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Palyno–morphological and isotopic characterization of monofloral and multifloral honeys from Lucknow, India

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ABSTRACT

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We describe and illustrate the detailed palyno–morphological attributes, based on light microscopy (LM) and field emission scanning electron microscopy (FESEM), as well as characterize the carbon stable isotopic ($\delta^{13}C$) values of filtered and unfiltered natural and commercial honey samples from Lucknow, India. The principal objective behind conducting the melissopalynological investigation is to gather relevant information about the pollen and nectar sources, foraged by honey bees in an area, which is a decade–old expansion of the city on an arable land. The pollen assemblages revealed one monofloral and two multifloral honey samples, ultimately provide insights into the variety of plants foraged by honey bees in an urban set–up, and is a potential archive for observing decadal changes in plant diversity. The carbon stable isotopic ($\delta^{13}C$) values of all the natural honeys varied from ~ –25 to –26‰, which relates to the regional vegetation types and environmental conditions. The difference between the filtered (without pollen) and unfiltered honey is ≤ 1.00‰. The $\delta^{13}C$ values of the three commercial honey, which are filtered /without pollen are same, i.e. –27‰. Hence, it is suggested that honey with pollen of diverse flora of the study area should be preferred by the consumers as a pure and also as a clinically safe food product.

Key–words—Melissopalynology, Microscopy techniques, Carbon stable isotope, Botany & Geography, Lucknow, India.

INTRODUCTION

MELISSOPALYNOLOGY, one of the applied branches of palynology dealing with the study of pollen grains and spores present in honey, provides significant information about the plants preferred by honey bees as sources of pollen and nectar for the production of honey in and around the area in question, ultimately determine the geographical and botanical origin of the honey (Louveaux *et al*., 1978; Barth, 2004; Von der Ohe *et al*., 2004; Cotte *et al*., 2004; Chauhan & Quamar, 2010; Ponnuchamy *et al*., 2014; Chauhan *et al*., 2017). The botanical and geographical origin of honey is associated with the floral sources, soil, environmental conditions, and mode of extraction and processing (El–Metwally, 2015; Varga *et al*., 2020; Sajtos *et al.,* 2022). In fact, bees store pollen in the combs or in hives (Santos, 1961) when honey is extracted and that stored food can be observed in the combs/hives (Barth & Melhem, 1988). Bees, in return, benefit plants by pollination (entomophily), hence, a profitable relationship exists between them (Pirani & Cortopassi–Laurino, 1993). Honey is a natural carbohydrate (especially fructose and glucose) –rich product produced by honey bees from the nectar of plants and utilised as a source of energy (Esti *et al*., 1997), however, proteins are provided by the pollen in honey (Turner, 1984; Lin *et al*., 1993). Moreover, fatty substances, minerals and vitamins are also present in honey (Gary, 1975), which act as the essential foods for raising the brood and for the longevity of the colony (Dietz, 1975; Schmidt *et al*., 1987). Honey possesses valuable nutritive, healing and prophylactic properties too (Pereira *et al*., 1995). Melissopalynological analyses provide knowledge

of important plants useful in promoting the production and clinically safe consumption of high–quality plant–specific labelled honeys, as some pollen proteins are allergenic to consumers.

In order to collect pollen, honey bees visit flowers and with the use of their jaws and front legs they, in fact, amass and accumulate the loose pollen from the anthers. The bees will roll over the flowers in some plant species, brushing their sides against the inflorescence to cover their hairy bodies with pollen. Then bees will moisten the grains with nectar and saliva, packed on their pollen basket (corbicula) in the form of pasty pellets, which are called pollen loads (Thorp, 2000). Humans have used pollen loads for traditional medicine and supplementary nutrition, as well as in alternative diets (Isla *et al*., 2001). Hidalgo *et al*. (1990) were of the opinion that the botanical and geographical origin of honey can be determined through microscopic analysis of the pollen loads or by direct observation of the physical characteristics of the loads. Further, Dimou and Thrasyvoulou (2007) suggested that the use of pollen traps is more accurate than field observations during the blooming periods and gives information about the available pollen sources and their contribution to the colony. This information could be helpful in developing apiaries and commercial honey production around the area of investigation. The present melissopalynological investigation is conducted on the honey samples collected from the Chinhat area (near Amity University Campus), Lucknow, with the principal aim of identifying the important source plants of honey in and around the region.

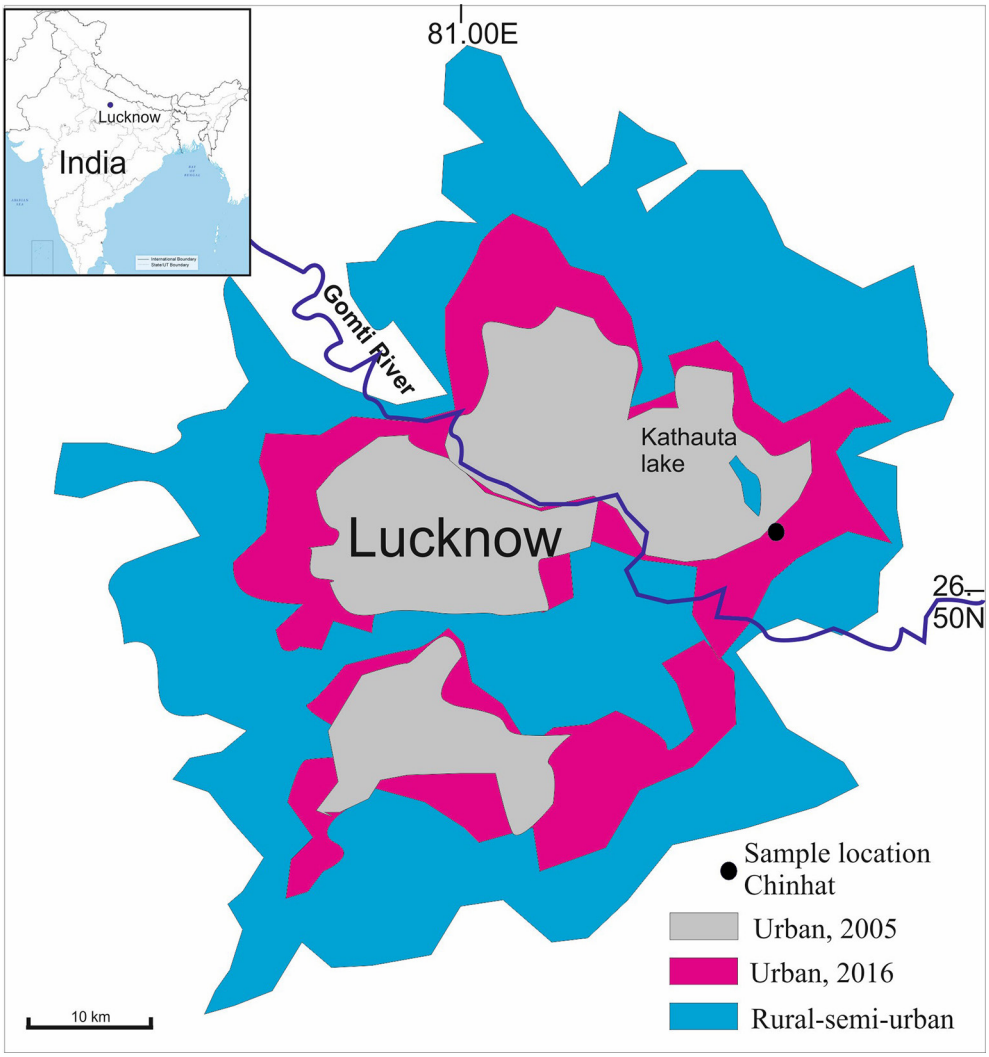


Fig. 1—Map showing the study area at the Chinhat in Lucknow (India) and also the urban extension of Lucknow City between 2005 and 2016 (after Shukla & Jain, 2019). Geographical map of India showing the District Lucknow (inset).

REGIONAL SETTING

Geographical setting and climate

The study area is Chinhat, which falls under the central Gangetic Plain and is the urban extension of Lucknow City located in the northern part of India at Latitude 26.8508º N and Longitude 81.049º E (Fig. 1). Two decades back, Chinhat area was an open agricultural land. One meter sedimentary profile from the Kathauta Lake deposited since ~400 years from now revealed palynological results comprising pollen of *Brassica* and other crop plants along with aquatic plants (Chauhan *et al*., 1990). A decade back the urban development started in this agricultural land fed by a large water body named Kathauta Tal (Lake). The development of this area has now reduced the expanse of Kathauta Lake and agricultural area in the vicinity due to concrete buildings and parks (Kumar *et al*., 2014; Shukla & Jain, 2019). At present, the lake covers only a small area.

Climate of the study area, in general, is humid sub–tropical and largely influenced by southwest monsoon. The temperature in winter season from November to February ranges between 7.6 ºC and 21 ºC. Occasionally it may go down to <1 ºC during cold months between December and January. April to June is the summer season characterized by dry winds. The temperature ranges between 27 ºC and 32.5 ºC but may reach 46 ºC in the month of June. The rainy season is from July to middle of September and about 75% of the average rainfall (100–120 cm) is received through south–west monsoon. Most of the plant species flower during spring season between February to April and is the convenient season for honey bees.

Vegetation

Open mixed dry deciduous forest type constitutes the vegetation around the study area. Anderson (1859) for the first time explored the vegetation of Lucknow District and enumerated indigenous and cultivated plants. Thereafter, Kapoor (1962), Patil (1963), and Balapure and Srivastava (1964) have studied floristics from ecological and phytogeographical point of view in this area. The natural vegetation, although scanty, consists of shrubs, grasses with sparsely distributed trees. Thus, the landscape presents a look of scrub forest. Patches of *Acacia arabica*, *Holoptelea integrifolia*, *Diospyros cordifolia*, *Cordia dichotoma*, *Syzygium cuminii*, *Capparis decidua*, *Butea monosperma*, *Mimosa* sp., *Albizia lebbek*, *Flacourtia indica*, *Ziziphus mauritiana*, *Carissa spinarum*, *Adhatoda vasica*, *Nyctanthes arbor–tristis*, *Lagerstroemia speciosa*, etc. are the basic components of tree and shrubby taxa, though scattered. However, a few stands of *Holoptelea integrifolia*, *Syzygium cuminii*, *Dalbergia sissoo*, *Bombax malabaricum* and *Acacia catechu* are also found in open areas. Along the banks of Gomti River, ravine thorn forest, consisting of *Capparis decidua*, *Aegle marmelos*, *Ziziphus mauritiana*, *Carissa spinarum*, *Calotropis gigantia* and *Adhatoda vasica* exist. Recently plantations of *Eucalyptus* sp., *Terminalia arjuna*, *Pongamia pinnata*, *Melia azadirach*, *Prosopis juliflora*, *Emblica officinalis*, *Syzygium cuminii*, *Acacia arabica*, *Parkinsonia* sp., *Gardenia sp.*, *Kalanchoe pinnata*, etc. have been raised. The herbaceous elements of terrestrial nature include *Ageratum conyzoides*, *Cichorum intybus*, *Euphorbia hirta*, *Euphorbia thymifolia*, *Oxalis acetosella*, *Chenopodium album*, *Chenopodium murale*, *Mazus japonicus*, *Melilotus alba*, *Justicia simplex*, *Justicia diffusa*, *Solanum nigrum*, *Solanum xanthocarpum*, *Heliotropium strigosum*, *Launaea nudicaulis*, *Portulaca oleracea*, etc. whereas along the margins of lakes, ditches, and in wet places *Polygonum plebeium*, *Polygonum glabrum*, *Rotala* sp., *Ammania baccifera*, *Alternanthera sessilis*, *Hydrocotyle* sp. and *Eriocaulon quinquangularis*, are quite common. Aquatic vegetation is luxuriant in the lakes, ponds, streams, and river. *Eichhornia crassipes* forms a thick mat throughout of water surface, often causing blockade of Gomti River. *Lemna polyrrhiza*, *Nymphaea* sp., *Nymphoides cristatum*, *Potamogeton indicus*, *Jussiaua repens*, *Ipomoea aquatica*, *Wolfia arrhiza*, *Vallisnaria spiralis*, *Hydrilla verticillata*, etc. are some of the frequent lake water plants (Chauhan *et al*., 1990).

Moreover, *Shorea robusta*, *Madhuca indica, Ailanthus excelsa*, *Cassia* spp., *Tinospora cordifolia*, *Morus alba*, *Delonix regia*, *Psidium guajava*, *Citrus lemon*, *Alangium salvifolium*, *Calistemon citrinus*, *Cajanus cajan* and members of the families Rutaceae, Malvaceae, Amaranthaceae, Brassicaceae and Asteraceae (sub–families–Asteroideae and Cichorioideae) also grow around the study area.

MATERIAL & METHODS

The materials for the present study comprise three honey samples (1, 2 and 3), which were procured from the Chinhat area (near Amity University Campus), Lucknow. The honey harvesting was done by local folks in the month of May, 2018 and was collected in glass bottles for the study. Three commercial honey samples (A, B and C) were obtained from market for analysing pollen assemblage and $\delta^{13}C$.

Pollen analysis

20 g each of the honey samples were dissolved in 20 ml distilled water and stirred gently to homogenise in the vial. The samples were sieved through 150-µm-mesh-size sieve and then the filtrate was treated with glacial acetic acid in order to dehydrate them. Thereafter, the samples were acetolysed using the acetolysis mixture (acetic anhydride and conc. sulphuric acid in the ratio of 9: 1) (Erdtman, 1943). The samples were again treated with glacial acetic acid before washing twice with distilled water to neutralize the effect of

Table 1—Plant pollen taxa recovered from the honey samples, their frequency classes, as well as their frequency percentages.

Pollen frequency class	Frequency (%)	Plant pollen taxa
Predominant pollen types	45 and/or > 45	*Psidium guajava*
Secondary pollen types	16–45	*Citrus lemon*, *Eucalyptus globulus*
Important minor pollen types	3–15	*Cassia fistula*, *Tinospora cordifolia*, *Prosopis specigera*, *Prosopis juliflora*, *Syzygium cuminii*, *Cassia siamea*, *Ceiba pentandra*, *Delonix regia*, *Terminalia* spp., *Ficus* spp.
Minor pollen types	< 3	Anacardiaceae, *Emblica officinalis, Holoptelea integrifolia*, *Alangium salvifolium*, Rutaceae, *Schleichera oleosa, Calistemon citrinus*, *Acacia* sp., *Madhuca indica*, *Shorea robusta*, *Morus alba*, *Bombax ceiba*, *Ixora* sp., *Diospyros melanoxylon*, *Manilkara zapota*, *Ailanthus excelsa*, *Strobilanthes* spp., *Ziziphus mauritiana*, *Ageratum conyzoides*, *Cichorum intybus*, *Cajanus cajan*, Cichorioideae (Liguliflorae: Asteraceae), Asteroideae (Asteraceae), Meliaceae, Brassicaceae, Amaranthaceae, *Astemisia* sp., *Xanthium strumarium*, *Solanum* spp. *Pimpinella tomentosa*, *Botryococcus* sp., *Alternaria* sp., *Meliola* sp., *Cookeina* sp. and Insect remains

acetolysis mixture. The samples were again sieved through 600-µm-mesh-size sieve and finally the residues were kept in vial having 50% glycerine along with phenol (a few drops) in order to avoid microbial contamination. Permanent slides were prepared in glycerine jelly and sealed with wax. Counting of palynomorphs and their photography was performed under an Olympus BX50 Microscope with an attached DP 25 Digital Camera. Further, the identification of pollen grains and spores (Pls 1–9), recovered from the honey samples, was made through consulting reference pollen slides available at the sporothek of the Birbal Sahni Institute of Palaeosciences (BSIP) Herbarium. Some published literature was also consulted to identify the palynomorphs, such as Chauhan and Bera, 1990; Chauhan and Quamar, 2010; Chauhan *et al*., 2017; Nayar, 1990. Terminology used for describing morphology of the pollen of various plant taxa is based on Erdtman (1952) and Faegri and Iversen (1964).

Furthermore, the pollen grains, after counting and identification, were categorized into the four pollen frequency classes, such as predominant pollen types (> 45%), secondary pollen types (16–45%), important minor pollen types (3–15%) and minor pollen types (< 3%) (Table 1) (Louveaux *et al*., 1978). Honey sample containing 45% or more than 45% pollen of a single pollen type is considered 'unifloral/ monofloral' honey (International Commission of Bee Botany: ICBB, 1970). The recovered pollen were placed into three major groups, such as arboreals (trees and shrubs), non–arboreals (herbaceous taxa), and non–pollen palynomorphs (NPPs). The non–arboreals were further categorised into terrestrial herbs, cultural plant pollen taxa, and wetland taxa (Fig. 2; Table 2).

Field Emission Scanning Electron Microscope (FESEM)

Scanning Electron Microscope (SEM) study of acetolysed pollen samples were carried out for identification of pollen at the species level. The acetolysed honey sample was dehydrated in a series of alcohol and was mounted on a smooth glass piece fixed on aluminum stubs. The samples were coated with Palladium–platinum for observation in FESEM (Model No. JEOL 7610F) (Farooqui *et al*., 2019).

Carbon stable isotope ($\delta^{13}C$)

Carbon stable isotope study was carried out in honey (1) natural honey retrieved from beehive (2) honey without pollen after filtration, and (3) with pollen concentrate (residue) in order to record the variations in $\delta^{13}C$ values of honey. For filtration of honey, the Nitex Cloth of pore size 10 µm (imported from Netherland) was used. This methodology was carried out in order to explore the variation of $\delta^{13}C$ values in filtered honey which is, in general, available for consumers in market and is without pollen. The plant source used for honey production by honey bees was correlated with the pollen assemblage of C3 and C4 photosynthesizing plants and its respective carbon stable isotope. It is known that $\delta^{13}C$ values of C3 plants is depleted and gives values ranging between–19 to–32 ‰ and the C4 plants give values ranging about–10 to–16 ‰. This provides the basis for the resource plants in the vicinity foraged by honeybees. Therefore, the correlation of the type of plants foraged by honey bees and its $\delta^{13}C$ values display the quality, utility and purity of honey.

A pin is dipped into the honey samples and the tip of the pin was touched on Tin boats which was packed for

measuring the TC%, of the samples along with its stable isotopic ratios ($\delta^{13}C$). The measurement was done by using Elemental Analyzer model "Vario Isotope Select" and their isotopic ratios are measured by using IRMS (Isotope Ratio Mass Spectrometer) Model "Isoprime precision" present in the Radiochronology and Isotopic Characterization Laboratory of the Birbal Sahni Institute of Palaeosciences, Lucknow.

Routine peak centering, autotuning, stability check were carried out for IRMS in connection with EA. Consistency of the data was checked by running lab standards then after the samples was fed in the Elemental Analyzer and scheduled to run automatically. Standards Ox–II, and lab standard Sulfanilamide, Caffeine is used to check the accuracy and precision of the data (Table 3).

RESULTS

Palynology

The honey samples analyzed were not very rich in terms of plant diversity and quantity as well, although, various plant pollen types were encountered in their permanent palynoslides under the microscope (Fig. 3). The pollen types encountered in each honey sample and their corresponding frequency classes have been listed in Table 1 (Louveaux *et al.*, 1978). A total of 47 pollen types (Pls 1–9) belonging to 29 families were identified in all the samples (Fig. 3; Table 2). The most frequent pollen is *Psidium guajava* (Myrtaceae family; 84.85%), followed by *Citrus lemon* (Rutaceae family; 36.11%), *Eucalyptus globulus* (Myrtaceae family; 16.55%), *Cassia fistula* (Fabaceae family; 12.8%) and *Tinospora cordifolia* (Cornaceae family; 10%) (Tables 1 & 2). Moreover, the details of pollen load are dealt separately below:

Honey sample 1—This sample shows the dominance of a single tree taxon–*Psidium guajava* (Myrtaceae family; 84.95%). However, other plant pollen taxa, such as tree taxa–*Terminalia* spp. (3.24%), *Eucalyptus globules* (2.55%), *Syzygium cuminii* (2.26%), *Citrus lemon* (2.06%), *Callistemon citrinus* (1.67%), *Schleichera oleosa* (1.17%), and *Tinospora cordifolia* (0.098%) were recorded in variable moderate to low frequencies; shrubby taxon–*Ziziphus mauritiana* (0.688%) and terrestrial herbs, such as *Ageratum conyzoides*

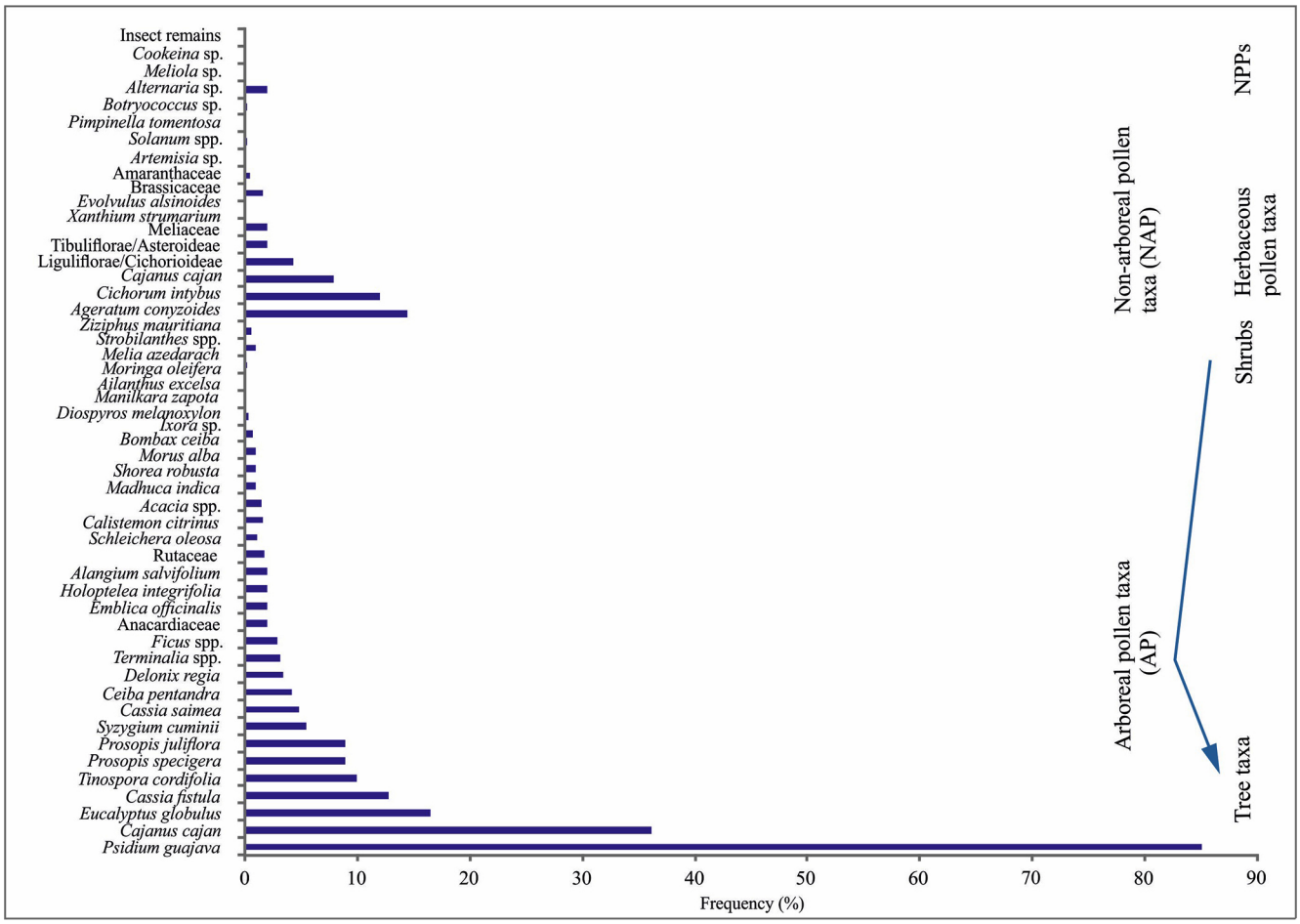


Fig. 2—Frequency percentages of all the pollen types recovered from the honey samples from Lucknow.

Table 2—Plants taxa recovered from the honey samples, their scientific name, common name/vernacular name, habit, family, flowering period and mode of pollination (EN–Entomophilous taxa; AN–Anemophilous taxa; AM–Amphiphilous taxa and unless otherwise stated).

Plant taxa/Scientific name	**Common name/ Vernacular name**	**Habit**	**Family**	**Flowering Period**	**Mode of pollination**	**Frequency (%)**
Psidium guajava L.	Guava, Amrood	Tree	Myrtaceae	Mar–Apr, Aug–Sep	EN	84.95
Citrus lemon (L.) Burm. F.	Lemon, Nimbu	Tree	Rutaceae	Feb–May	EN	36.11
Eucalyptus globulus Labill.	Indian laburnum, Gum tree, Neelgiri	Tree	Myrtaceae	Dec–Apr	AM	16.55
Cassia fistula L.	Amaltas	Tree	Fabaceae	Jan–Oct	EN	12.8
Tinospora cordifolia (Willd. Miers	Heart–leaved moonseed	Tree	Menispermaceae	Apr–May	EN	10
Prosopis specigera L. (Syn. *P. cineraria*)	Amrita, Khejri, Gurgo	Tree	Fabaceae	Dec–Mar	EN	9
Prosopis juliflora (Sw.) DC.	Prosopis, Mesquite	Tree	Fabaceae	Aug–May	EN (bees)	9
Syzygium cuminii (L.) Skeels	Malabar plum, Java/Black plum, Jamun	Tree	Myrtaceae	Feb–Apr	AN, EN (bees, flies)	5.46
Cassia siamea Lam.	Siamese cassia, Kassod tree	Tree	Fabaceae	Aug–May	EN (bees)	4.9
Ceiba pentandra (L.) Gaertn.	Kapok tree, Silk–cotton tree	Tree	Malvaceae	Feb–May	EN (bees)	4.2
Delonix regia (Hook.) Raf.	Royal Poinciana, flame tree, Flamboyant	Tree	Fabaceae	Mar–Apr	EN (bees)	3.5
Terminalia spp. (cf. *T. arjuna*) (Roxb. ex DC.) Wight et Arn.	Arjun	Tree	Combretaceae	Apr–May	AM, EN (honey bees, butterflies, wasps, flies, ants and sun birds)	3.24
Ficus spp. L.	Fig trees, Figs	Tree	Moraceae	Inconspicuous flower	EN (wasp)	3
Anacardiaceae L.	Mango or Cashew family	Tree	Anacardiaceae	Nov–Jan	EN (insect)	2
Emblica officinalis Gaertn.	Gooseberry, Aaonla	Tree	Phyllanthaceae	Feb–April	EN (bees)	2.1
Holoptelea integrifolia Planch.	Chilbil	Tree	Ulmaceae	Jan–Mar	AN	2.1
Alangium salvifolium (L. f.) Wang.	Sage–leaved alangium	Tree	Cornaceae	Feb–Jun	EN (bees) Ornithophily (birds)	2.01
Rutaceae Juss.	Citrus family	Tree	Rutaceae	————————	EN	1.8

Schleichera oleosa (Lour.) Oken.	Ceylon Oak	Tree	Sapindaceae	Mar–Apr	EN	1.17
Callistemon citrinus (Curtis) Skeels. R. Br.	Bottle brush	Tree	Rutaceae	Feb–May	EN	1.7
Acacia spp. (cf. *Pithecelobium dulce*) (Roxb.) Benth.)	Acacias, Wattles	Tree	Fabaceae	Feb–Apr	EN	1.49
Madhuca indica J. F. Gmelin	Mahua	Tree	Sapotaceae	Mar–Apr	Cheiropteriphily (bats)	1
Shorea robusta Gaertn. f.	Sal	Tree	Dipterocarpaceae	Feb–Apr	AN, EN	1
Morus alba L.	Shahtoot, White mulberry	Tree	Moraceae	Feb–Jun	AM	1
Bombax ceiba L.	Semul	Tree	Malvaceae	Feb–May	EN	0.74
Ixora sp. L.	West Indian Jasmine	Tree	Rubiaceae	Mar–May	EN (small moths)	0.4
Diospyros melanoxylon Roxb.	Tendu, Ebony tree	Tree	Ebenaceae	Apr–Jun	EN (large bees)	0.1
Manilkara zapota (L.) P. Royen	Chickoo, Sapodilla, Sapota, Noseberry	Tree	Sapotaceae	Apr–May	EN (bees)	0.1
Ailanthus excelsa Roxb.	Maharukh, Mahaneem	Tree	Simaroubaceae	Jan–Mar	EN	0.2
Butea monosperma (Lam.) Taub.	Flame–of–the–forest, Bastard teak, Palash	Tree	Fabaceae	Feb–Mar	EN, Ornithophily (birds), three–striped squirrel	0.1
Moringa oleifera Lam.	Moringa, Drumstick tree	Tree	Moringaceae	Jan–Mar (May)	EN (bees)	0.1
Melia azedarach L.	Pride of India, Indian lilac, White cedar, China berry tree	Tree	Meliaceae	Mar–Jun	EN (Planalto hermit)	0.1
Strobilanthes spp.(cf. *S. angistifrons* C.B. Clarke)	————	Shrub	Acanthaceae	Sep–Oct	————	0.688
Ziziphus mauritiana Lam.	Indian plum, jujube, Chinese date/apple	Shrub	Rhamnaceae	Jul–Oct	EN	1
Ageratum conyzoides L.	Tropical whiteweed	Terrestrial herb	Asteraceae	Jan–Dec (Throughout the year)	EN	14.46
Cichorum intybus L.	Blue daisy, Cichory, Succory	Terrestrial herb	Asteraceae	Jul–Oct	EN (bees)	12
Cajanus cajan (L.) Millsp.	Tur, Tuver, Arhar, pigeon pea	Terrestrial herb	Fabaceae	Dec–Jan	EN	7.984
Cichorioideae/Liguliflorae	Aster, Daisy, Sunflower	Terrestrial herb	Asteraceae Bercht. & J. Prest.	Highly variable: Jul–Sep/Oct	EN (bees, flies)	4.3

Asteroideae/Tubuliflorae	Aster, Daisy, Sunflower	Terrestrial herb	Asteraceae Bercht. & J. Prest.	Highly variable: Oct–Feb/Mar	EN (Coleoptera, Diptera, Hymenoptera groups))	2.1
Meliaceae Juss.	Mahogany family	Terrestrial herb	Meliaceae	May–Jun	EN	2.01
Alium cepa L.	Common Onion, Pyaz, Bulb Onion	Terrestrial herb	Amaryllidaceae	Jan–Mar	EN (insects, bees)	0.1
Coriandrum sativum L.	Coriander, Dhaniya	Terrestrial herb	Apiaceae	Jun–Jul	EN (honey bees of Apidae family)	0.1
Ocimum basilicum L.	Common Basil, Sweet Basil, Ram tulsi, Tropical Basil	Terrestrial herb	Lamiaceae	Aug–Nov	EN	0.1
Brassicaceae (cf. *Brassica compestris* L.)	Mustard, Sarson	Cultural plant pollen taxa/ Terr. herb	Brassicaceae	Nov–Apr	EN	1.7
Amaranthaceae (cf. *Amaranthus* L.)	Bathua, Chaulai	Cultural plant pollen taxa/ Terr. herb	Amaranthaceae	Oct–Apr	AN	0.53
Artemisia sp. L.	Mugwood, Sagebrush, Wormwood, Mugwort	Cultural plant pollen taxa/ Terr. herb	Asteraceae	————	AN	0.06
Xanthium strumarium L.	Common Cocklebur	Terrestrial herb	Asteraceae	Aug–Mar	AN	0.06
Evolvulus alsinoides L.	Dwarf Morning Glory	Terrestrial herb	Convolvulaceae	Aug–Dec	EN (honey bees) and snails	0.1
Solanum spp. Schrad et Wendell	*Solanum*	Wetland (Marshy) texa	Solanaceae	Jan–Dec (Throughout the year)	EN	0.2
Pimpinella tomentosa (Dalzell & Gibson) ex. C.B. Clarke	Hairy Hogweed	Wetland (Marshy) texa	Apiaceae	Sep	EN (honey bees)	0.04
Botryococcus sp.	————	Algal remains	Botryococcaceae	————	————	0.2
Alternaria sp.	————	Fungal spores	Pleosporaceae	————	————	2.018
Meliola sp.	————	Fungal spores	Meliolaceae	————	————	0.1
Cookeina sp.	————	Fungal spores	Sacoscyphaceae	————	————	0.1
Insect remains	————	Animal remains	————	————	————	0.068

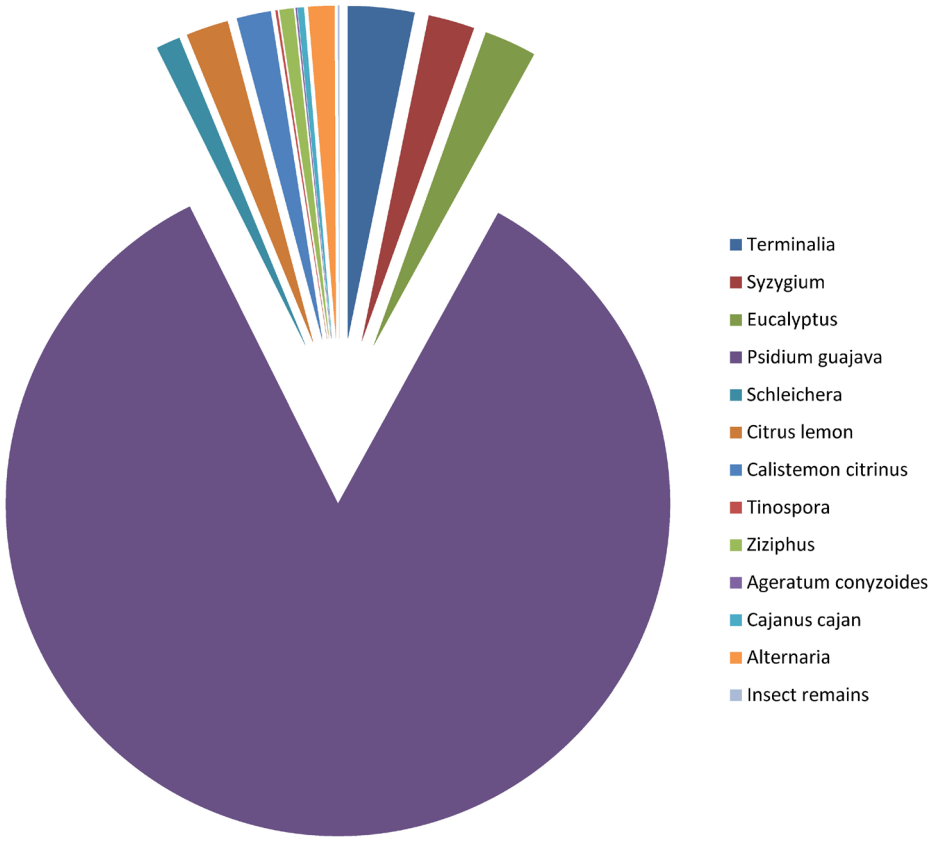


Fig. 3—Frequency percentages of the pollen types recovered from the honey sample 1 from Lucknow.

(0.069%) and *Cajanus cajan* (0.294%), as well as non–pollen palynomorphs (NPPs), such as *Alternaria* sp. (a fungal spore; 1.27%) and insect remains (0.068%) were also recovered in low values (Fig. 3).

Honey sample 2—This sample comparatively shows diversified pollen types in the pollen assemblages. Amongst the tree taxa, *Citrus lemon* (34.5%) forms the dominant component with relatively higher frequency than others retrieved in the sample and is very much evident in the pollen assemblages (Fig. 4). *Eucalyptus globules* (16.02%) and *Syzygium cuminii* (15.7%) have high frequencies in the pollen assemblages, however, *Ceiba pentandra* (4.2%), *Tinospora cordifolia* (3.84%), *Holoptelea integrifolia* and *Emblica officinalis* (2.1% each), *Cassia siamea* (1.9%), *Cassia fistula* (1.8%), *Acacia* spp. (1.49%), *Bombax ceiba* (0.74%), *Calistemon citrinus* and *Terminalia* spp. (0.3% each), *Ailanthus excelsa* (0.2%), *Diospyros melanoxylon*, Anacardiaceae, *Alangium salvifolium*, *Melia azedarach*, *Moringa oleifera* and *Manilkara zapota* (0.1% each) have moderate to low frequencies in the pollen assemblages. *Strobilanthes* spp. is the sole representative of the shrubby taxa, which have less value (1% pollen only) in the pollen assemblages. Amongst the herbaceous taxa, *Ageratum conyzoides* (9.4%), *Cajanus cajan* (7.69%), Liguliflorae/Cichorioideae (4.3%; Asteraceae family), other members of Tubuliflorae/Asteroideae (2.1%; Asteraceae family), Malvaceae and *Evolvulus alsinoides* (0.1% each) are the terrestrial herbaceous taxa, whereas Brassicaceae (1.7%), Amaranthaceae (0.53%) and *Artemisia* sp. (0.06%) are the cultural plant pollen taxa, which have variable high to moderate and less values in the pollen assemblages. Moreover, *Botryococcus* sp. (0.2%) the algal spore, as well as *Alternaria* sp. (0.74%), *Meliola* sp. and *Cookeina* sp. (0.1% each) are the fungal spores and are also recorded in less values (Fig. 4).

Honey sample 3—This sample also shows diversified pollen types in the pollen assemblages, but in terms of quantity, it has comparatively (compared to Honey sample 2) lesser frequencies of plant pollen taxa. *Eucalyptus globulus* (14%), *Prosopis juliflora* and *P. specigera* (9% each) and *Tinospora cordifolia* (6%) are the dominating tree taxa, which have been recorded in high frequencies in the pollen assemblages. However, *Cassia pentandra* (5%), *Delonix regia* (3.5%), *Syzygium cuminii* (3.2%), *Acacia* spp., *Cassia fistula*, *C. siamea* and *Ficus* spp. (3% each), Meliaceae (2%), Anacardiaceae (1.9%), Rutaceae (1.8%), *Madhuca indica*, *Shorea robusta* and *Morus alba* (1% each) and *Ixora* sp. (0.4%) have moderate to less values in the pollen assemblages. *Cichorum intybus* (Cichorioideae sub–

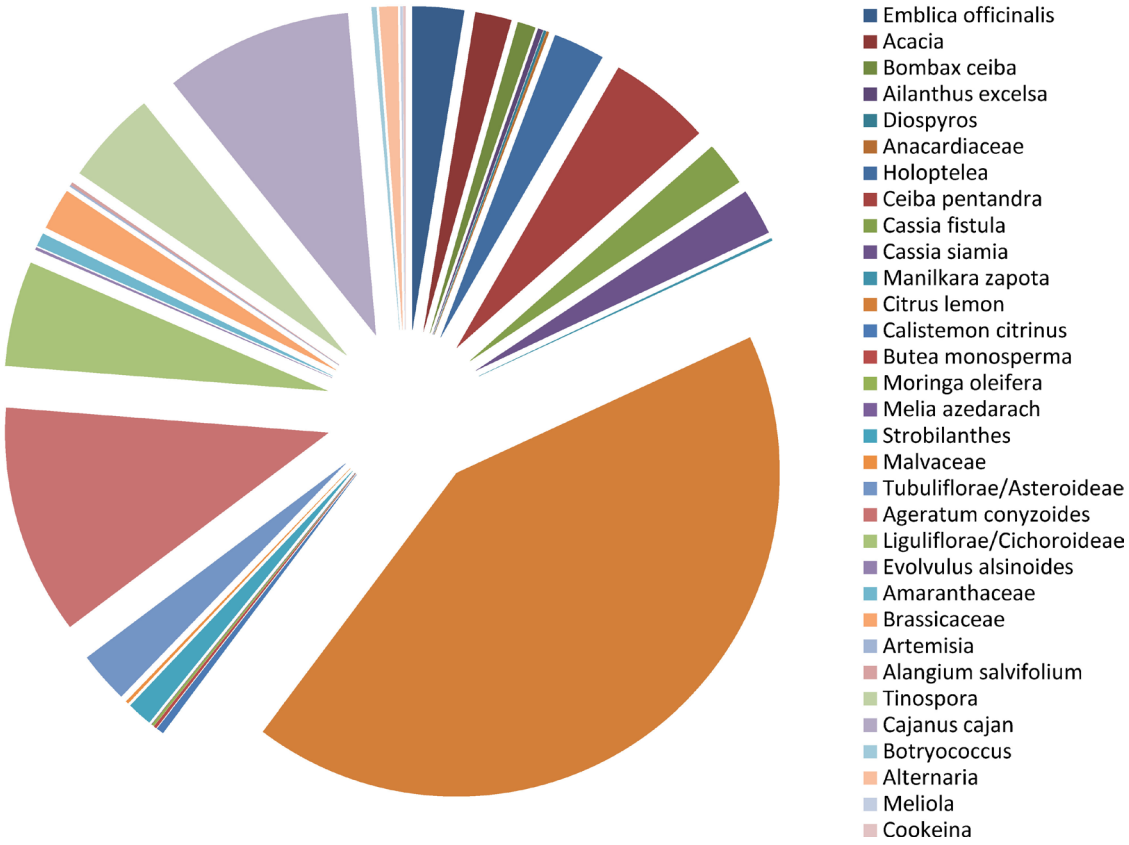


Fig. 4—Frequency percentages of the pollen types recovered from the honey sample 2 from Lucknow.

family of the Asteraceae family; 12%), *Ageratum conyzoides* (Asteroideae sub–family of the Asteraceae family; 5%) and *Xanthium strumarium* (Asteraceae family; 0.06%) are the terrestrial herbaceous taxa, which have high to less values in the pollen assemblages. Further, *Solanum* spp. (0.2%) and *Pimpinella tomentosa* (0.04%) are the wetland taxa (marshy taxa), which are recorded scantily in the pollen assemblages (Fig. 5).

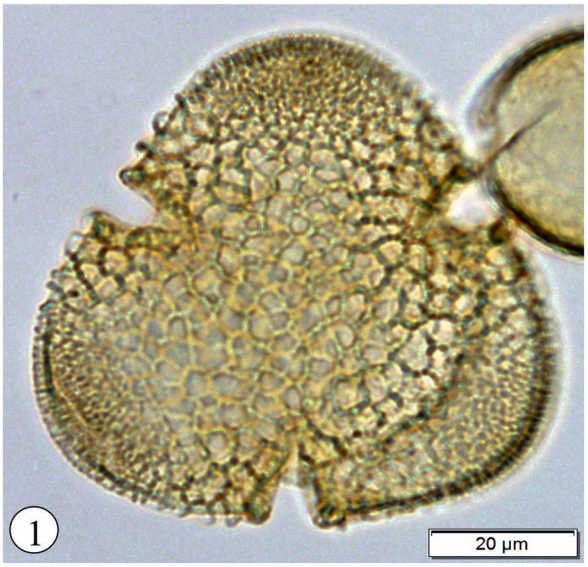
The detailed pollen morphological characteristics (Tables 1 & 2), based on the observations from LM and FESEM, of some of the recovered plant pollen taxa, which are illustrated in plates 1–9, are discussed below:

1. *Bombax ceiba* L. Pl. 1. figs 1–4. Pollen grain 3 colporate, planaperturate, amb triangular (45 × 30 µm), exine 2.5 µm thick, sexine thicker than nexine, reticulate pattern, distinctly heterobrochate, brochus also distinct although the body of the grain, tegillate.
2. *Pithecelobium dulce* (Roxb.) Benth. (cf. *Acacia* spp.) Pl. 1. figs 5–10. Polyad, 16–celled, size (45 × 40 µm), exine 2 µm thick, sexine thicker than nexine, granulate.
3. *Syzygium cuminii* (L.) Skeels Pl. 2. figs 1–6. Pollen 3 colporate, parasyncolpate, amb triangular, oblate (13 × 19 µm), colpi long and broad, os lalongate (1.6 × 1.9 µm), exine 2.5 µm thick, sexine thicker than nexine, obscure/psilate.

PLATE 1

1–4. *Bombax ceiba:* 1, 2. LM microphotographs; 3, 4. FESEM images; 1. General view showing general aspect and aperture especially brevicolpi; 2. reticulation focussed, showing big brochus; 3. Complete view showing brevicolpi, macro and microreticulation; 4. Brochus focussed.

5–10. *Pithecelobium dulce*: 5–7. LM microphotographs: polyad condition focussed; 8–10. FESEM images: 8. monad, 9. polyad, 10. granules focussed.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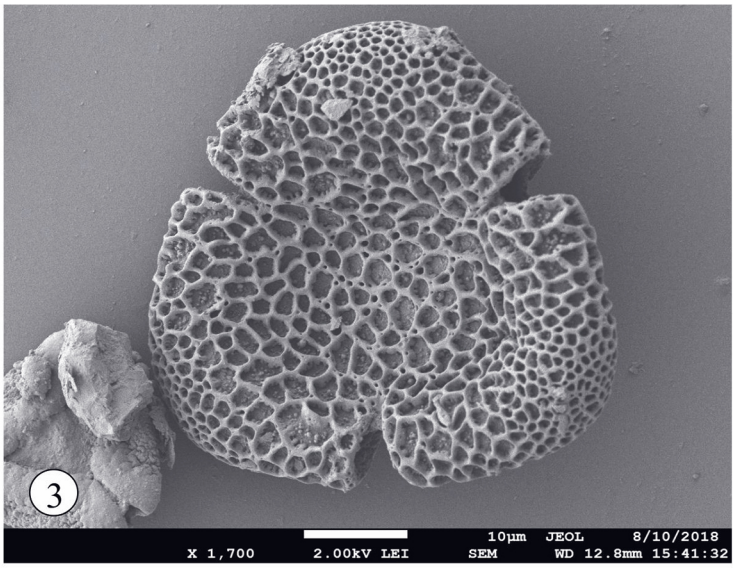
20 µm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10µm JEOL 8/10/2018
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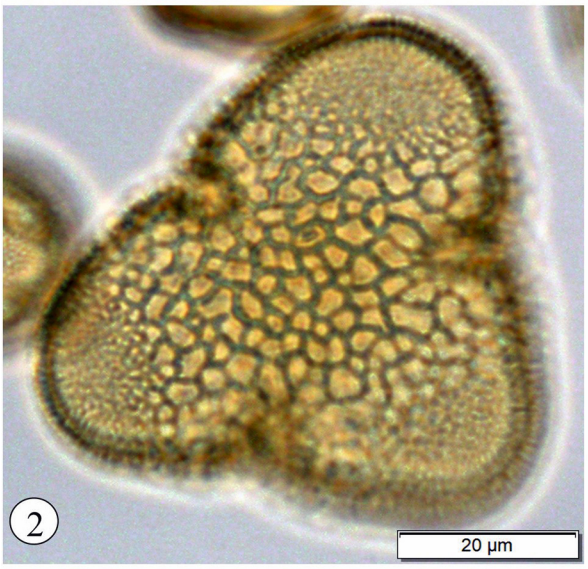
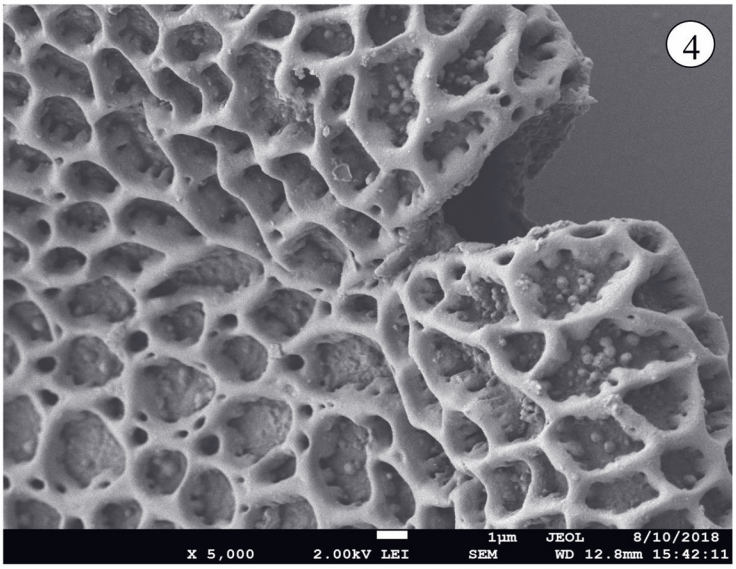

2
20 µm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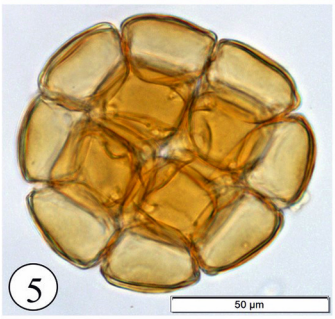
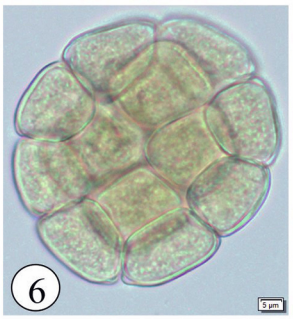
1µm JEOL 8/10/2018
X 5,000 2.00kV LEI SEM WD 12.8mm 15:42:11


5
50 µm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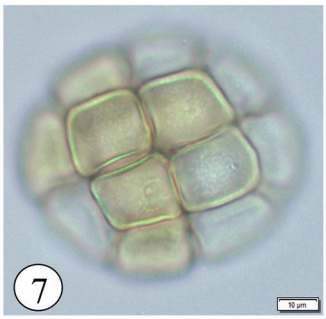
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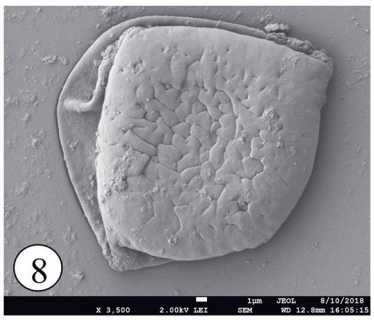
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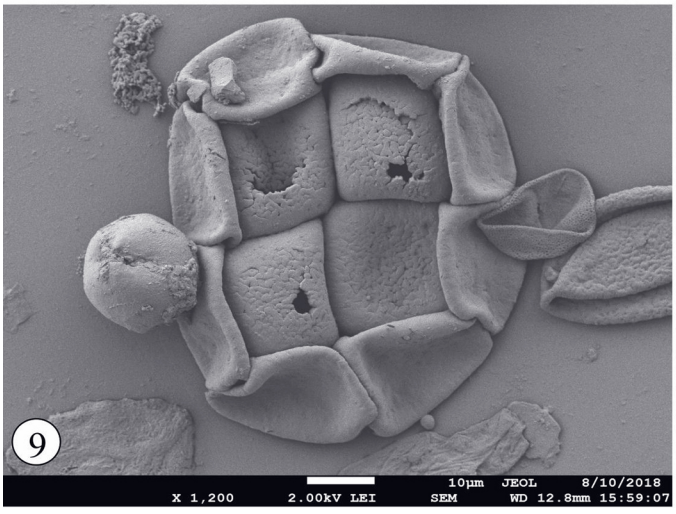
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10µm JEOL 8/10/2018
X 1,200 2.00kV LEI SEM WD 12.8mm 15:59:07

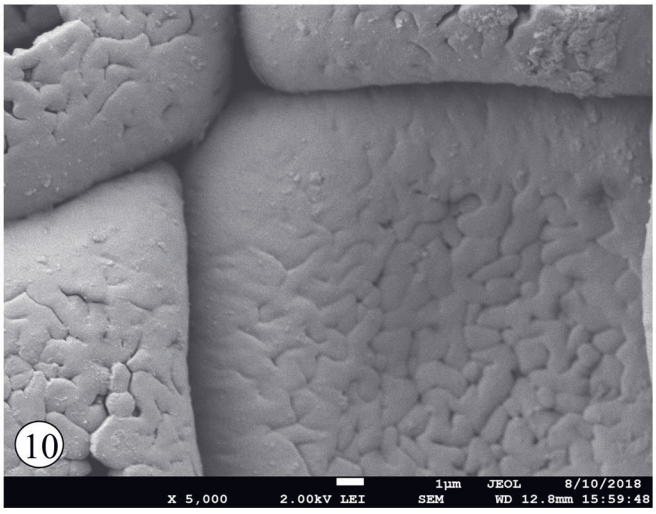

10
1µm JEOL 8/10/2018
X 5,000 2.00kV LEI SEM WD 12.8mm 15:59:48

PLATE 1

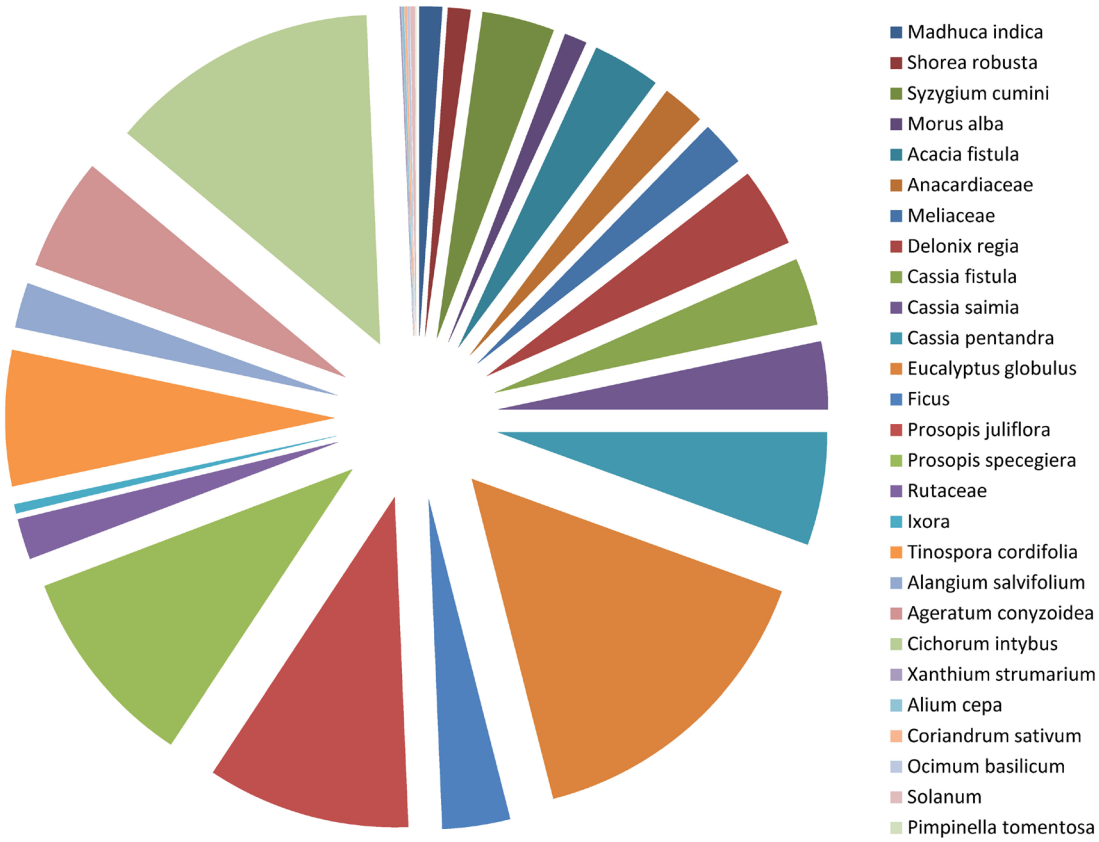


Fig. 5—Frequency percentages of the pollen types recovered from the honey sample 3 from Lucknow.

4. *Eucalyptus globulus* Labill. Pl. 2. figs 7, 9, 13–15. Pollen grain 3 colporate, syncolpate, prolate (21 × 13 µm), amb triangular, exine 1.5 µm thick, sexine as thick as nexine, psilate pattern.
5. *Psidium guajava* L. Pl. 2. figs 8, 11, 12. Pollen grain 3 colporate, colpi long (11 µm), ora lalongate oblate (14 × 26 µm), amb triangular, exine 1.5 µm thick, sexine as thick as nexine, granular pattern.
6. *Ziziphus mauritiana* Lam. Pl. 2. fig. 10. Pollen grain 3 colporate, colpi long (9 µm) and narrow elliptic, tips acute, prolate (24 × 16 µm), amb triangular, exine 2 µm thick, sexine as thick as nexine, rugate pattern.
7. *Schleichera oleosa* (Lour.) Oken. Pl. 3. figs 1, 3, 4. Prolate (27 × 19 µm), bilateral, isopolar, amb triangular
8. *Terminalia arjuna* (Roxb. Ex DC.) Wight et Arn. Pl. 3. fig. 2. Pollen grain 3 colporate, heterocolpate, prolate (19 × 15 µm), colpi 14 µm long, os lalongate (2 × 1.5 µm), exine 1.5 µm thick, sexine thicker than nexine, obscure/psilate pattern.
9. *Tinospora cordifolia* (Thun.) Miers Pl. 3. figs 5–7. Pollen grain trizonocolporoidate, colpi almost linear, margins thick and wide (0.75 µm long and 1 µm wide), os not distinct, exine 1.5 µm thick, reticulate pattern.
10. *Amaranthus* spp. L. Pl. 4, figs 1, 3, 4. Pollen grain pantoporate, sub–oblate (20 × 20 µm), pore almost

27 µm, tricolporoidate–syncolpate, colpi long, tips acute, ora circular. Exine 1.5 µm thick, sexine as thick as nexine. Ornamentation striate.

PLATE 2

1–6. *Syzygium cuminii*: 1–4. FESEM microphotographs; 5 & 6. LM images; 1. General view showing apertures and parasyncolpate condition; 2–4. parasyncolpate condition and psilate pattern; 5, 6. showing parasyncolpate condition.

7, 9, 13–15. *Eucalyptus globulus:* 7, 13, 14. LM microphotographs; 9, 15. FESEM images: 7, 13, 14.–parasyncolpate condition, exine focussed; 9, 15. colpi and parasyncolpate condition focussed

8, 11, 12. *Psidium guajava*: 8, 11, 12. colporate condition seen and exine focussed; 10. *Ziziphus mauritiana,* syncolpate condition and exine focussed.

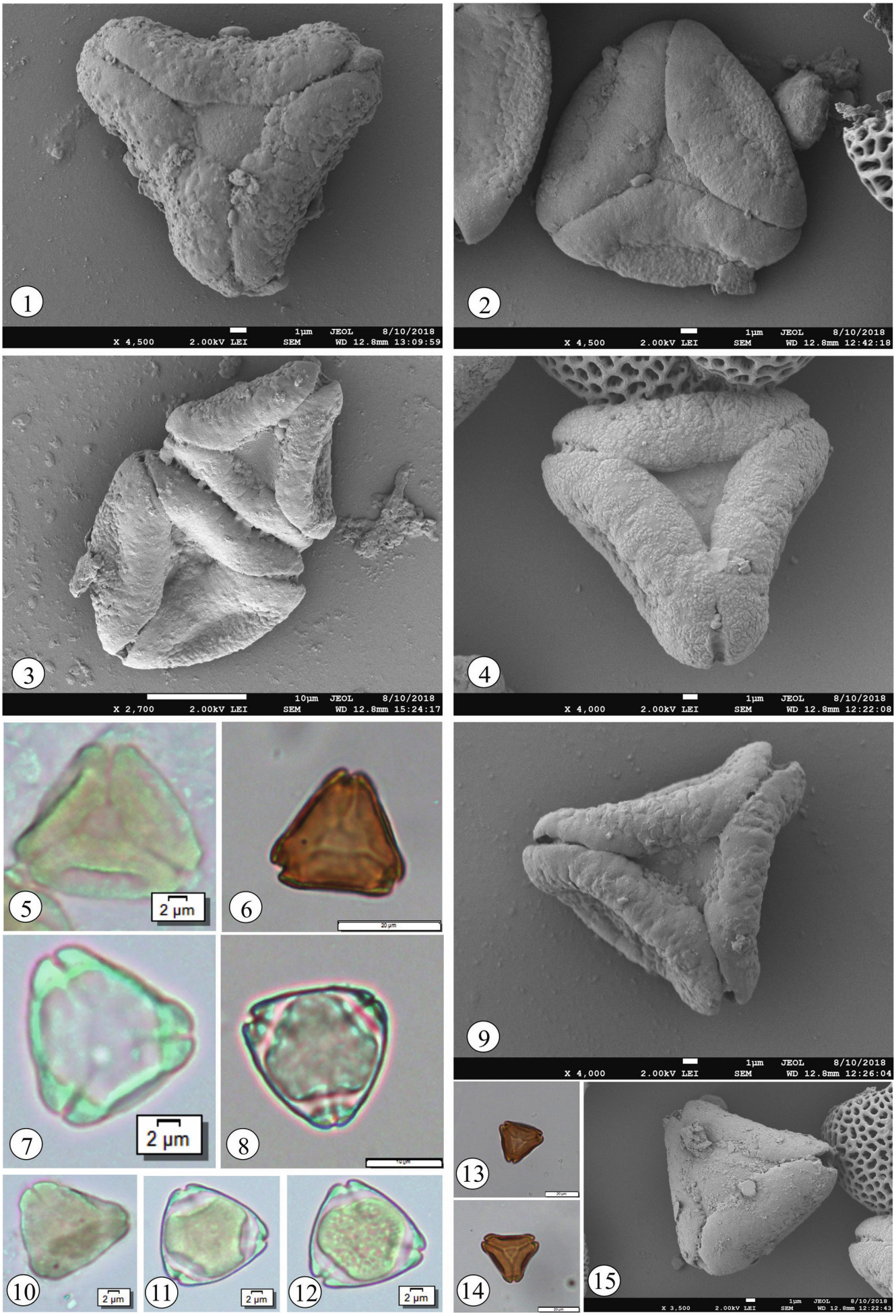

1
X 4,500 2.00kV LEI 1µm JEOL SEM 8/10/2018 WD 12.8mm 13:09:59
2
X 4,500 2.00kV LEI 1µm JEOL SEM 8/10/2018 WD 12.8mm 12:42:18
3
X 2,700 2.00kV LEI 10µm JEOL SEM 8/10/2018 WD 12.8mm 15:24:17
4
X 4,000 2.00kV LEI 1µm JEOL SEM 8/10/2018 WD 12.8mm 12:22:08
5
2 µm
6
7
2 µm
8
9
X 4,000 2.00kV LEI 1µm JEOL SEM 8/10/2018 WD 12.8mm 12:26:04
10
2 µm
11
2 µm
12
2 µm
13
14
15
X 3,500 2.00kV LEI 1µm JEOL SEM 8/10/2018 WD 12.8mm 12:22:43

PLATE 2

circular or oblate, pore diameter (2 × 2 µm), exine 2.5 µm thick, psilate or scabrate pattern.

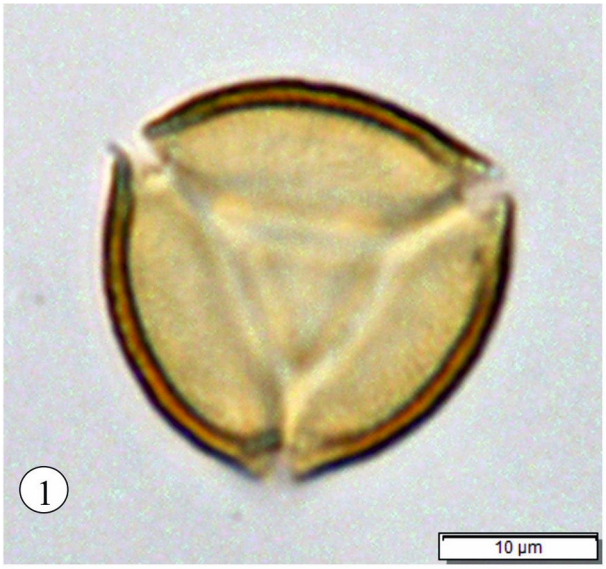
11. *Ageratum conyzoides* L. Pl. 4, figs 2, 5, 6. Pollen grain 3–colporate, sub–oblate (14 × 16 µm), colpi long and broad (length 8 µm, width 2 µm), apices acute; margin smooth; os distinct, lalongate (2 × 1 µm), exine 1 µm thick, sexine slightly thicker than nexine, pattern echinate (spinules length 2 µm, width 1 µm), tegillate.
12. *Cichorum intybus* L. Pl. 5. figs 1–4. Pollen grain trizonoporate, prolate spheroidal (23 x 25 µm), polar and equatorial view circular, pore circular (4 × 4 µm) and covered by ridges, exine 5.5 µm thick, echinocolphate pattern.
13. *Cajanus cajan* (L.) Millsp. Pl. 5. figs 5, 6. Pollen grain 4 colporate (tetracolporate), vestibulate condition, colpi long and broad (7 µm long and 2 µm width), pores almost circular (3.5 × 3.5 µm), psilate pattern.
14. *Moringa oleifera* Lamarck Pl. 5. fig. 7. Pollen grain trizonocolporate, colpi long and broad (length 7 µm, width 1.5 µm), outline almost circular (20 × 20 µm), side of colpi tapering towards the poles, tips acute, os circular (1.5 × 1.5 µm), distinctly costate pattern.
15. *Ailanthus excelsa* Roxb. Pl. 5. fig. 8. Pollen grain trizonocolporate, polar view triangular, obtuse, convex, colpi linear (length 13 µm, width 1.5 µm), exine 2 µm thick, psilate pattern or faintly perforate.
16. *Brassica compestris* L. Pl. 5. fig. 9. Pollen grain 3 colpate, colpi 13 µm long and 1.5 µm wide, outline circular (21 × 21 µm), reticulate pattern.
17. *Butea monosperma* (Lamk.) Taub. Pl. 5. fig. 10. Pollen grain 3 colporate, prolate spheroidal (39 × 35 µm), os mostly circular (5 × 5 µm), grain protruded at the equator, exine 1.5 µm thick, sexine slightly thicker than nexine, granulate pattern.
18. *Citrus lemon* (L.) Burm. F. Pl. 6. figs 1–3. Pollen grain tetrazonocolporate, colpi long and broad (length 25 µm, width 1.5 µm), amb circular (47 × 47 µm), reticulate pattern.
19. *Cassia fistula* L. Pl. 6. figs 4–6. Pollen grain 3 colporate, colpi long and wide (27 µm long, 2 µm wide), prolate (29 × 20 µm), amb triangular, obtuse convex, tips acuminate, ora lolongate, exine 2 µm thick, sexine thicker than nexine, reticulate pattern.
20. *Manilkara zapota* (L.) P. Royen Pl. 6. fig. 7. Pollen grain 3 colporate, colpi 13 µm and 2 µm wide), amb circular (18 × 18 µm), exine 1.5 µm thick, pattern psilate or homobrochate.
21. *Coriandrum sativum* L. Pl. 6. figs 8, 12. Pollen grain 3 colporate, synorate condition, colpi long and broad (29 µm long, 1.5 µm wide), os lalongate, exine 1.5 µm thick, granulate pattern.
22. *Alium cepa* L. Pl. 6. fig. 9. Pollen grain monosulcate, prolate (25 × 18 µm), sulcus long and broad (13 µm long, 1.5 µm wide), exine 1.5 µm thick, psilate pattern or granulate.
23. *Morus alba* L. Pl. 6. figs 10, 11. Pollen grain 2 porate (biporate), pores (1.5 × 1.5 µm), amb almost circular (30 × 30 µm), psilate pattern.
24. *Cassia siamea* Lam. Pl. 7. figs 1, 3. Pollen grain 3 colpate, syncolpate, prolate (45 × 30 µm), amb triangular, exine 2 µm thick, sexine as thick as nexine, reticulate pattern.
25. *Prosopis juliflora* (Sw) DC. Pl. 7. figs 2, 4, 7. Pollen grain 3 colporate, syncolpate colpi tapering towards end, tips acute, ora lalongate, subprolate (38 × 30 µm), amb circular, exine 2.9 µm thick, sexine thicker than nexine, reticulate pattern.
26. *Melia azedarach* L. Pl. 7. figs 5, 6. Pollen grain tetrazonocolporate, vestibulate grain, colpi long and broad (27 µm long, 1.5 µm wide), exine 2 µm thick, psilate or microreticulate pattern.
27. *Callistemon citrinus* (Curtis) Skeels. R. Br. Pl. 7. fig. 8. Pollen grain 3 colporate, syncolpate, colpi 8 µm long, 0.6 µm wide, tips acute, ora lolongate, oblate (13 × 18 µm), amb triangular, exine 2.5 µm thick, sexine thicker than nexine, psilate pattern.
28. *Cassia fistula* L. Pl. 7. figs 9, 10, 13. Pollen grain 3–colporate, sub–prolate (30 × 26 µm), colpi long and broad (length 24 µm, width 2 µm), apices acute, margin smooth, os lalongate (2 × 1 µm), exine 1 µm thick, sexine slightly thicker than nexine, pattern distinctly reticulate, homobrochate, tegillate.
29. *Alangium salvifolium* L. Wang. Pl. 7. figs 11, 12. Pollen grain tetrazonoporate, outline circular, pores not clear, exine 3.7 µm thick, verrucate pattern.

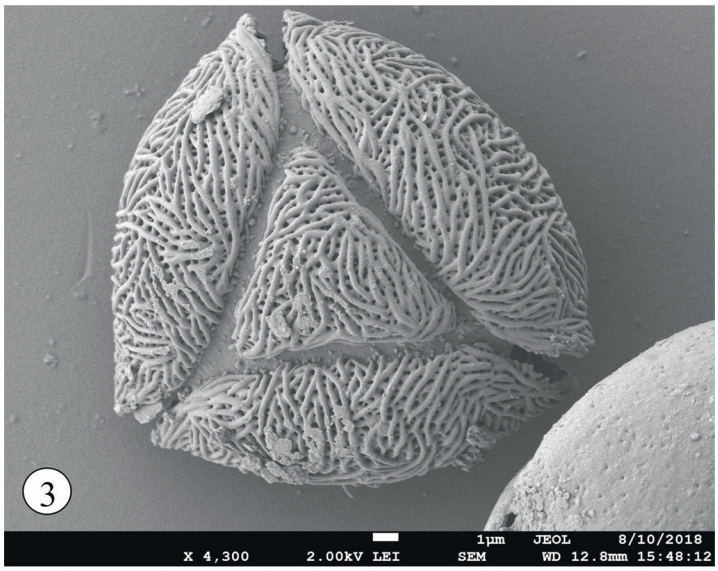
PLATE 3

1, 3, 4. *Schleichera oleosa*: 1. LM microphotograph; 3 & 4. FESEM images. 1. General view showing exine, parasyncolpate condition and striate pattern; 3. Complete view showing parasyncolpate condition, striate pattern; 4. Striation focussed.

2. *Terminalia arjuna* Polar view: LM microphotograph showing the heterocolpate condition and exine.

5–7. *Tinospora cordifolia:* 5, 6. LM microphotographs showing trizonocolporoidate condition, reticulate pattern, 7. trizonocolporoidate and reticulation focussed.


1
10 µm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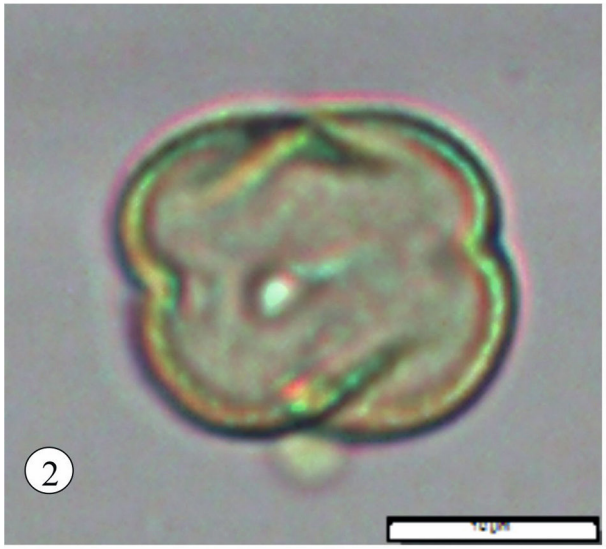
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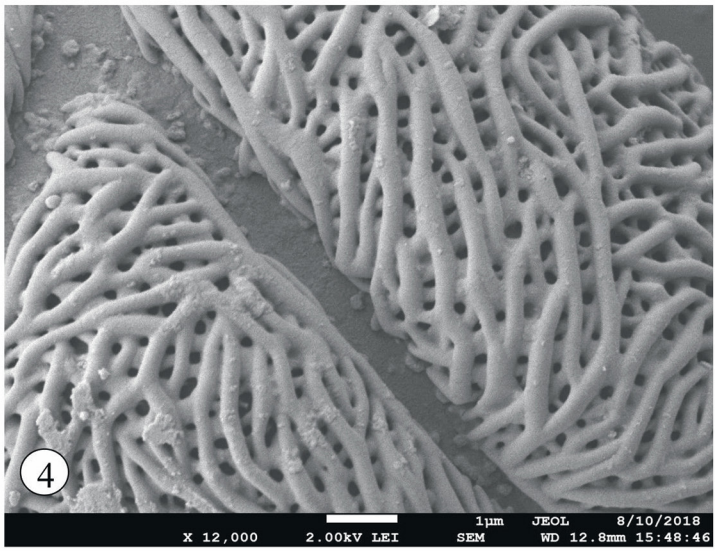
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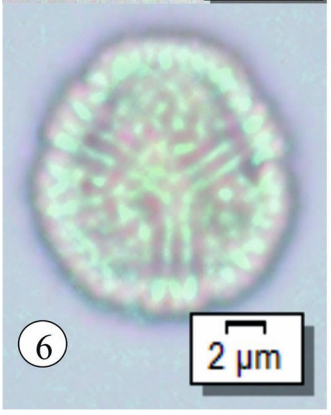
5
10 µm


6
2 µm

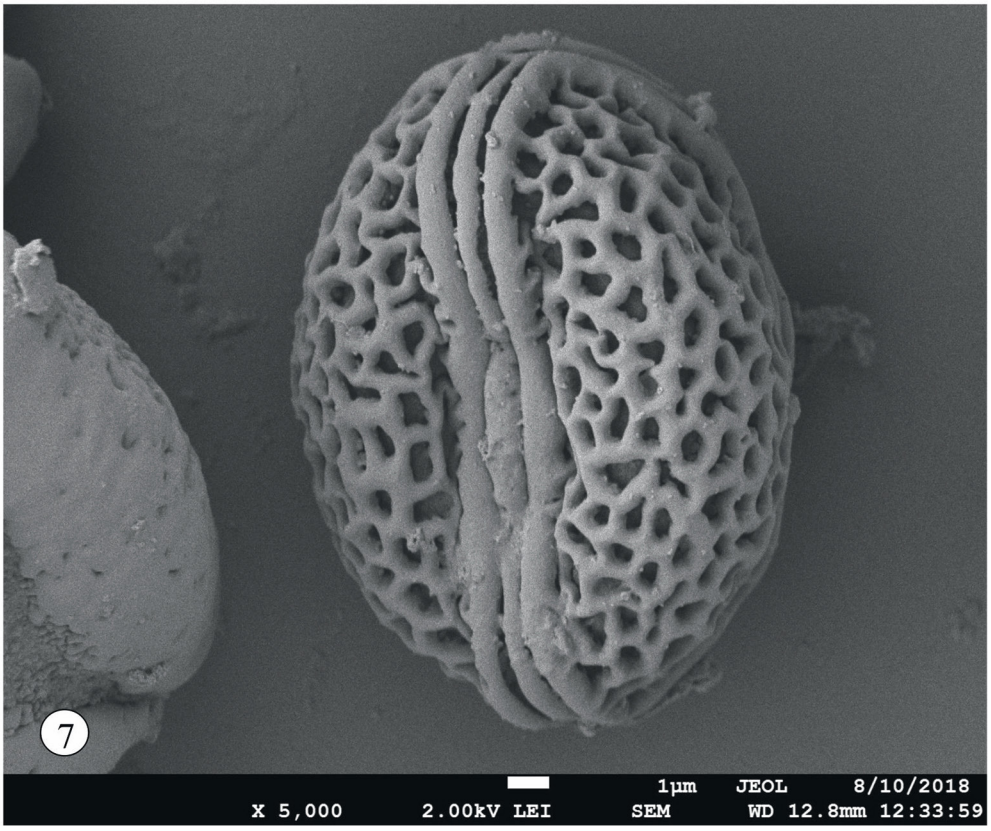

7
1µm JEOL 8/10/2018
X 5,000 2.00kV LEI SEM WD 12.8mm 12:33:59

PLATE 3

30. *Evolvulus alsinoides* L. Pl. 8. fig. 1. Pollen grains octazonocolpate, outline circular, subprolate (59 × 47 µm), exine 3.9 µm thick, psilate or foveolate pattern.
31. *Ocimum basilicum* L. Pl. 8. figs 2, 3. Pollen grain octazonocolpate, colpi linear (17 µm long and 1.7 µm wide), outline circular (59 × 59 µm), reticulate pattern.
32. *Emblica officinalis* Gaertn. (cf. *Phyllanthus angustissimus* L.) Pl. 8. figs 4–6. Pollen grain 4 colporate, oblate spheroidal (30 × 30 µm), colpi long and broad (length 23 µm, width 3 µm), os circular, exine 2 µm thick, sexine thicker than nexine, distinctly reticulate pattern, heterobrochate.

Carbon stable isotope

The carbon stable isotopic ($\delta^{13}C$) values of all the natural honey varied within ~ –25.14 to –26.70‰ (Table 3). The filtered samples were observed under microscope and no pollen was observed in honey. In 'sample 1' it changed from –26.16 to –25.62‰. In sample 2 and 3 it changed from –25.14 to –24.40‰ and –26.70 to –25.90‰, respectively. In all the cases, the values enhanced rather than depletion and the difference is 0.54 to 0.80‰. The residue which contained only bulk pollen provided enhanced values as –24.81, –24.10 and –25.40‰ in samples 1, 2 and 3, respectively. These values show a difference of ~ 1.04 to 1.35‰ as compared to unsieved honey. The $\delta^{13}C$ values of honey obtained from market is same in all three samples, i.e. –27‰. All these commercial honey samples were observed under light microscope and no pollen was recorded as these are supposed to be filtered before packing as mentioned in the label on bottles.

DISCUSSION

The present melissopalynological investigation provides new insights into the pollen composition in the honey samples from Lucknow. The pollen assemblages in each honey sample suggest the diversity of nectar contributing taxa in all the three honey samples. Further, the study helps in understanding the kind of honey, i.e. whether they are monofloral/unifloral, bifloral, and/or multifloral honey (as per the guidelines of the International Commission of Bee Botany: ICBB, 1970) and could also in assessing their fidelity i.e. whether the samples are pure or adulterated by careful examination of the pollen composition in each honey sample.

Table 3—Values of $\delta^{13}C$ in filtered/ unfiltered natural honey from hive and commercial honey.

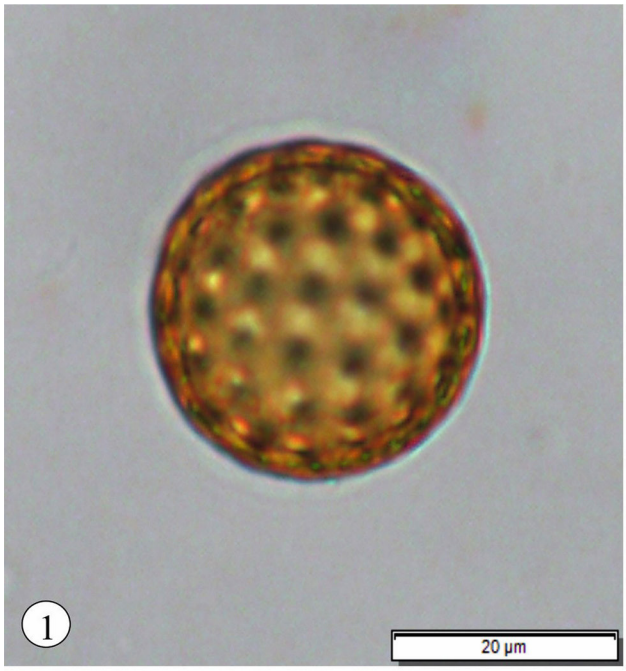
Standards		$\delta^{13}C$
Ox–II	Average	–17.80
	Stdev	0.48
Sulfanilamide	Average	–28.02
	Stdev	0.12

Sample type	Unfiltered	$\delta^{13}C_{PDB}$	Difference ‰
	A		
Sample 1	Natural Honey	–26.16	
Sample 2	Natural Honey	–25.14	
Sample 3	Natural Honey	–26.70	
	B		
	Sieved (filtrate–without pollen)		A–B
Sample 1	Natural Honey	–25.62	0.54
Sample 2	Natural Honey	–24.40	0.74
Sample 3	Natural Honey	–25.90	0.80
	C		
	Sieved (residue–bulk pollen)		A–C
Sample 1	Natural Honey	–24.81	1.35
Sample 2	Natural Honey	–24.10	1.04
Sample 3	Natural Honey	–25.40	1.3
	Commercial Honey (filtered and without pollen)		
Sample 1	Commercial Honey 1	–27	
Sample 2	Commercial Honey 2	–27	
Sample 3	Commercial Honey 3	–27	

PLATE 4

1, 3, 4. *Amaranthus* sp.*:* 1. LM microphotograph; 3, 4. FESEM images; 1. General view showing pantoporate condition, 2. pantoporate condition and granules focussed, 4. single pore focussed and granules also seen frequently

2, 5, 6. *Ageratum conyzoides:* 2. LM microphotograph showing the colporate condition and echineae; 5, 6. FESEM images: 5. echineae (bulbous spines) focussed, 6. complete grain with echineae)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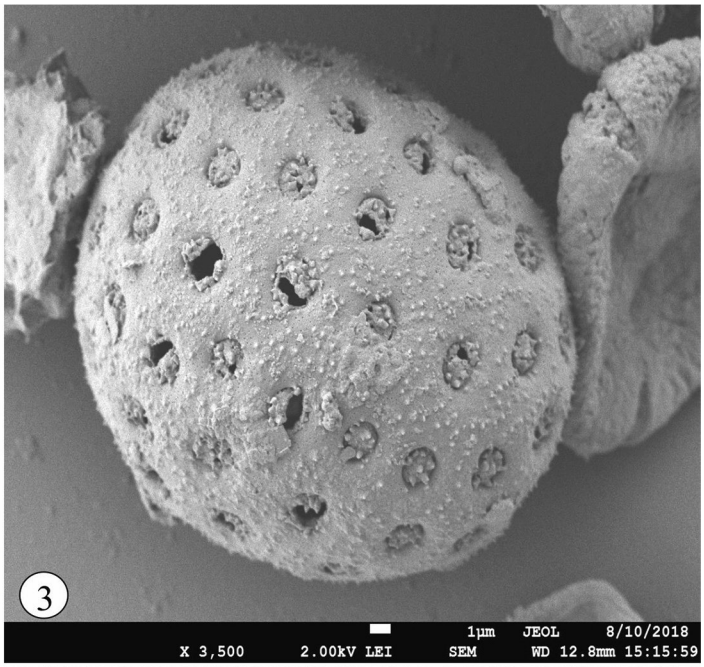
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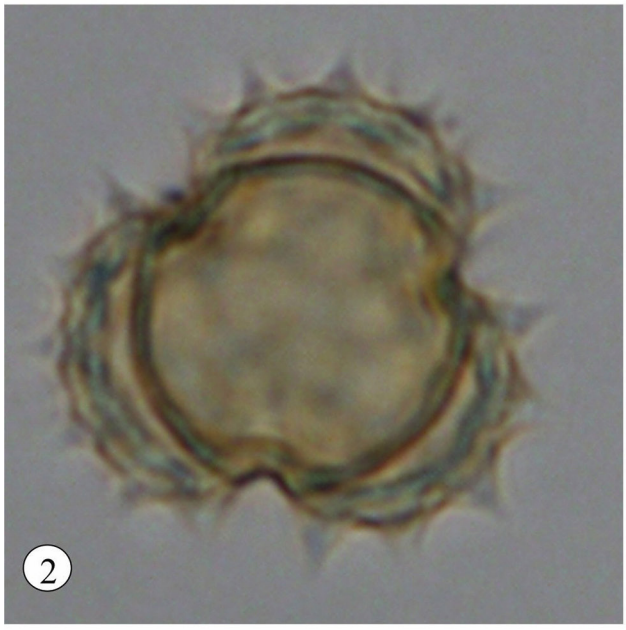
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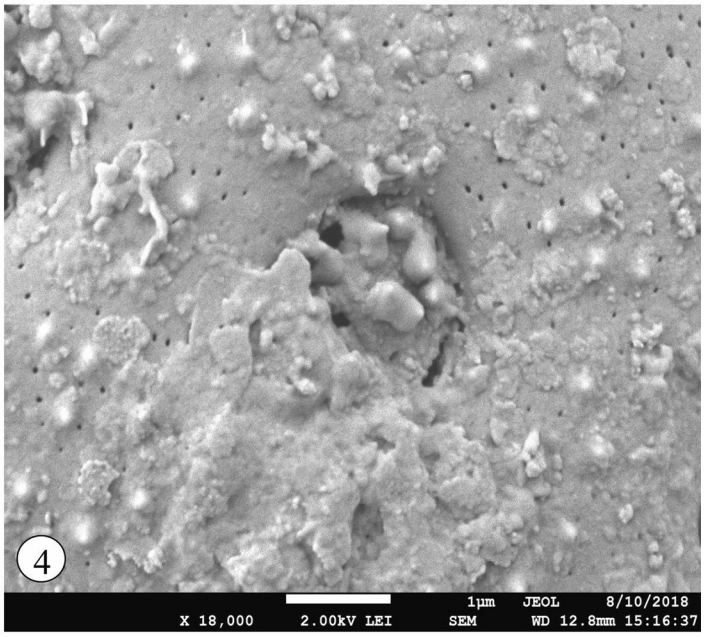
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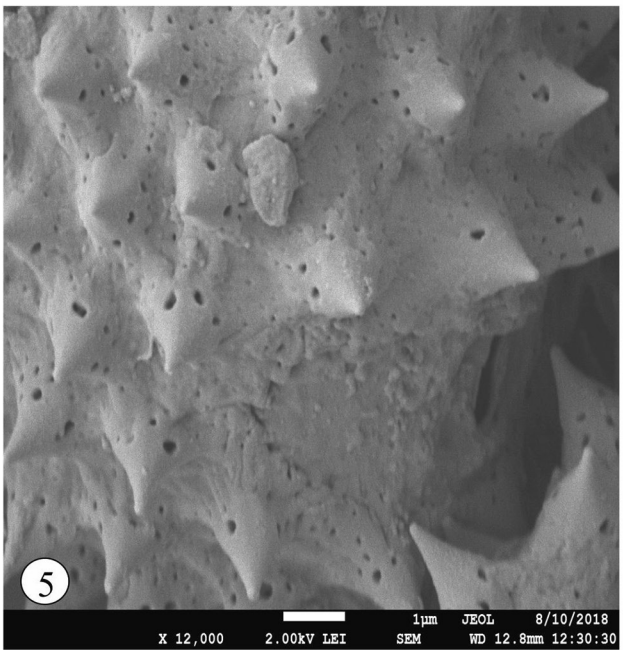
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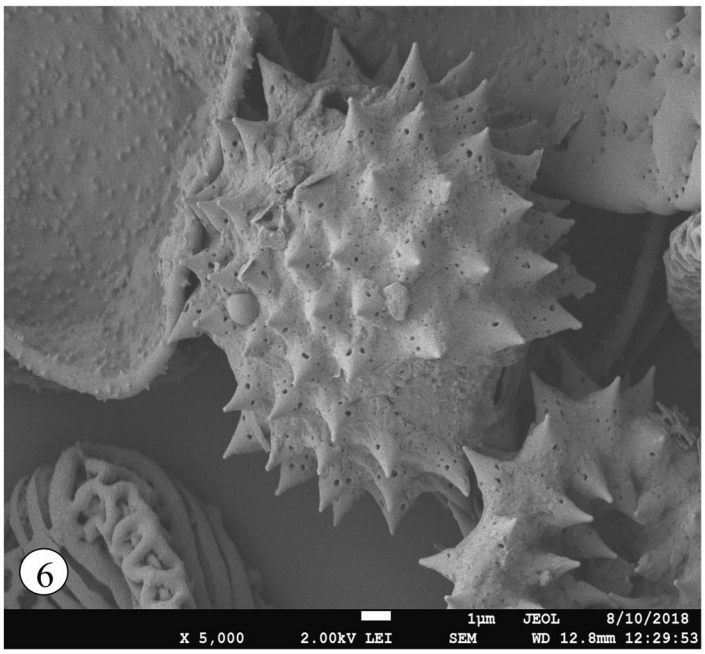

6
1µm JEOL 8/10/2018
X 5,000 2.00kV LEI SEM WD 12.8mm 12:29:53

PLATE 4

Honey sample 1 has exhibited that the pollen of *Psidium guajava* has excessively high values (~85%), which suggests that this plant is the main source (predominant pollen type) of pollen and nectar of this honey sample and, hence, the honey is monofloral/unifloral (ICBB, 1970). *Terminalia* spp., *Eucalyptus globulus*, *Syzygium cuminii*, *Citrus lemon*, *Callistemon citrinus*, *Schleichera oleosa* and *Tinospora cordifolia* are the other tree taxa (important minor pollen types), which could have served as the secondary source of forage by the bees around the study area. *Ziziphus mauritiana*, *Ageratum conyzoides* and *Cajanus cajan* are the minor pollen types, which are extremely rare and which could have occasionally exploited by the bees for nectar and pollen. The spores of *Alternaria* sp. and insect remains were also recorded (Table 1). Honey sample 2 has shown that *Citrus lemon* (34.5%) and *Eucalyptus globulus* (16.02%), *Syzygium cuminii* (~16%) (Secondary pollen types); *Ageratum conyzoides*, Liguliflorae/ Cichorioideae, *Cajanus cajan*, *Ceiba pentandra*, *Tinospora cordifolia* (important minor pollen types), as well as *Holoptelea integrifolia*, *Emblica officinalis* and Tubuliflorae/Asteroideae, *Cassia siamea*, *Cassia fistula*, Brassicaceae, *Bombax ceiba*, *Terminalia* spp., *Ailanthus excelsa*, *Diospyros melamoxylon*, *Butea monosperma*, *Melia azedarach*, *Moringa oleifera*, Anacardiaceae, *Manilkara zapota*, *Calistemon citrinus*, Malvaceae, *Evolvulus alsinoides*, *Artemisia* sp., Amaranthaceae and *Alangium salvifolium* (minor pollen types) are the plant taxa visited by bees for nectar and pollen. The pollen floral assemblages of this honey

PLATE 5

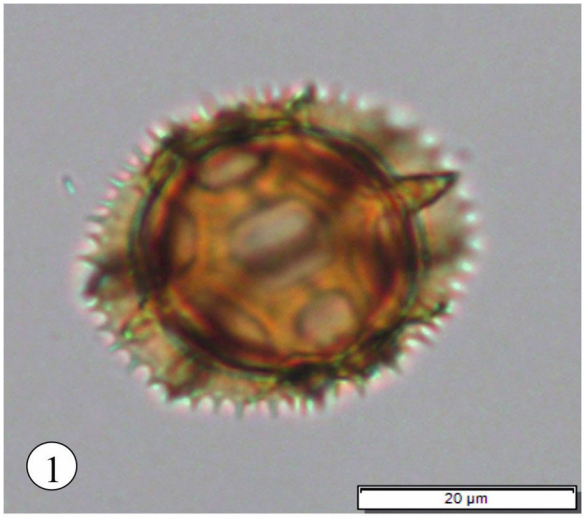
1–4. *Cichorum intybus:* 1, 2. LM microphotographs; 3, 4. FESEM images showing general view of the grain, trizonoporate condition and echinocolphate pattern focussed.
5, 6. *Cajanus cajan:* 5. LM microphotograph; 6. FESEM image showing 4 tetracolporate and vestibulate condition, psilate pattern focussed.
7. *Moringa oleifera:* LM image showing Pollen grain trizonocolporate condition and distinctly costate pattern.
8. *Ailanthus excelsa:* LM microphotograph showing trizonocolporate, colpi focussed, psilate pattern or faintly perforate.
9. *Brassica compestris:* FESEM microphotograph showing colpate condition, reticulate pattern focussed.
10. *Butea monosperma:* LM microphotograph showing general view, exine, colpi and circular os focussed).

PLATE 6

1–3. *Citrus lemon:* LM microphotographs showing tetrazonocolporate condition, reticulate pattern.
4–6. *Cassia fistula:* LM microphotographs showing colporate condition and reticulate pattern and homobrochate condition.
7. *Manilkara zapota:* LM microphotograph demonstrating the colporate condition, psilate or homobrochate pattern.
8, 12. *Coriandrum sativum:* 8. LM microphotograph showing colporate and synorate condition; 12. FESEM image showing colporate and synorate condition, granules also focussed.
9, 11. *Alium cepa:* 9. LM microphotograph showing the sulcus; 11. FESEM microphotograph showing the sulcus and granules.
10. *Morus alba:* LM microphotograph showing the exine and pores, as well as psilate pattern.

PLATE 7

1, 3. *Cassia siamea:* LM microphotographs showing the exine and syncolpate condition, microreticulate and homobrochate condition also focussed.
2, 4, 7. *Prosopis juliflora:* LM microphotographs showing colporate condition, syncolpite condition and exine focussed, microreticulate pattern and homobrochate condition also seen focussed).
5, 6. *Melia azedarach:* 5. polar view; 6. equatorial view– LM images showing tetrazonocolporate, vestibulate grain, psilate or microreticulate pattern.
8. *Callistemon citrinus:* LM microphotograph showing colporate condition, exine and syncolpate condition focussed.
9, 10, 13. *Cassia fistula:* FESEM images showing colporate condition, exine and lolongate os focussed.
11, 12. *Alangium salvifolium:* FESEM microphotographs showing tetrazonoporate, verrucate pattern).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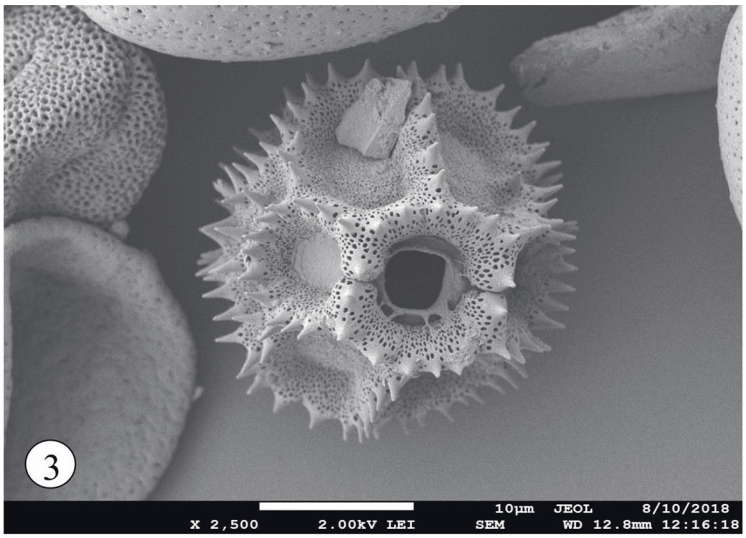
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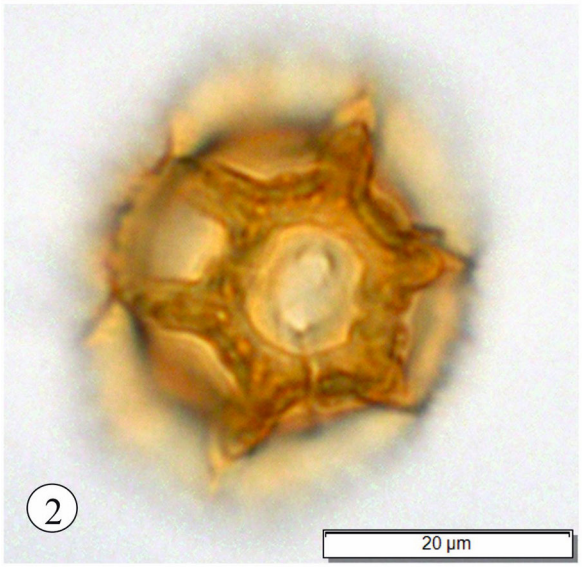
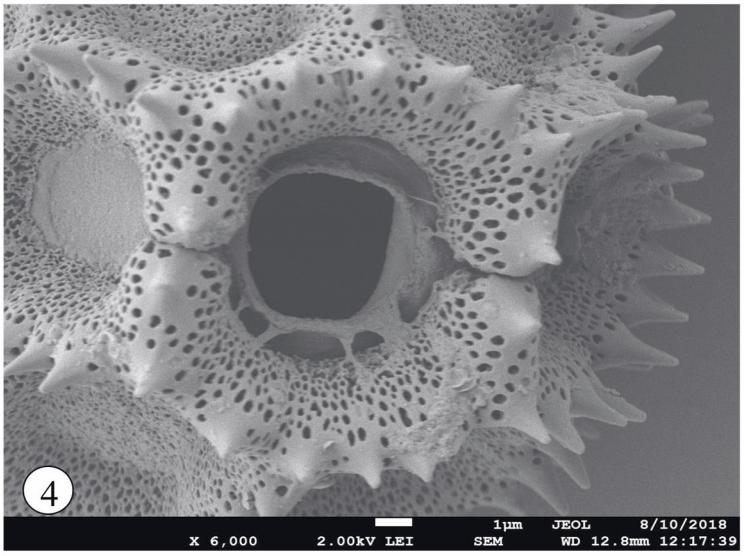

2
20 µm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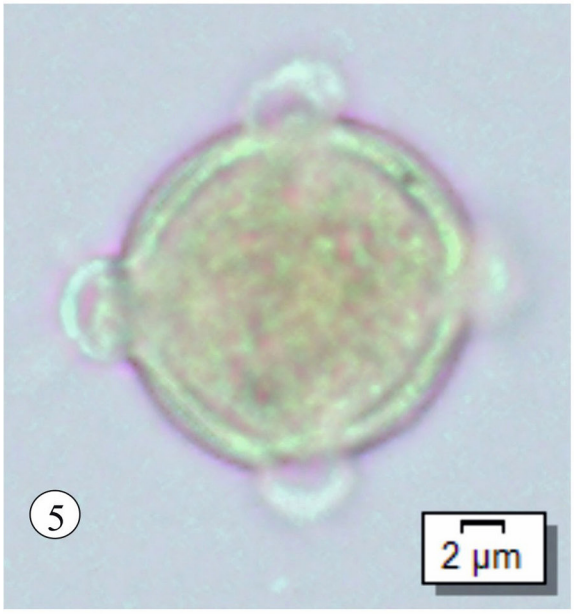
X 6,000 2.00kV LEI SEM WD 12.8mm 12:17:39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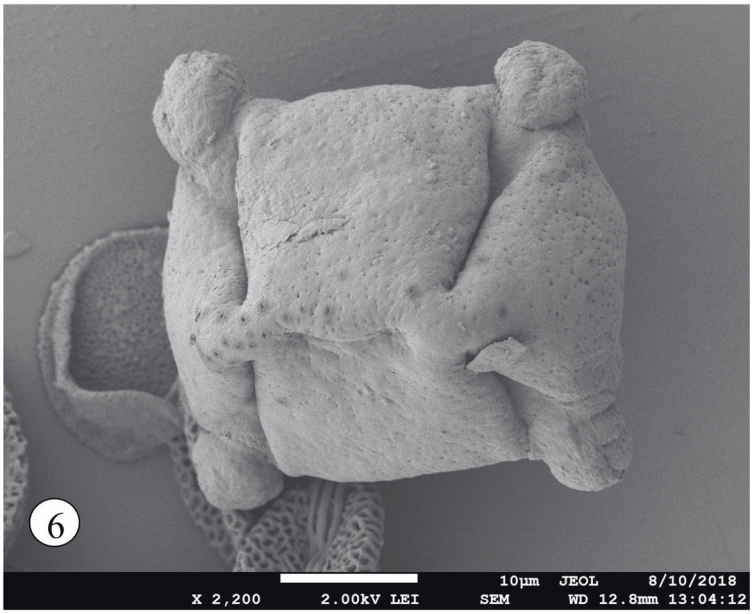
2 µm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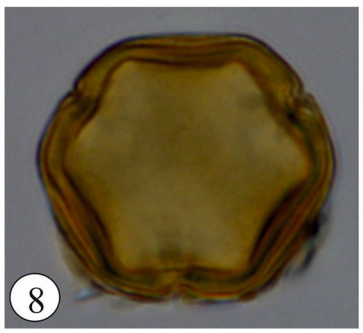
10µm JEOL 8/10/2018
X 2,200 2.00kV LEI SEM WD 12.8mm 13:04:12


7
20 µm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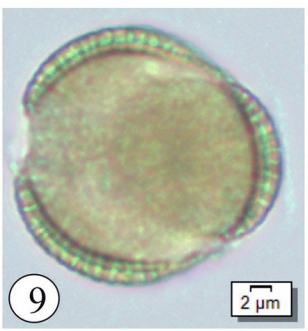
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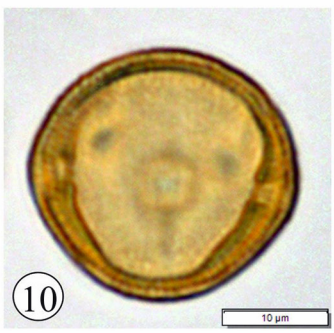
2 µm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10 µm

PLATE 5

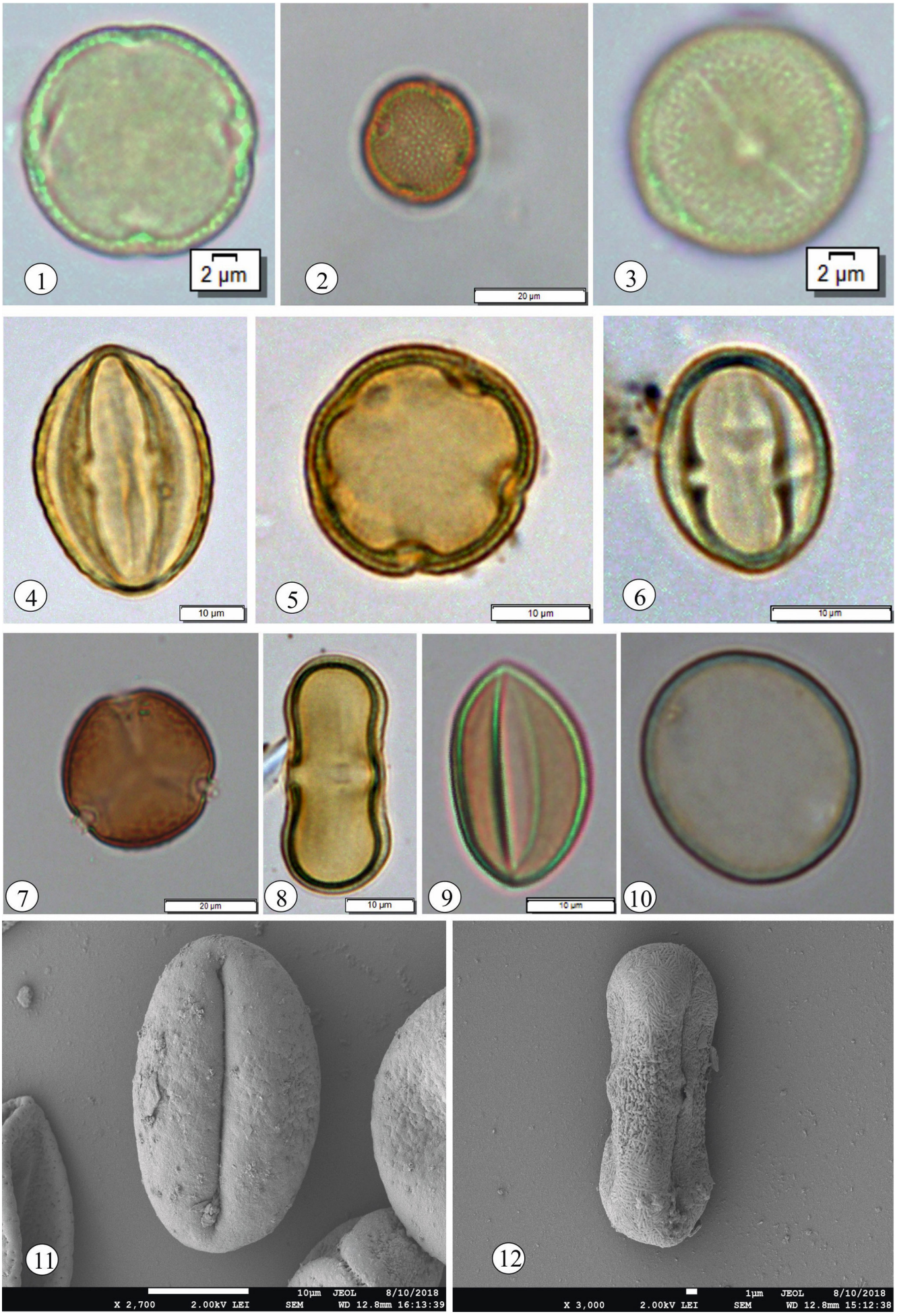

1
2 µm
2
20 µm
3
2 µm
4
10 µm
5
10 µm
6
10 µm
7
20 µm
8
10 µm
9
10 µm
10
11
10µm JEOL 8/10/2018
X 2,700 2.00kV LEI SEM WD 12.8mm 16:13:39
12
1µm JEOL 8/10/2018
X 3,000 2.00kV LEI SEM WD 12.8mm 15:12:38

PLATE 6

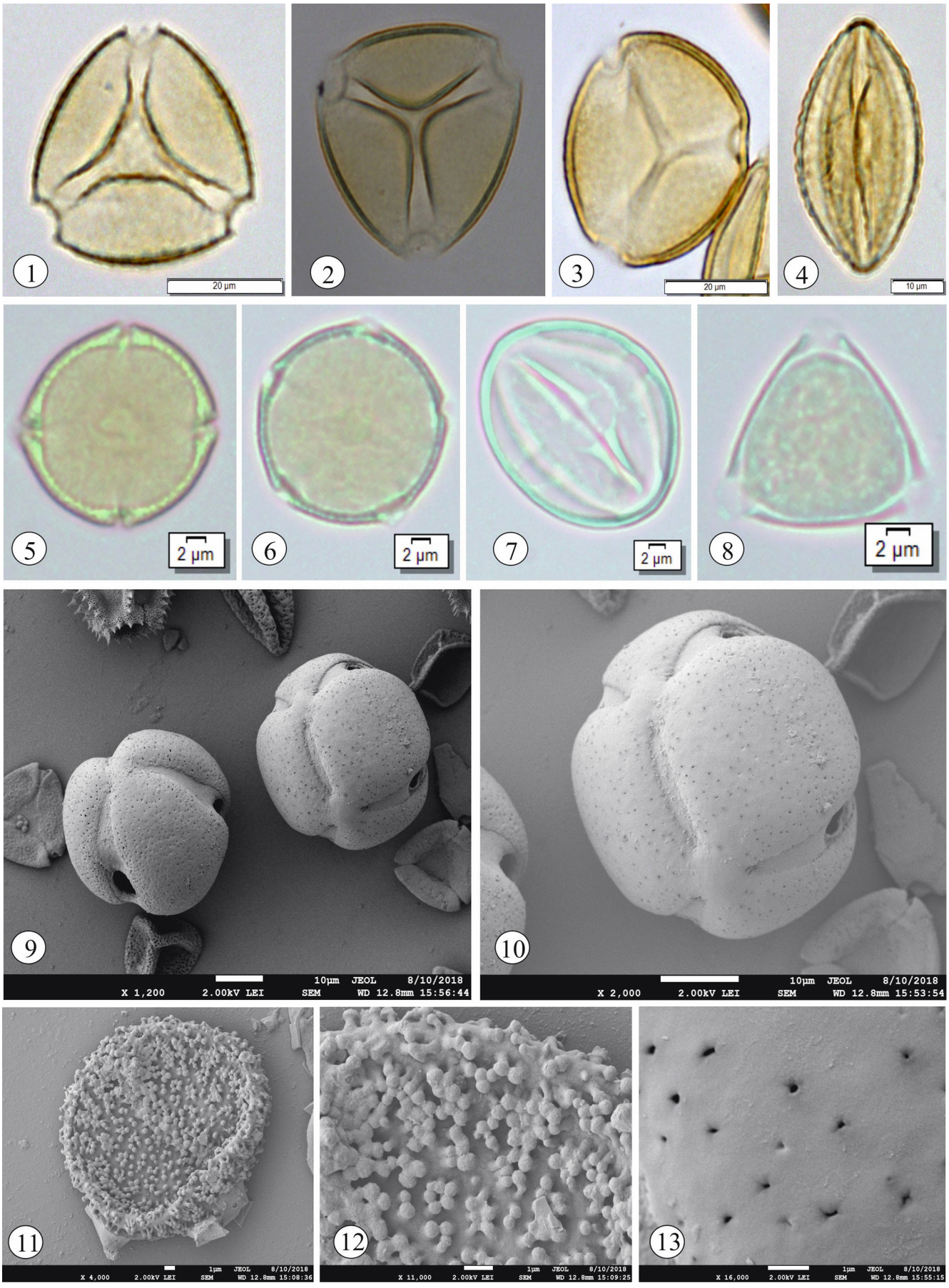

1
20 µm
2
3
20 µm
4
10 µm
5
2 µm
6
2 µm
7
2 µm
8
2 µm
9
X 1,200 2.00kV LEI 10µm JEOL SEM 8/10/2018 WD 12.8mm 15:56:44
10
X 2,000 2.00kV LEI 10µm JEOL SEM 8/10/2018 WD 12.8mm 15:53:54
11
X 4,000 2.00kV LEI 1µm JEOL SEM 8/10/2018 WD 12.8mm 15:08:36
12
X 11,000 2.00kV LEI 1µm JEOL SEM 8/10/2018 WD 12.8mm 15:09:25
13
X 16,000 2.00kV LEI 1µm JEOL SEM 8/10/2018 WD 12.8mm 15:55:15

PLATE 7

sample suggest that this honey is multifloral (ICBB, 1970). However, *Botryococcus* sp., *Alternaria* sp., *Meliola* sp. and *Cookeina* sp., the fungal spores, were also encountered in this honey sample, but have lesser values, suggesting that the bees could have visited these taxa also (Table 1). On the other hand, the honey sample 3 also demonstrated that *Eucalyptus globulus* (14%), *Cichorum intybus* (12%), *Prosopis specigera* and *Prosopis juliflora* (9% each), *Tinospora cordifolia*, *Cassia pentandra*, *Delonix regia*, *Syzygium cuminii*, *Acacia* spp., *Cassia fistula*, *Cassia siamea*, *Ficus* spp., (important minor pollen types), *Alangium salvifolium* and Meliaceae), Anacardiaceae, Rutaceae, *Madhuca indica*, *Morus alba*, and *Shorea robusta* (minor pollen types) are the important plant taxa visited by the bees for nectar and pollen. The overall pollen floral assemblages are suggestive of its being multifloral in nature. Moreover, the bees would have visited *Xanthium strumarium*, *Alium cepa*, *Coriandrum sativum*, *Ocimum basilicum*, *Solanum* spp., and *Pimpinella tomentosa* too (minor pollen types) for collection of nectar and pollen (Table 1).

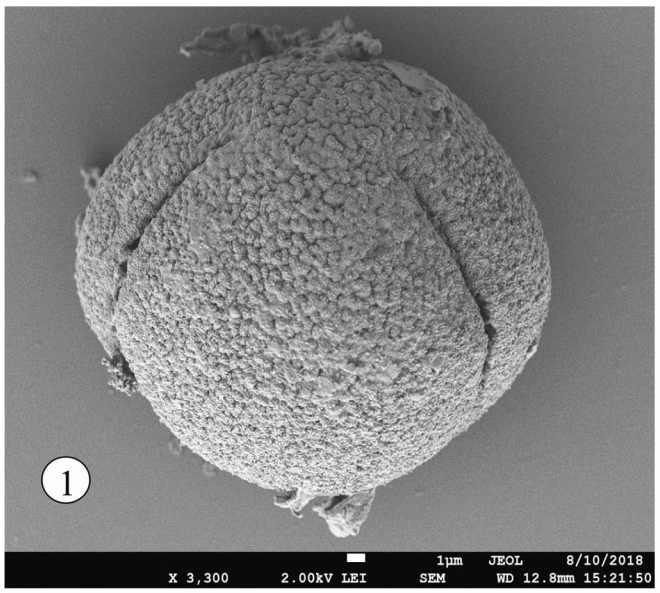
From the perusal of the aforementioned discussion, it is evident that the honey bees (*Apis cerana indica*, *Apis dorsata*, *Apis florae* and *Apis mellifera*: India's indigenous bees), in fact, preferred mainly the tree taxa , such as *Psidium guajava* (~85%), *Citrus lemon* (34.5%), *Eucalyptus globulus* (~33%), *Syzygium cuminii* (21%) of the arboreal plant (AP), followed by the herbaceous taxa (NAP), such as *Ageratum conyzoides*/Tubuliflorae/Asteroideae (18%) and *Cichorum intybus*/Liguliflorae/Cichorioideae (16%) for collecting nectar and pollen around the area of investigation (Table 1). The flowering periodicities, as well as their pollinators could also be ascertained with the help of the present study (Table 2). Further, the honey bees would not have visited to farther places, as the plant pollen taxa recovered in these honey samples grow in and around the immediate surrounding study area in Lucknow. The study could be helpful in developing apiary and apiculture industry, as the knowledge and understanding of the pollen flora of an area, which has been provided in this study, is a basic tool for the development of apiculture, as well as for the quality control of bee pollen for human consumption. Despite the excessive biodiversity around the area of investigation, the apiculture industry does not still exist officially in and around the study area, although, at some places temporary apicultural settlements can be found around the study region. These results will assist in the successful establishment of an apicultural industry and also increase the yield of apicultural products within the state. In addition, the present melissopalynological investigation also provides insights into the identification of various plant pollen taxa from the tropical parts of India, which aid in reconstruction of vegetation dynamics and associated climate change. The study is also significant from the aerobiological view point, as many recovered plant pollen taxa act as aeroallergens. *Ageratum conyzoides* (Asteroideae/Tubuliflorae, sub–family of the Asteraceae family), an obnoxious invasive weed, is responsible for causing asthma or rhinitis (Killian and McMichael, 2004). *Prosopis* spp. also cause allergy in humans. There are many plant, the pollen grains and spores of which have been identified as potential allergens that are regarded as a prospective cause of certain allergic disorders, such as bronchial asthma, hay fever (pollinosis/ allergic rhinitis), dermatitis and other disorders, such as naso–bronchial allergy and other respiratory disorders along with conjunctivitis, contact dermatitis, eczema, food allergies and other health hazards (Quamar & Chauhan, 2011; Quamar and Bera, 2016 and references cited therein). The pollen grains, in fact, release proteins (Ig E–binding proteins) that may be responsible for immediate hypersensitivity reactions in sensitive patients (Mandal *et al*., 2008). The understanding of pollen and spores present in our surroundings or environment could be helpful in assessing the allergenicity of various pollen grains/spores present in the area of investigation. Further, identification, isolation and molecular characterization of allergens are useful for the allergologists too in establishing a correct diagnosis and treatment of patients suffering from allergic disorders, ultimately enabling an improved quality of life for the inhabitants of the area of investigation (Altungolu *et al*., 2010). In addition, this information could help in spreading vigilance amongst the local populace regarding the aforementioned allergic disorders and other health hazards caused by pollen especially by the non–government organisations (NGOs) (Quamar & Bera, 2016).

Comparative account

Melissopalynological study conducted from the RDSO area of Alambag in Lucknow suggested that the honey was of multifloral nature as, although, *Syzygium cuminii* was the major source (37.38%) of nectar, but *Prosopis juliflora* (22.7%) and *P. spicigera* (10%) were also frequently visited by the bees for nectar and forage. The study further suggests that these plants were in full bloom during the course of

PLATE 8

1. *Evolvulus alsinoides:* FESEM microphotographs showing the pantozonocolpate condition.
2, 3. *Ocimum basilicum:* FESEM microphotographs showing octazonocolpate condition, reticulate pattern; 3. big brochus focussed.
4-6. *Emblica officinalis* (cf. *Phyllanthus angustissimus*): FESEM images showing colporate condition and circular os, macroreticulate reticulate pattern focussed.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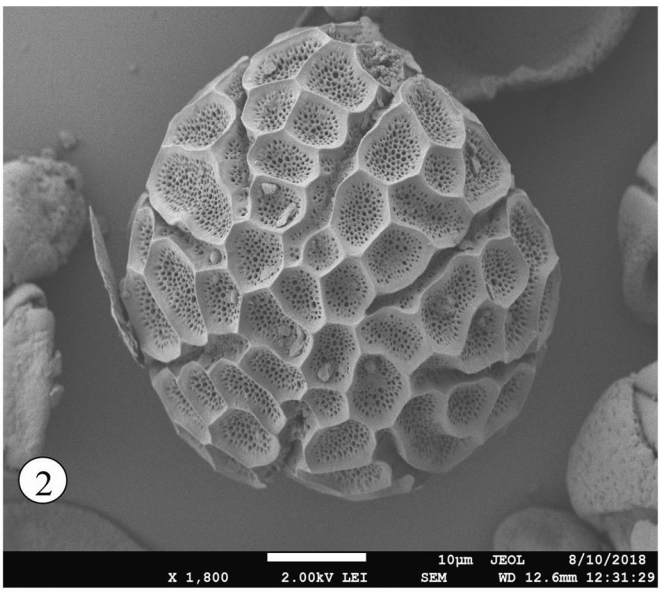
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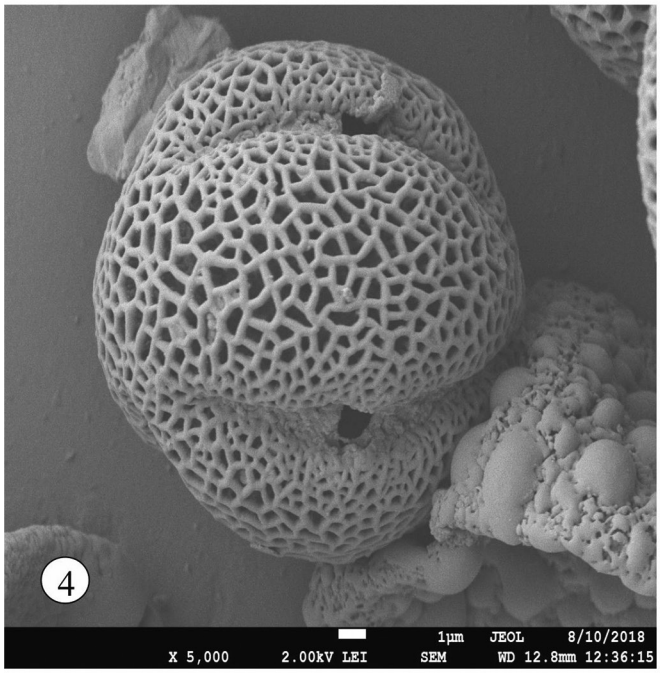
4
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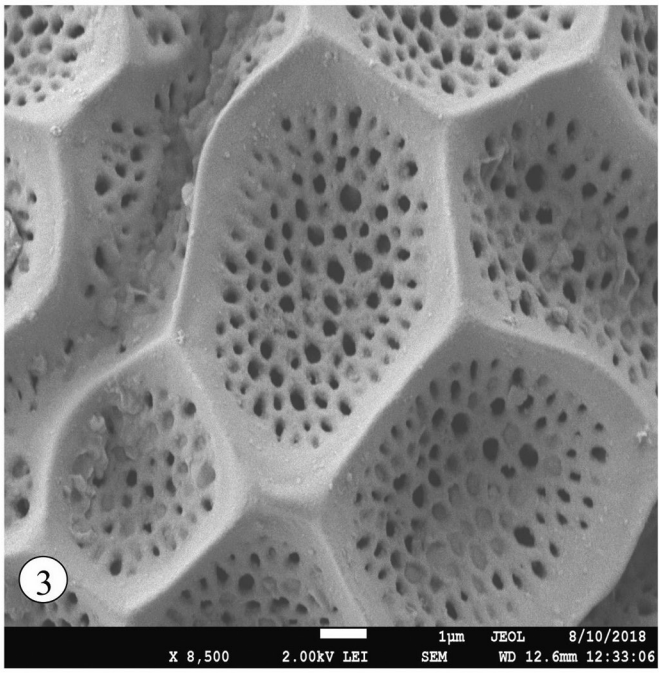
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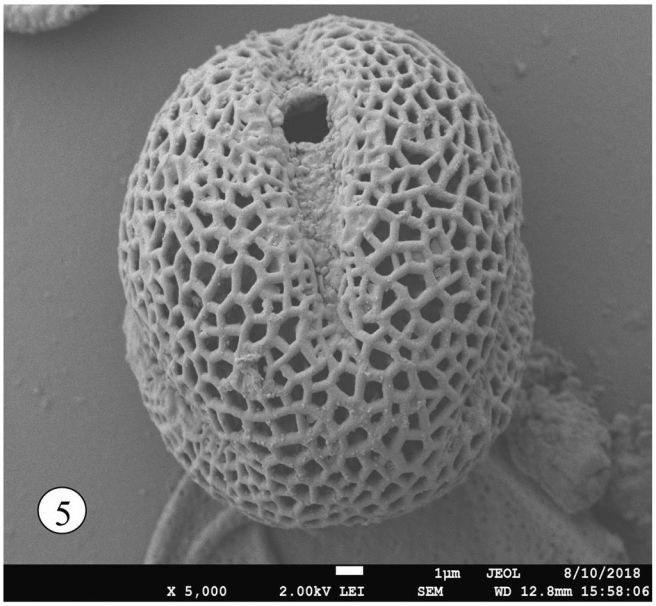
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1µm JEOL 8/10/2018
X 5,000 2.00kV LEI SEM WD 12.8mm 15:58:06

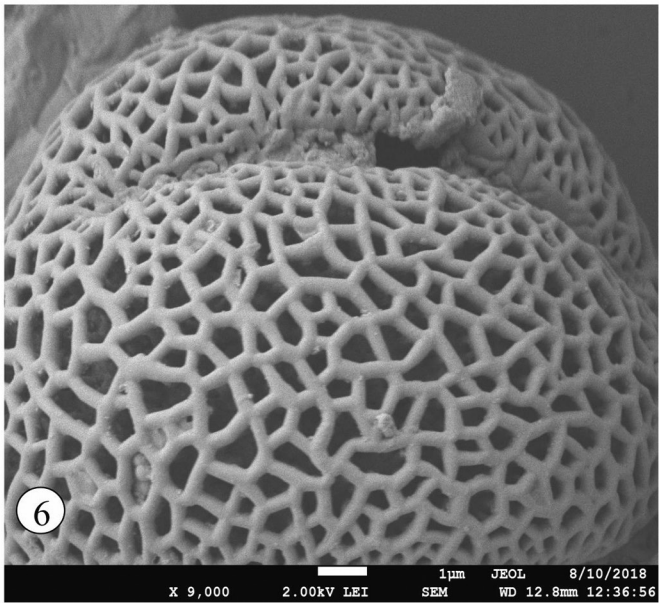

6
1µm JEOL 8/10/2018
X 9,000 2.00kV LEI SEM WD 12.8mm 12:36:56

PLATE 8

honey production. Moreover, *Pithecellobium dulce*, *Tinospora cordifolia* and *Moringa oleifera* were the minor sources of nectar and bee forage as indicated by relatively low frequencies of their pollen in the honey sample. The recovery of scanty pollen of nectar less plants, such as *Chenopodium album* and Poaceae indicate that these plants got trapped in the honey by wind or involuntarily carried by the honeybees to the hives (Chauhan & Trivedi, 2011). Chauhan *et al*. (2015) studied eight honey samples from different parts of Uttar Pradesh (U.P.) and suggested the impact of urbanization and deforestation in foraging pattern of honey bees. The study revealed that monofloral honey was there in Jhansi and Girar areas (south western region) as *Syzygium cuminii* contributed about 49.7% pollen in the honey samples; multifloral in Trilokpur and Bahraich areas (eastern region) as obnoxious weed *Ageratum conyzoides* (35.9%) and crop *Brassica campestris* (17.5%) with low percentage of exotic *Eucalyptus globulus* (3.45%) contribute more in the honey compared to the indigenous Sy*zygium cuminii* (25.3%), which is common in arboriculture. However, high percentage of *Prosopis* pollen in these areas in honey is due to its extensive commercial plantation for land reclamation. Moreover, like eastern U.P., the honeys produced in central region of U.P., such as Ashakhera, Malihabad, Mallawan and New Hyderabad (Lucknow) are also of multifloral type. *Ageratum conyzoides* (20–46%) is the dominant pollen contributor taxa, which is attributed to large expanse of arable land and wastelands in rural areas, such as Ashakhera, Malihabad and Mallawan; however, high percentage of *Prosopis* species (7–40%) and other indigenous taxa in urban areas such as New Hyderabad are related to either plantation of exotic trees or few pockets of natural vegetation in the conserved areas in the vicinity.

Chauhan and Singh (2010) conducted melissopalynological investigation from the Unnao District of U.P., and suggested the honey was of multifloral nature. The pollen assemblage has revealed an enormous quantity of pollen predominated by *Brassica campestris*, *Brassica* sp., *Syzygium cuminii* approximately 20–32% each and *Eucalyptus* 14% pollen sp., constituting the major fractions of the pollen load. Moreover, the honey–bees have frequently visited these plant taxa to glean the nectar at time of their full bloom during late winter to spring seasons since these plants attain peak flowering during this period. In addition, *Prosopis juliflora*, *Terminalia*, *Ageratum conyzoides* and *Solanum* were the secondary source of nectar as these taxa were recorded intermittently in the pollen assemblage. Sahney *et al.* (2016) conducted melissopalynological study on six honey samples of *Apis dorsata* from the Varanasi District, Uttar Pradesh. Based on the pollen recovered from the honey samples, it was suggested that two honey samples were unifloral and four were multifloral in nature. The predominant pollen types in unifloral honeys were *Brassica campestris* and *Ageratum conyzoides*, whereas in multifloral honeys *Brassica campestris*, *Ageratum conyzoides*, *Callistemon citrinus*, *Parthenium hysterophorus*, *Holoptelea integrifolia* and *Lathyrus aphaca* were the secondary pollen types. Moreover, *Brassica campestris*, *Ageratum conyzoides*, *Callistemon citrinus*, *Coriandrum sativum*, *Eucalyptus globulus*, *Carica papaya*, *Citrus* sp., *Crotalaria juncea* and Poaceae were very frequent in all the honey samples, and *Brassica campestris*, *Ageratum conyzoides*, *Callistemon citrinus* and *Coriandrum sativum* are the major bee source plants of the region. Pollen analysis of 20 honey samples from the Allahabad (Prayagraj) District of U.P. suggested that thirteen honey samples were unifloral in nature, whereas the remaining seven samples were multifloral. *Brassica campestris*, *Ageratum conyzoides*, *Bombax ceiba* and *Citrus* sp. were the predominant pollen types. Moreover, *Brassica campestris*, *Ageratum conyzoides*, *Bombax ceiba*, *Citrus* sp. and *Coriandrum sativum* were very frequent pollen types as they were recovered in > 50% of the honey samples of Allahabad (Sahney *et al*., 2018). Shukla and Rao (2021) studied 18 honey samples from Prayagraj (Allahabad) District of U.P., and suggested that five of the 18 honey samples were unifloral and 13 were multifloral. *Syzygium cuminii*, *Azadirachta indica* and *Coriandrum sativum* were the predominant pollen types. The Asteraceae family showed the highest frequency of occurrence. *Ageratum conyzoides*, *Bombax ceiba*, *Azadirachta indica* and *Syzygium cuminii* were recorded in more than 50% of the honey samples of Prayagraj District.

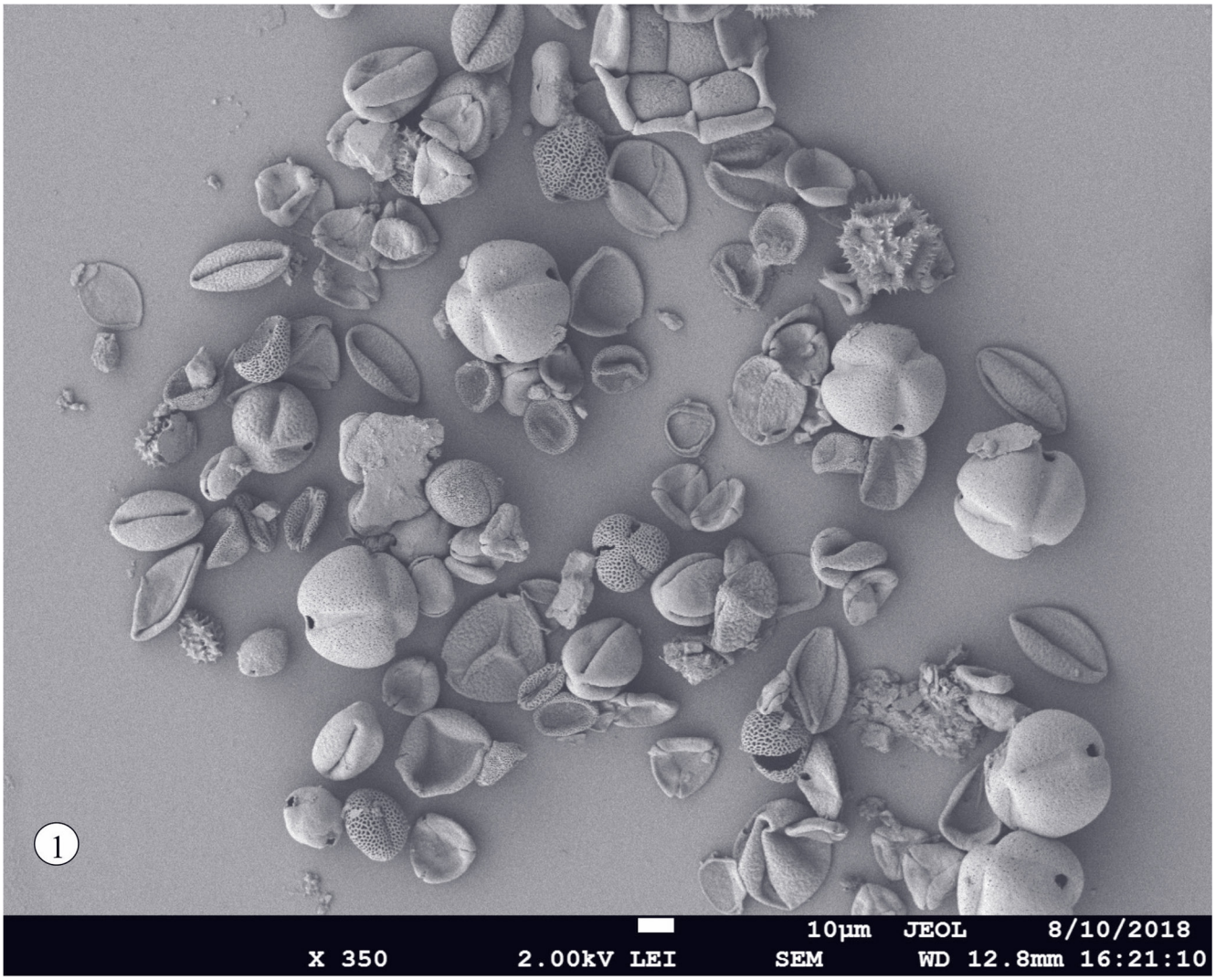
Challenges and applications of pollen and $\delta^{13}C$ in honey

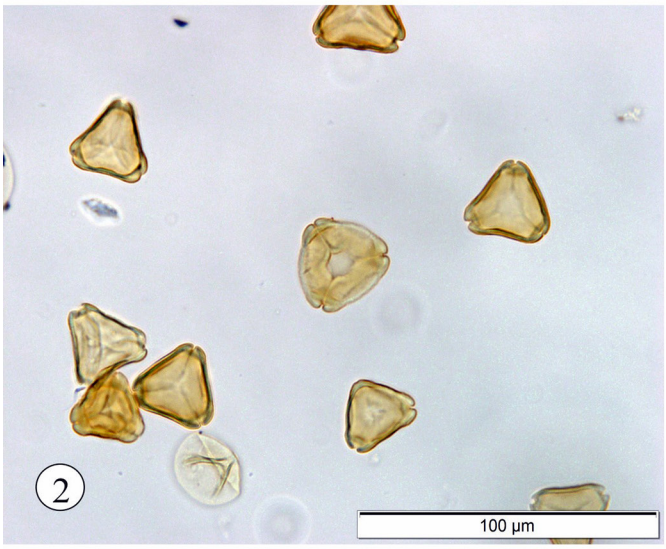
Significant findings on pollen stable isotopic composition reflecting C3 and C4 photosynthetic pathways have been used for climate and environmental controls (Amundson *et al*., 1997; Nelson *et al*., 2007, 2008). A correlation evident between climate conditions and stable isotope compositions is

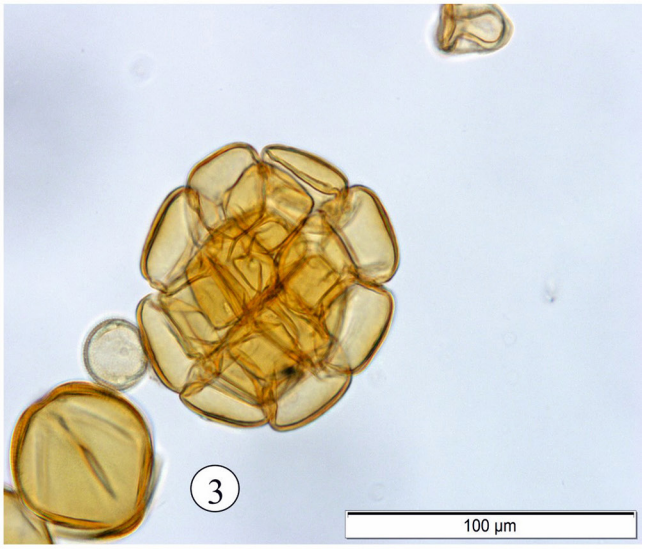
PLATE 9

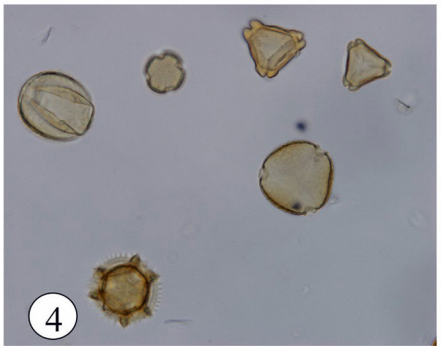
Clusters of pollen comprising:

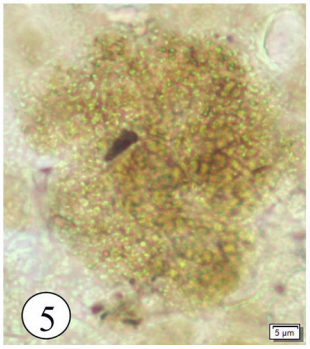
1. *Pithecellobium dulce*, *Cichorum intybus*, *Eucalyptus globulus*, *Syzygium cuminii*, *Alium cepa*, *Coriandrum sativum.*
2. *Eucalyptus globulus*, *Syzygium cuminii*, etc.
3. *Pithecellobium dulce*, *Butea monosperma.*
4. *Eucalyptus globulus*, *Syzygium cuminii*, *Cichorum intybus*, *Moringa oleifera.*
5. *Botryococcus* sp.
6. *Cookeina* sp.
7. *Alternaria* sp.

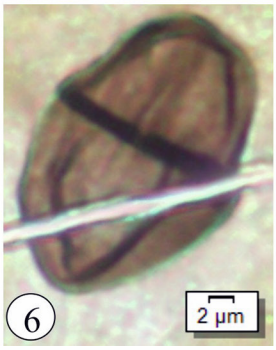

1
X 350
2.00kV LEI
10µm JEOL
SEM
8/10/2018
WD 12.8mm 16:21:10


2
100 µm


3
100 µm


4


5


6
2 µm


7
2 µm

PLATE 9

specific to different plants (Loader & Hemming, 2004). About 175 different species were analysed for $\delta^{13}C$ in pollen, leaf and stem tissue and it was found that $\delta^{13}C$ of pollen correlated strongly with the parent leaf material (Jahren, 2004). The $\delta^{13}C$ of flower and pollen also correlates with difference of ≤ 1.0 to 2.0‰ with the leaf samples of parent plant (Farooqui *et al*., 2021). Therefore, $\delta^{13}C$ of bulk pollen (more likely to be preserved rather than leaf/stem in the sediments) could provide clue to pollen assemblages in the sedimentary archives where species response can vary the values significantly representing past climatic conditions and its equilibrium with the vegetation (Fardusi *et al*., 2016; Farooqui *et al*., 2021). There is a growing interest in understanding the morphology and geochemistry of pollen for climate–vegetation changes and environmental informations supported by species–level identification (Pappas *et al*., 2003; Julier *et al*., 2016; Zimmermann *et al*., 2016). Since our results too show a strong relationship of $\delta^{13}C$ values with the pollen assemblages in honey concentrated after filtration, it is suggested that the species diversity in the region obtained through melissopalynology has strong relationship with the climate–vegetation of an area dominated by deciduous to mixed C3 vegetation indicating the source of honey from a high seasonality zone. Thus, melissopalynological studies with specific pollen identification (FESEM study) serve as potential indicators of urban and rural vegetation depicting the culture, development and agricultural strategies of humans. The $\delta^{13}C$ values of identified bulk pollen in honey indicate the type and floral source of honey which could be clinically valued by sensitive consumers who are allergic to certain type of pollen protein. *Ageratum conyzoides* weed is one among the highly foraged plant by honey bees in most of the region as it grows widely in fallow lands and, therefore, the knowledge of pollen content in honey is of utmost important and should be preferred over filtered honey in market. The honey without pollen and $\delta^{13}C$ values related with the standard value (–27‰) recommended for honey provides insufficient information.

Pollen protein causes more than 30% of allergenic reactions in humans (Singh & Mathur, 2012). Allergenic properties of *A. conyzoides* pollen and other members of Asteraceae and Poaceae are responsible for ~60–75% of seasonal rhino sinusitis, asthma and dermatitis (Jaggi & Gangal, 1987; Stanley & Linskens, 1974; Fernández *et al*., 1993; Ghosh *et al*., 2017; Singh, 2017). *Cichorium intybus* var. *sativum* (blue flowers) is commonly known as Chicory and is grown for its medicinal use in producing inulin and a substitute for coffee produces pollen which has allergenic properties causing asthma and other oral /cutaneous problems in sensitive patients (Cadot *et al*., 1996; Morita *et al*., 2007) commonly called as Chicory belong to the Asteraceae family (Compositae) and its roots are used to produce inulin and roasted to produce a substitute for coffee. The allergenic properties of protein found in chicory causes asthma and other oral and cutaneous manifestations which was first reported in 1989 (Cadot *et al*., 1996; Morita *et al*., 2007). Abnoxious growth of exotic weeds like *A. conyzoides* or culture of *C. intybus* by humans in urban and industrial development has forced honey bees to change their preference for foraging nectar from the available plants in the region. Except for sensitive consumers, *C. intybus* is useful for production of honey in apiculture as it is a high nectar producing plant (Leonora, 2017). Earlier melissopalynological study of ~51 samples of honey until 2008 did not reveal *C. intybus* and *T. cordifolia* pollen. However, the present samples of honey (2018) from urban set up are rich in these pollen and that the honey bees have preferred for foraging nectar and honey production. Therefore, these two plants are excellent choice by humans for use in pharmacological industry and food production which also provides information related to anthropogenic activities in the region.

CONCLUSIONS AND PROSPECTS

1. The pollen assemblages suggest that monofloral and multifloral honeys are found in the decade old area of investigation.
2. The urban expansion has shown that plant diversity has increased over an arable land.
3. Melissopalynology indicates dominance of fruit bearing trees, medicinal herbs, such as *Cichorum intybus* and *Tinospora cordifolia* along with flower bearing avenue trees planted for aesthetic value.
4. Gleaning of nectar from *Ageratum conyzoides,* an exotic weed, was significantly high suggesting its preference by honey bees as primary or secondary plant for foraging.
5. Honey bees prefer both tree taxa (AP) followed by the herbaceous taxa (NAP).
6. The pollen assemblages portray the dominance of deciduous and mixed types of vegetation common in high seasonality region.
7. Isotopic ($\delta^{13}C$) values of all the natural honey varied within ~ –25 to –26‰, which relates to the regional vegetation types and environmental conditions.
8. The $\delta^{13}C$ of bulk pollen in honey relates to the local plant community foraged by honey bees and, therefore, provides the locality of honey production. This knowledge can be used in forensic science as well.
9. Evidence of *C. intybus* and *T. cordifolia* pollen in honey indicates its introduction since the last decade perhaps due to its use in pharmacological industry.
10. Knowledge and understanding of the local flora could help in establishing the apiculture industry and labelling plant community specific honey for medicinal and clinically safe use by consumers who are sensitive to pollen specific protein.
11. The study could help understand the plant taxa, which could be the cause of allergic disorders amongst the consumers. The knowledge of pollen specific honey

may facilitate correct diagnosis and treatment of patients suffering from allergic disorders after consuming honey.

A systematic study with large number of honey samples (both natural and commercial products) is further required to be carried out in order to have a complete knowledge and understanding of the rich and diverse sources of pollen in honey from varied plant communities, occurring in equilibrium with regional climate and environmental conditions. Such systematic study could have the potential not only to develop apiary, but also to serve commercial honey units officially.

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