*Lagerstroemia* (6.53%), Sapotaceae (5.18%), *Bahuhinia* (4.77%)	Poaceae (1.59%), *Barringtonia* (1.13%)	
5	**H5**	Multifloral	Squeezed			*Syzygium* (36.67%), Solanaceae (25.09%)	*Terminalia* (14.42%), Asteroideae (10.23%), *Lagerstroemia* (9.21%)	Anacardiaceae (2.78%)	Sapotaceae (1.6%)

Discussion:

The number of pollen morphotypes present in each honey sample varied, with sample H-1 having the maximum number of pollen morphotypes, while H-2 and H-5 had the least. The pollen analytical data of each honey sample (H-1 to H-5) were discussed in detail according to their abundance in the samples. The study also identified whether the honey samples were unifloral or multifloral. The results showed that samples H-1, H-3, and H-4 were unifloral, while H-2 and H-5 were multifloral.The study also identified some of the scantly found pollen types present in the samples. The palynoassemblage of most honey samples mainly consisted of pollen from summer blooming tree taxa such as *Syzygium*, *Schleichera*, *Terminalia*, *Lannea*, *Lagerstroemia*, and Ancardiaceae, indicating the occurrence of a tropical moist deciduous forest in the region with high monsoonal conditions (core monsoon zone). The dominant occurrence of core arboreal taxa in these honey samples suggests that they originated and sourced from the forest-stand.

During the honey-pollen investigation, the discovery of a predominant pollen type (Asteroideae; 56.35%) and minor pollen types (Cichorioideae) of the Asteraceae family in a solitary honey sample (H-1) is significant. The Asteraceae family is cosmopolitan and triggers various allergenic diseases, especially those related to the respiratory system, in a wide range of the population. This discovery highlights the need to investigate the allergenicity of different pollen grains found in the area of investigation that may cause asthma, hay fever, dermatitis, rhinoconjunctivitis and other allergic disorders. Knowledge of the triggers of allergic diseases is essential to avoid, control, and minimize symptoms(Altungolu et al., 2010; Guvensen et al., 2005; Tripathi et al., 2018).. The study recommends further investigation of the allergenicity of pollen grains found in the area to identify triggers of allergic diseases. The study willbe helpful in formulating a correct diagnosis, ultimately improving the quality of life for the permanent inhabitants of the study region.

The occurrence of pollen from nectarless plants such as grasses (Poaceae) and Chenopodiaceae indicates that these pollen were trapped in the hive fortuitously by wind or unintentionally transported by honeybees. Regular monitoring of honey samples for toxic pollen and fungal elements is also necessary. Research on potential health benefits of unifloral honey with high core arboreal taxa levels can be conducted, and the study can be expanded to cover a wider area to understand honey distribution and sources. Further studies on environmental factors' impact on honey quality and quantity can be conducted. Collaboration with local beekeepers can also improve honey production practices.Collaboration with local beekeepers can also improve honey production practices. The study provides valuable insights into honey sample quality and sources, serving as a basis for future research and monitoring.

CONCLUSION :

The study concludedthat most of the honey samples from the investigated region were of good quality and suitable for human consumption, with predominance of unifloral honeys without any toxic pollen grains and scarce fungal elements. The palynoassemblage of most honey samples indicated the occurrence of a tropical moist deciduous forest in the region with high monsoonal conditions. However, the discovery of a predominant pollen type of the Asteraceae family in a solitary honey sample highlights the need to investigate the allergenicity of different pollen grains found in the area of investigation to identify the triggers of allergic diseases. The study provides valuable insights into the quality and sources of honey samples in the region and can serve as a basis for further research and monitoring of honey samples.

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Morphological Characterization of Pollen Grains

Morphological features of XX pollen morphotypes belonging to XX families of angiosperms and XX families of gymnosperms recovered from analysis of XX honey samples procured from XX rural localities and XX urban localities of Allahabad district are presented below.

Out of XX pollen morphotypes XX morphotypes were identified upto species level, XX morphotypes upto generic level while some morphotypes could be identified upto family level only viz. Cyperaceae and Poaceae. Due to morphological similarity in pollen grains of Amaranthus sp. and Chenopodium sp. these are grouped in one type.

Table-1: Morphological features of pollen morphotypes recovered from honey samples ANGIOSPERMS

Dicotyledons

Acanthaceae

Pollen Types	**Shape**	**Size (µm)**	**Amb**	**Aperture Type**	**Surface Pattern**
Adhatoda zeylanica (Pl.5, Fig. a)	Prolate	Large (61.27x 37.08 µm)	Elliptical	2- zonocolporate, os-Lalongate	Finely reticulate
Hygrophi/a auricu/ata (Pl.5, Fig. b)	Prolate	Large (52.5x 37.5 µm)	Circular	polyzonoheterocolpate	Reticulate
Justicia simplex (Pl.5, Fig. d)	Prolate	Small (17.Sx12. µm)	Elliptical	3- zonocolporate, colpi bi-operculate, os- lalongate	Reticulate, reticulation larger around equator
Rungia repens (Pl.5, Fig. c)	Prolate	Small (17.5x 13.2 µm)	Elliptical	2- zonocolporate, os-Circular	Finely reticulate
Thunbergia grandiflora (Pl.5, Fig. e)	Spheroidal	Large (56 µm)	Circular	Spiraperturate	Finely granulose

Amranthaceae

Pollen Types	**Shape**	**Size (µm)**	**Amb**	**Aperture Type**	**Surface Pattern**
Acyranthes sp. (PI.S, Fig. t)	Spheroidal	Small (1Sµm) - I	Circular	Pantoporate	Scabrate
Alternanthera sessilis f Pl.5, Fig. g)	Spheroidal	Small (17.Sµm)	± Circular	Pantoporate, 5-7 pores, muri pentagonal	Baculate
Amaranth us/ Chenopodium sp. (Pl.5, Fig. h)	Spheroidal	Small to medium (15-30 µm)	Circular	Pantoporate	Psilate

Anacardtaceae

Mangifera indica (Pl.5, Figs. i & i)	Oblate spheroidal occasionally suboblate	Small (20.5x22.5 µm)	Triangular with convex sides	3- zonocolporate os- lalongate	**Striate**

Apiaceace

Criandrum Sativum (PLS, Fig.K	Perprolate	Medium (30 x12.5µm)	Circular	3- zonocolporate os- lalongate	Coarsely granulose

Apocvnaceae

Catharanthus sp. (Pl.5, Figs. I & m)	Prolate spheroidal	Large (67.5 X 65 µm)	Circular	3- zonocolporate	Psilate

Asteraceae

Ageratum conyzoides (Pl.5, Fig. n)	Oblate spheroidal	Small (18.75x 20µm)	Circular to slightly triangular	3- zonocolporate, angulaperturate, os- almost circular to lalongate	Spinulose
Calendula officinalis (Pl.5, Fig. o)	**Oblate** spheroidal	Medium (22.Sx 26.25µm)	Circular to slightly triangular	3- zonocolporate, os- lolongate	Echinate
Chrysanthemum sp. (Pl.5, Figs. p & q)	Oblate **spheroidal**	**Medium (30x32.5µm)**	± Circular	3- zonocolporate, circulaperturate, os- circular	Baculate spinulose
Helianthus annus (Pl.5, Fig. r)	**Oblate** spheroidal	**Medium** (32.5x35µm)	Circular	3- zonocolporate, os- circular	Tectate **spinulose**
Parthenium hysterophorus (Pl.5, Fig. s)	**Oblate** spheroidal	Small (15.7Sx16.25µm)	Triangular with convex sides	3- zonocolporate, angulaperturate, os- circular	Spinulose
Sonchus arvensis (PI.S, Fig. t)	Oblate spheroidal	Small (22.5x25µm)	Circular	3- zonocolporate, os- circular	Echinate
Spilanthes paniculata (Pl.6, Fig. a)	**Oblate** spheroidal to spheroidal	Small **(20x22.5µm)**	Circular	3- zonocolporate os- lalongate	Echinate
Tagetes erecta (Pl.6, Fig. b)	Oblate spheroidal	Small (13.Sx 15µm)	Triangular with convex sides	3- zonocolporate	Spinulose
Tridax procumbens (Pl.6, **Fig.** c)	Oblate spheroidal	Small (22.5x25 **µm)**	Circular	3- 4 zonocolporate os- circular	Echinate

Balsamlnaceae

Pollen Types	**Shape**	**Size** (uml	**Amb**	**Aperture Type**	**Surface Pattern**
Impatiens balsamina f Pl.6, fig. e)	Bilateral oblate	Medium (21.25x31 .Sµm)	Rectangular	4- zonocolpate angulaperturate, colpi elliptical	Reticulate

Blenomaceae

Spathodea campanu/ata (Pl.6,Figs. f & g)	Subprolate	Medium (46.Sx35.7 Sµm)	Circular	3- zonocolpate, circulaperturate	Retipilate
Tecoma stans (Pl.6,Figs. h & i)	Oblate spheroidal	Medium (31.25x33. Sµm)	± Circular	3- zonocolporate, circulaperturate, os- faint	Finely reticulate

Bombacaceae

Bombax ceiba (Pl.6,Fig. j)	Oblate	Large (37.SxSS. Sµm)	Triangular with rounded angles	3- zonocolpate, Planaperturate	Reticulate,reticula tions fine at the angles of the grains

Brassicaceae

Brassica campestris (Pl.6,Figs. K & 1)	Prolate spheroidal	Medium (32.Sx30. 7Sµm)	± Circular	3- zonocolpate, circulaperturate	Reticulate
Coronopus didymus (Pl.6,Figs. **M** & n)	Prolate spheroidal	Small (22.5x20 µm)	± Circular	3- zonocolpate, circulaperturate	Finely reticulate

lberis amara (Pl.6,Figs. o & p)	Prolate spheroidal	Small (23.7Sx21 .75 um)	± Circular	3- zonocolpate, circulaperturate	Finely reticulate
Raphanus sativus (Pl.6,Figs. q & r)	Spheroidal to prolate spheroidal	Small (22.Sx20 µm)	±Triangular	3-zonocolpate, angulaperturate	Reticulate
Rorippa dubia (Pl.6,Fig. s)	Oblate spheroidal	Small (23.75x2 4.25 µml	± Circular	3-zonocolpate, circulaperturate	Finely reticulate

Cannabinaceae

Cannabis sativa (Pl.6,Fig. t)	Suboblate	Small (18 x23 µm)	Circular	3-zonoporate, occasionally 4-zonoporate,circulap erturate, pore circular, annulate	Granulose

Capparidaceae

Crataeva narvala (Pl.7, Figs. a & b)	Suboblate	Small (15.SxlB. 75µm)	Circular	3- zonocolporate, circulaperturate, os-circular	Reticulate

Caryophyllaceae

Dianthus sp. (Pl.7, Fig.)	Suboblate	Medium (36.25 · 50 m	Circular	Panto porate	Puntitegillate with scattered spinules

Combretaceae

Ferminalia arjuna (Pl.7, Fig.d)	Spheroidal	Small (12.5-1Sµm)	Circular	3- zonocolporate, angulapertunate, colpi alternating with 3 pseudocolpi, os- lalon ate	Psilate

Convolvulaceae

Pollen Types	**I Shape**	**Size furn)**	**Amb**	**Aperture Type**	**Surface Pattern**
Convolvulus arvensis (Pl.7, Fig. e)	Oblate shperoidal	Medium (45x47.S µm)	± Circular	3- zonocolpate, circulaperturate	Faveolate

Cucurbitaceae

Benincasa hispida (Pl.7, Fig. f)	Suboblate	Large (40.75x52 .75µm)	Circular	3- zonocolporateos-lalongate	Reticulate
Coccinia grandiflora (Pl.7, Figs. G & h)	Oblate spheroidal	Medium (43.Sx45 µm)	Almost circular	3- zonocolporateos-lolongate	Reticulate
Cucumis sativum (Pl.7, Fig. i)	Spheroidal	Medium (37.Sx37. Sum)	Sub- triangular	3- zonoporate	Finely reticulate
Cucurbita maxima (Pl.7, Fig. j)	Spheroidal	Very large (165µm)	Almost circular	Pantoporate, spine long broad at base tips blunt	Echinate
luffa acutangula (Pl.7, Fig. k)	Prolate spheroidal	Large (72.Sx69 µm)	Circular	3- zonocolporateos-lalongate	Reticulate
Momordica charantia (Pl.7, Figs. I & m) *I*	Sub prolate	Medium(38.7Sx33. Sµm)	Almost circular	3- zonocolporate, os- circular to lolongate	Reticulate

Euphorbiaceae

Acalypha indica (Pl.7, Fig. n)	Prolate	Small (15x8.75μm)	Circular	3- zonocolporate, os-circular	Psilate
Chrozophora rottleri (Pl.7, Fig. o)	Sub prolate	Large (62.5x53.75 um)	Circular	Pantoporate, pores circular	Reticulate
Gelonium multijlorum (Pl.7, Fig. p)	Spheroidal	Large (SSμm)	Circular	Pantoporate	Croton pattern
Jatropha gossypiifolia (Pl.7, Fig. q)	Spheroidal	Large (67.Sμm)	Circular	Inaperturate	Crotonpattern
Phyllanthus emblica (Pl.7, Fig. r)	Spheroidal	Small (20μm)	Circular	4-5 zonocolporate, circulaperturate, os-lalongate	Reticulate
Ricinus communis (Pl.7, Figs s & t)	Oblate spheroidal	Medium (26.25><27.5 μm)	Triangular with convex sides	3- zonocolporate, angulapertunate, os-lalongate	Finely reticulate

Fabaceae(Caesalpiniaceae)

Cassia fistula (Pl.8, Fig. a)	Oblate spheroidal	Medium (37.Sx40μm)	± Circular	3-zonocolporate,circulaperturate, os- lalongate	Puntitegillate
Cassia occidenta/is (Pl.8, Figs. b & c)	Prolate spheroidal	Medium (3Sx32.5μm)	± Circular	3- zonocolporate,os-lalongate	Faintly reticulate
Leucaena leucocephala (Pl.8, Figs. D & e)	Sub oblate	Medium (41x46.75 μm)	± Circular	3-zonocolporate,circulaperturate, os-lalongate	Granulose

Pollen Types	**Shape**	**Size** hun)	**Amb**	**Aperture Type**	**Surface Pattern**
Delonix regia (Pl.8, Figs. f & g)	Prolate spheroidal	Medium (49.Sx46.5 um)	Circular to subtriagular	3- zonocolpate, Circulaperturate	**Reticulate**
Peltophorum pterocarpum. (Pl.8, figs. h & i)	Oblate spheroidal	Large (48x51.5μm)	± Triangular with rounded angles	3- zonocolporate, Planaperturate,os-circular	**Reticulate,** reticulations smaller near colpi
Tamarindus indica (Pl.8, Figs. i & k) .	-Oblate spheroidal	Medium (31.25x32.5 μm)	Circular	3-zonocolporate,circulaperturate, os-lolongate	Rugulate

Fabaceae (Mimosaceae l

Acacia ni/otica (Pl.8, Fig.I)	Polyads of 16 cells	Medium (30x32.5μm)	-	lnaperturate	Finely reticulate
Cal/iandra sp. (Pl.8, Fig. m)	Pyriform polyads of8 cells	Large **(94x** 60 um)	-	lnaperturate	Obscure
Pithece/lobium dulce (Pl.8, Fig. n)	Polyads of 16 cells	Large (65x60μm)	-	lnaperturate	Coarsely granular
Prosopis juliflora (Pl.8, Figs. o & p)	Suboblate	Medium (25.80x29.9 Oμm)	± Circular with rounded angles	3-zonocolporate,circulaperturate, os- lalongate	Finely reticulate

Fabaceae Papilionaceae

Butea monosperma (Pl.8, Figs. q & r)	Oblate spheroidal	Medium (32.Sx35µ	Triangular with convex	3- zonocolporate,	Obsure pattern
		m)	sides		
Cajanus cajan (Pl.8, Figs. s & t)	Oblate spheroidal	Medium **(39x45µm)**	Circular	3- zonocolporate, angulapertunate, os-	Reticulate
				circular	
Cicer arietinum (Pl.9, Fig. a)	Prolate spheroidal	Medium (30x27.5µ m)	Sub- triangular	3- zonocolporate,os-circular to lolongate	Faintly reticulate
Dolichos lab/ab (Pl.9, Figs. b & c)	Oblate spheroidal	Medium (30.Sx32µ m)	Sub- triangular	3- zonocolporate,os-almost circular	Faintly reticulate
Lathyrus odoratus (Pl.9, Fig. d)	Subprolate	Medium (44.Sx36.75 µm)	Circular	3- zonocolporate, circulaperturate, os-lalongate	Faintly reticulate
Lathyrus sativum (Pl.9, **Fig.** e)	Subprolate	Medium (47.Sx38.25 µm)	Circular	3- zonocolporate ,circulaperturate, os-lalongate	Faintly reticulate
Viciafaba	Prolate to perprolate	Medium (42.Sx22.25 µm)	Sub- triangular	3- zonocolporate,os-circular to lalongate	Faintly reticulate
(Pl.9, Fig. g &_h)	Subprolate	Medium (47.Sx40µ m)	Circular	3- zonocolporate, circulaperturate, os-lalongate	Foveolate

Lamiaceae

Pollen Types	**Shape**	AMB		**Aperture Type**	Surface Pattern
Ocimum sancatum (Pl.9, Fig. i)	Suboblate	Medium (27.38x35.7 5wn1	Circular	6- zonocolpate, Circulaperturate	Reticulate
Salvia dorrii (Pl.9, Fig.i)	Suboblate	Medium (28.25><36.2 Sum)	Elliptical	6- zonocolpate	Reticulate

Linaceae

linum usitatissimum (Pl.9, Figs. k & 1)	Oblate spheroidal	Large (49.25><55 µm)	Triangular with convex sides	3- zonocolporate, angulapertunate	Pilate

Loranthaceae

Dendrophthoe sp. CPl.9, Fig. m)	Oblate	Medium (22.5><41.25 µm)	Triangular with concave sides	3- zonocolpate, angulapertunate, syncolpate	Granulose

Malvaceae

Hibiscus rosa-sinensis (Pl.9, Fig. n)	Spheroidal	Very large (125 µm)	Circular	Pantoporate	Spinose, spine with blunt ends
Sida cordifolia (Pl.9, Fig. o)	Spheroidal	Large (65.5 µm)	Circular	Pantoporate	Spinose, spines with pointed ends
Thespesia sp. (Pl.9, Fig. p)	Spheroidal	Large (57.5 µm)	Circular	Pantoporate	Spinose, spine with broad base

Meliaceae

Azadirachta indica (Pl.9, Figs. q & r)	Prolate spheroidal	Large (52.Sx50 µm)	Circular	4-5 zonocolporate,circula perturate, os- lalongate	Finely reticulate
Melia azedarach (Pl.10, Fig. a)	Spheroidal to prolate spheroidal	Medium (38 x37.Sµm)	Circular to quadrangula	4- zonocolporate,os-lalongate	Finely reticulate
Toona ciliata (Pl.9, Figs. s & t)	Oblate spheroidal	Small (21.25><22.5 µm)	Circular	4-5 zonocolporate,circula perturate, os- lalongate	Reticulate

Memsoermaceae

Tinospora cordifolia (Pl.10, Figs. b & c)	Prolate spheroidal	Small (17.Sx15 µm)	Triangular with rounded angles	3- zonocolporoide	Reticulate

Moraceae

Morusalba (Pl.10, Fig. d)	Oblate spheroidal	Small (11.25x13. 75µm)	Ellipsoidal	2- zonoporate, sometime 3- zonoporate, circulaperturate	Psilate

Morinzaceae

Moringa oleifera (Pl.10, **Figs. e** & f)	**Prolate** spheroidal	Medium **(35x30** µm)	Circular	3- zonocolporate, circulaperturate, os-lalongate	Psilate

Myertaceae

Pollen Types	**Shape**	AMB	**Aperture Type**	Surface Pattern

Callistemon citrinus (Pl.10, Fig. g)	Oblate	Small (14.25 X 19.25 µm)	Triangular	3-parasyncolporate, os- lalongate	Granulose
Eucalyptus citriodora (Pl.10, Fig. h)	Suboblate	Small (14.75 X 18 um)	Triangular	3- zonocolporate, angulaperturate	Granulose
Psidium guajava (Pl.10, Fig. i) *I*	Suboblate	Small (13.75><18.5 µm)	Triangular, sometimes quadrangular	3-zonocolporate, occasionally 4, angulaperturate	Granulose
zygium cumini (Pl.10, Fig.i)	Suboblate	Small (12.5 X 18 µm)	Triangular, occasionally quadrangular	3-syncolporate, occasionally 4-syncolporate, angulaperturate, os lalongate	Psilate

Nvctaz1naceae

Bougainvillea sp. (Pl.10, Fig. k)	Oblate	Medium (22.Sx30 µm)	Circular	3- zonocolpate, circulaperturate	Retipilate

Oleaceae

Nyctanthus arbor trist (Pl.10, Figs. L & m)	Spheroidal	Large (57.5 µm)	Circular	3- zonocolporate, circulaperturate, colpi long & broad	Retipilate

Papaveraceae

Argemone maxicana (Pl.10, Figs. N & o)	Oblate spheroidal	Medium (30.5x32.5 µm)	Circular	3- zonocolpate, angulaperturate	Finely reticulate

Pedaliaceae

Sesamum indicum (Pl.10, Fig. p)	Spheroidal	Large (72-81µm)	Circular	Polyzonocolpate	Finely reticulate

Polemoniaceae

Phlox drummondii (Pl.10, Fig. q)	Spheroidal	Medium (30 µm)	Circular	Pantoporate	Reticulate

Poligonaceae

Antigonon leptopus (Pl.10, Figs. r & s)	Prolate spheroidal	Large (62.5x61.2 5 µm)	Circular	3- zonocolporate, os-lalongate	Reticulate
Polygonum plebeium (Pl.10, Fig. t)	Prolate	Small (17.5x12.5 µm)	Circular	3- zonocolporate, os-lalongate	Finely reticulate
Rumex dentatus (Pl.11, Fig. a)	Oblate spheroidal	Medium (26.75x 29 µm)	Circular	3- zonocolporate, os-slightly lolongate to circular	Finely reticulate

Portulacaceae

Portulaca grandiflora (Pl.11, Fig. b)	Spheroidal	Medium to large [45-60 µm)	Circular	Pantocolpate, colpi arranged in tetra to hexagonal manner	Scabrate

Punicaceae

Pollen Types	**Shape**	Size (UM)	AMB	**Aperture Type**	Surface Pattern
Punica granatum (Pl.11, Figs. c & d)	Prolate spheroidal	Small (17.5x15.5 µml	Circular	3- zonocolporate, os-circular	Psilate

Rhamnaceae

Zyziohus *jujuba* 1.11, Fig. e & f)	Prolate spheroidal	Small (22.5x20µ m)	Tringular	3- zonocolporate, angulaperturate, os-circular	**Psilate**

Rutaceae

Aegle marmelos (Pl.11, Figs. i & j)	Oblate spheroidal	Medium **(22.Sx26.5** µm)	± Circular	4- zonocolporate, sometime 5-zonocolporate, circulaperturate, os-lalongate	Reticulate
Citrussp. (Pl.11, Fig. k)	Oblate spheroidal	Medium (30x31.25 µm)	± Circular	4-5 zonocolporate, circulaperturate, os-lalongate	Reticulate

Sapotaceae

Madhuca indica (P:l.11 Fig. 1)		Prolate spheroidal	Medium (42.5x37.5 m	Almost circular	4-5 zonocolporate, circulaperturate, os- lalon ate	Finely reticulate

Spindaceae

Schleichera oleosa (Pl.11, Fig. m)	Oblate spheroidal	Small (12.5x15μ m)	Sub- triangular	3- zonoporate, Parasyncolporate,	Striate

Simaroubaceae

Ailanthus exce/sa (Pl.11, Fig. n)	Oblate spheroidal	Medium (30x32.5 μm)	Sub- triangular	3- zonocolporate, angulaperturate, os-lalongate	Finely reticulate

Solanaceae

Datura stramonium (Pl.11, **Fig.** o)	Oblate spheroidal	Medium **(32.Sx35.5** uml	Circular	3- zonocolporate,os-lolongate	Striate
Lycopersicon esculentum (Pl.11, Figs. p & q)	**Oblate** spheroidal	Small (17.SxlB.75 um)	Circular to sub triangular	3- zonocolporate,os-lolongate	Granulose
Solanum nigrum (Pl.11, Figs. r & s)	Oblate spheroidal	**Medium (31.25x32.5** μm)	Triangular with convex sides	3- zonocolporate, sometime 4-zonocolporate angulaperturate, os-lalongate	Granulose

Ulmaceae

Pollen Types	**Shape**	Size (UM)	AMB	**Aperture Type**	Surface Pattern
Holoptelea integrifolia (Pl.11, **Fig.** t)	Suboblate	Small (18.4x21.7 5 um)	Circular	4-5 zonoporate, circulaperturate	Coarsely granulose

Verbinaceae

Lantana indica (Pl.12, Figs. a & b)	Oblate spheroidal	Medium (30x32.5μ **m)**	Triangular	3- zonocolporate,	Psilate

MONOCOTYLEDONS

Arecaceae

Phoenix sylvestris (Pl.12, Fig. c)	Elliptical	Medium (longest diameter 37.25μm)	-	1-ana- colpate	Faintly granulose

Cyperaceae

Cyperaceae (PJ.12, Fig. d)	Pear shaped	Medium (32.Sx25μ m)	-	1-ana- porate, pore on broader side	Psilate

Nvmpibaeaceae

Nymphea sp. **(Pl.12,** Fig. e)	Spheroidal	Medium (42.S μm)	Circular	1-zonosulculate	Warts & spinules

Joaceae

Zeamays (Pl.12, Fig. f)	Spheroidal	Large (9Sμm)	Circular	1-ana- porate, pore ± circular, operculate	Psilate
Other Poaceae (Pl.12, Fig. g)	Spheroidal	Small to large (22.25-so	Circular	1-ana- porate, pore ± circular, operculate	Psilate

		µm)			

Typhaceae

Typha angustata (Pl.12, Fig. h)	Variously shaped but more or less spheroidal	Small (21.Sµm)	Circular	1-ana- porate	Finely reticulate

GYMNOSPERMS
Cuuressaceae

Thuja occidentalis (Pl.12, Fig. i)	Spheroidal	Small to medium f22-49µm)	-	lnaperturate	Flecked with granules

Cvcadaceae

Cycas sp. (Pl.12, Fig. j)	Ovate	Small (longest diameter 21.7µm)	± Circular	1-anacolpate	Psilate

Pinaceae

Pinus roxburghaii (Pl.12, Fig. k)	Bi-saccate	Large (SOxSSµm)	-	1-furrow on ventral surface	Reticulate on sacci appearing as predominant ridges on inner surface

Pollen morphotypes in honey with regard to size & form:

In the present investigation out of 112 pollen morphotypes identified from the microscopic examination of honey samples·maximum number of pollen types is 3- zonocolporate (SO types) followed by 3-zonocolpate (12 types), pantoporate (11 types), 4-5 zonocolporate (6 types), inaperturate (5 cypes), 1-anaporate (4 types), 2-
zonocolporate (2 types), 3-4 zonocolporate (2 types), 3-zonoporate (2 types), 6- zonocolpate (2 types), 3-parasyncolporate (2 types) and one each of spiraperturate, 4-zonocolpate, polyzonoheterocolpate, 2-3 zonoporate, 4-5 zonoporate, 3- zonocolporoide, 3-4 syncolporate, polyzonocolpate, 1-zonosulculate and saccate.

With regard to size of pollen grains the maximum pollen morphotypes are medium in size (51 types) followed by those of small size (36 types), large size (21 types) and very large size (2 types). With regard to ornamentation, pollen types having reticulate ornamentation (SO types) are most abundant followed by spinulose (13 types), granulose (13 types), psilate (12 types), striate (3 types), foveolate (3 types), retipilate (3 types), with crotonoid pattern (2 types), obscure (2 types), puntigellate (2 types), regulate (1 type),warts (1 type), scabrate (1 type) and baculate (1 type).

Among the various morphological features size and surface features of pollen grains are related to mode of pollination. Entomophilous pollen grains are heavier, larger in size, heavily ornamented with various type of exine pattern amongst which reticulate ornamentation is most common. They are sticky in nature due to presence of electron dense pollen kitt in their exine. This feature of exine helps them in adherence to the appendages of pollinator.

In the present investigation majority of pollen grains are entomophilous (66.37%) followed by amphiphlous (17.69%) and anemophilous (15.92%).

3- zonocolpate pollen morphotypes belonging to Brassica campestris formed the major component of the pollen spectra of Allahabad followed by 3-zonocolporate (Coriandrum sativum, Ageratum conyzoides) and 1-ana porate (Poaceae). Pollen grains of Brassica campestris are medium in size with reticulate exine, Coriandrum
Morphological Characterization of Pollen Grains Recovered from Pollen Analysis 48 sativum are also medium size with granulose exine, Ageratum conyzoides are small with spinulose exine and Poaceae are small to large in size with psilate exine.

Other pollen types found in dominance viz. Bombax ceiba, Amaranthus / Chenopodium sp., Cajanus cajan, Callistemon citrinus, Citrus sp., Eucalyptus citriodora, Holoptelea integrifolia, Lathyrus sativus, Phyllanthus emblica, Psidium guajava, Syzygium cumini and Tinospora cordifolia are small to medium in size except Bombax ceiba which are large in size. Exine is psilate in Poaceae, Amaranthus
/ Chenopodium sp., Psidium guajava and Syzygium cumini, Spinulose in Ageratum conyzoides while Bombax ceiba, Cajanus cajan, Citrus sp., Lathyrus sativus, Phyllanthus emblica and Tinospora cordifolia comprising with reticulate exine pattern.