



POLLEN MORPHOLOGY OF SOME SELECTED BEE FLORAL RESOURCES OF *Apis cerana* F. AND *Apis mellifera* L. USING SCANNING ELECTRON MICROSCOPY FROM HIMACHAL PRADESH, INDIA

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AUTHOR'S CONTRIBUTION

The sole author designed, analysed, interpreted and prepared the manuscript.

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ABSTRACT

Pollen morphology of ten nectariferous and polleniferous bee floral resources of honeybees *Apis cerana* F. (Asian honeybee) and *Apis mellifera* L. (European honeybee) was studied by using scanning electron microscope. Pollen grains of *Foeniculum vulgare*, *Taraxacum officinale*, *Jacaranda mimosifolia*, *Sechium edule*, *Emblica officinalis*, *Ocimum sanctum*, *Lagerstroemia indica*, *Cedrela toona*, *Eucalyptus tereticornis* and *Moringa oleifera* belonging to different bee favored families were examined for the morphological characterization. The pollen grains were analyzed for aggregation, shape, shape class, size, aperture, polarity, symmetry and exine ornamentation. All the pollens observed in the study had solitary grains, isopolar and have radial symmetry. The shape and shape class of pollen grains varies among different families. The size of pollen grains studied ranged from small, medium to large sized among different plant families. Most of pollens observed were 3-colporate, except pollens of *Sechium edule* which were 9-colpate, *Emblica officinalis* and *Lagerstroemia indica* were 3-4-zonocolporate, *Ocimum sanctum* were 6-colpate, *Cedrela toona* and *Moringa oleifera* were 3- zonocolporate. Variation was also observed in surface pattern of different pollens belonging to different families.

Keywords: *Apis cerana*; *Apis mellifera*; SEM; bee forage; pollen morphology.

1. INTRODUCTION

Honeybees are wonderful creatures and are able to forage on wide diversity flowering plants having

varying honey potentiality. There are more than four hundred species of plants which are either major or minor sources of pollen and nectar to Indian hive bee, *Apis cerana* F. and Italian bee, *Apis mellifera* L.

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[1,2,3]. Honeybees while foraging on the flowers of different entomophilous plants for collecting nectar, also gather some pollen with it. The pollen is retained in the ripened honey which is subsequently stored in the honey combs.

Pollen grains are the male microgametophytes produced by the flowering plants for the purpose of fertilization [4]. Although, pollens are very small entities but has numerous applications in various studies such as plant taxonomy, palynology, paleobotany, paleontology, archeology, agricultural sciences, forensics and melissopalynology. The morphological features of pollen grains such as shape, size, aperture, surface pattern and thickness of exine and intine etc. are helpful in taxonomic identification of plants [5,6]. Thus, pollen morphology serves as important tool in resolving the taxonomic disputes of plant families, class, order, genus and species. In melissopalynology [7], by studying the pollen in a sample of honey, it is possible to gain evidence of the geographical location by observing the honey samples for the presence of a combination of pollen that is typical only to that particular location [8]. It is also possible to identify taxonomically the genera of the plants the honeybees visited, although honey may also contain airborne pollen from anemophilous plant species, spore and dust due to electrostatic charge of the worker bee. Information gained from a given honey sample is useful when substantiating claims of a particular honey source and is also of great importance for quality control and helps to ascertain whether honey is adulterated or not [9,8,10]. Studies also indicated that pollens suspended in air they give insights about the local flora [11,12]. Similarly, past flora and climate can also be determined as pollens get deposited in strata/sediments of any time period [13,14,15]. Therefore, such studies also help to explore the past life history of animal and human beings [16,17]. Palynology has significant importance in forensics as well. Pollens can adhere to clothes, shoes, body parts or any other object of suspect, thus providing evidence [18,19]. Keeping in view the above applications, recognition and identification of pollen grains is valuable in many multidisciplinary approaches.

Moreover, such studies have wide array of implications as scanning electron microscopy gives better depth of focus and facilitates to evaluate the pollen features with absolute accuracy, which is not possible through simple compound microscope [20]. Therefore, palynology enables to resolve many taxonomic problems. These studies will also prove beneficial in melissopalynology, providing accurate identification of pollens in honeys. Moreover, it also helps to prepare floral calendar in a particular locality,

which will be helpful to manage bee forage resources especially during dearth periods.

2. MATERIALS AND METHODS

These studies were undertaken (field and laboratory studies) during 2009 to 2012 as a part of Ph.D Work. But, extensive field work was also done between the years 2012 to 2016 to study the honey plants from different agro-climatic zones of Himachal Pradesh.

The pollen grains/anthers of mature identified pollen taxa were collected in glass vials (within 5 km from apiary) and preserved at sub-zero temperature. The reference slides were prepared by standard acetolysis method suggested by Erdtman [21,22]. The pollen slides were then observed under light microscope. However, for the scanning electron microscopic studies, an adhesive (a plastic dissolved in a volatile solvent) is applied to the smooth metal surface of the microscope stage, and a small quantity of pollen residue obtained above or of pollen collected directly from the plant is placed on the adhesive. The specimen is placed in a vacuum evaporator, coated first with carbon and then with gold, after which it is ready for observation [23]. Or after dehydration on a silica gel drier, small quantities of pollen grains were mounted on scanning electron microscopy (SEM) stubs and coated with gold-palladium (Ion Sputter JFC-1100) and examined at accelerating voltage of 15 to 20 KV in a Scanning Electron Microscope (SEM- JSM 6100) at CIL/SAIF, Punjab University, Chandigarh.

In this study different pollen characters were observed viz. aggregation, shape and size of pollen, type and number of aperture and ornamentation of exine. The descriptive terminology is followed as by Sawyer [24] and Vorwohl [25].

3. RESULTS AND DISCUSSION

1. *Foeniculum vulgare* Mill.: Monad, elongated/elliptical and per-prolate, small grains, size ranges from 18.1µm x 8.52µm, tricolporate, isopolar, radially symmetric. Tectum was striate-reticulate.
2. *Taraxacum officinale* Weber: Monad, circular and spheroidal, medium grains, size ranges from 32.1µm x 30.9µm, tricolporate, isopolar, radially symmetric. Tectum was echinate and perforate.
3. *Jacaranda mimosifolia* D. Don: Monad, circular /oval and sub-prolate, large grains, size ranges from 48.8µm x 43.2µm, tricolporate, isopolar, radially symmetric. Tectum was psilate.
4. *Sechium edule* SW.: Monad, circular and sub-oblate, large grains, size ranges from 53.5µm x

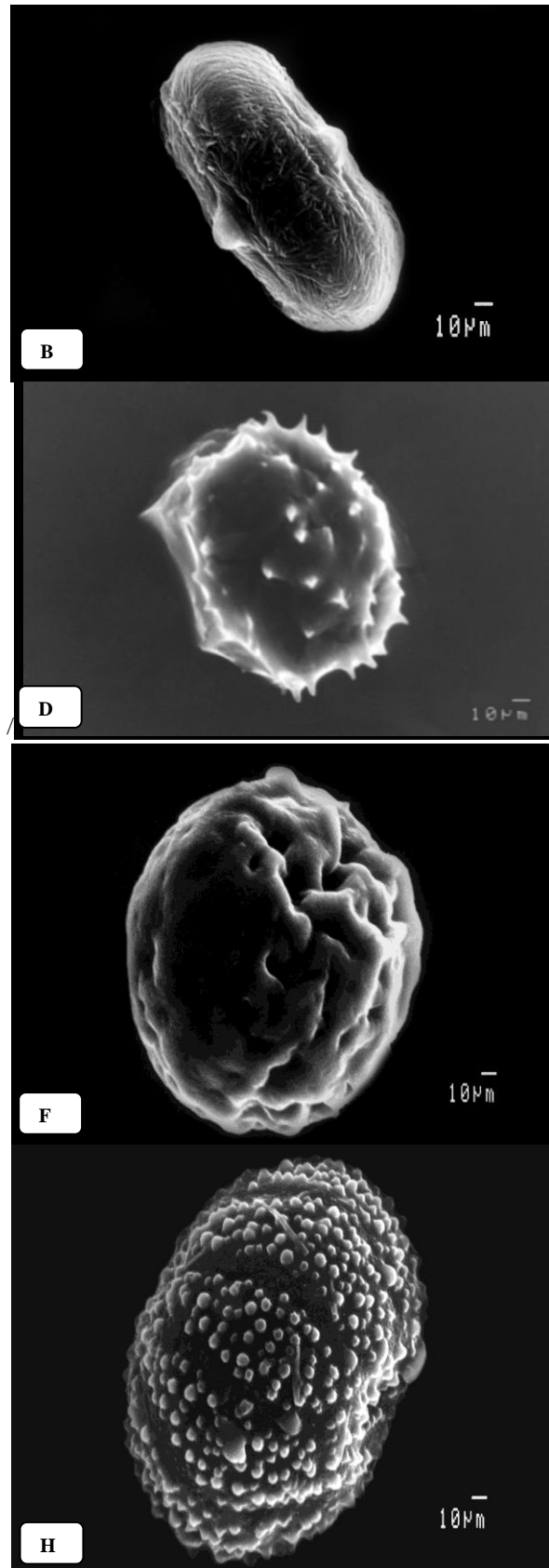
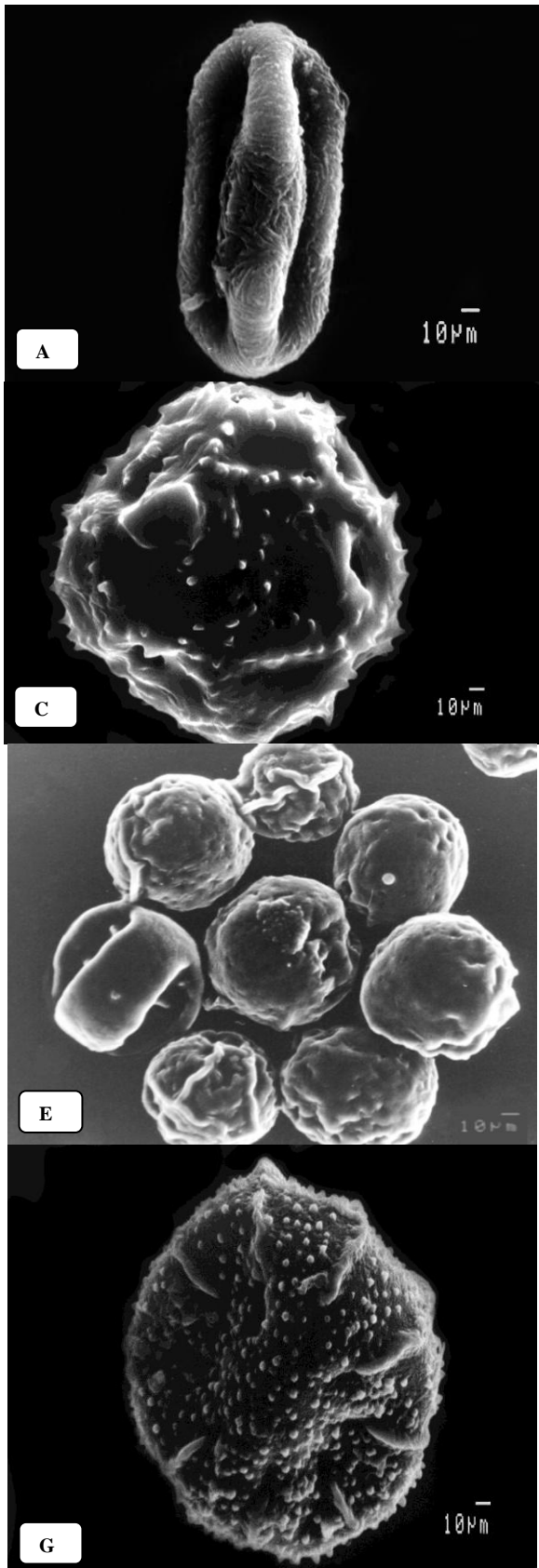
- 49.5µm, 9-colpate, isopolar, radially symmetric. Tectum was echinate/gemmate.
5. *Emblica officinalis* Gaetrn.: Monad, circular and prolate-spheroidal to subprolate, small grains, size ranges from 16.4µm x 14.9µm, 3-4-zonocolporate, isopolar, radially symmetric. Tectum was finely reticulate.
 6. *Ocimum sanctum* L.: Monad, circular /oval and sub-oblate, medium sized grains, size ranges from 46.2µm x 44.5µm, hexacolpate, isopolar, radially symmetric. Tectum was reticulate
 7. *Lagerstroemia indica* Linn.: Monad, circular /oval and prolate-spheroidal, medium sized grains, size ranges from 32.9µm x 28.6 µm, 3-4 zonocolporate, isopolar, radially symmetric. Tectum was scabate, granulate.
 8. *Cedrela toona* Roxb. ex. Rottl. & Willd.: Monad, circular and oblate-spheroidal, small grains, size ranges from 17.6µm x 16.4µm, 3- zonocolporate, isopolar, radially symmetric. Tectum was psilate.
 9. *Eucalyptus tereticornis* Sm.: Monad, triangular and oblate-spheroidal, small sized grains, size ranges from 19.2µm x 16.6µm, tricolporate, isopolar, radially symmetric. Tectum was psilate on edges/rugulate in centre.
 10. *Moringa oleifera* Lamk.: Monad, circular/oval and prolate-spheroidal, small grains, size ranges from 20.5µm x 17µm, , 3-zonocolporate, isopolar, radially symmetric. Tectum was psilate.
- Ten pollen grains belonging to different bee favored families have been shown in (Tables 1 & 2, Fig. 1). The pollen morphology varies among different families having variation in size, shapes and other pollen characters viz. aperture and exine ornamentation.

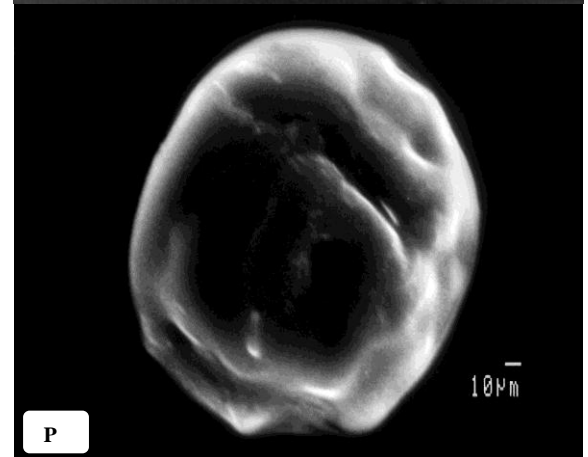
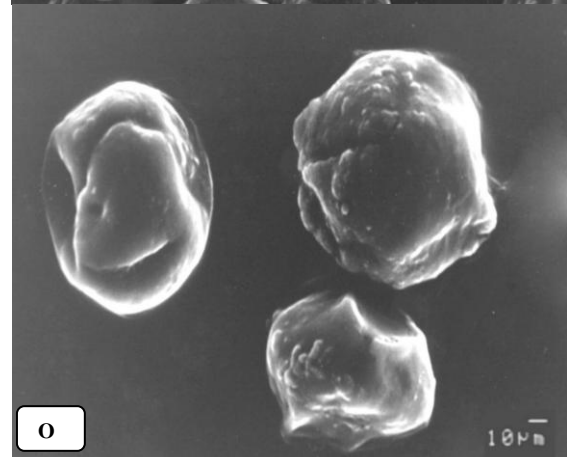
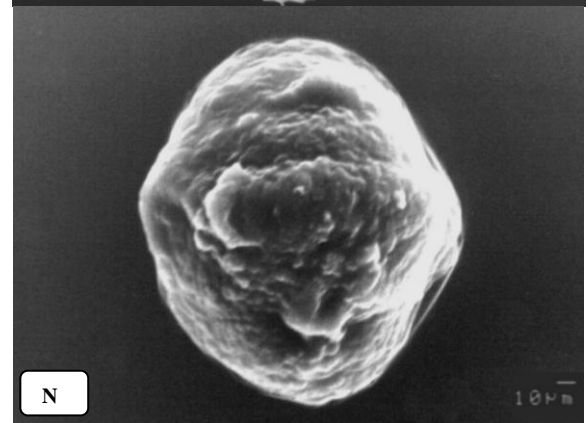
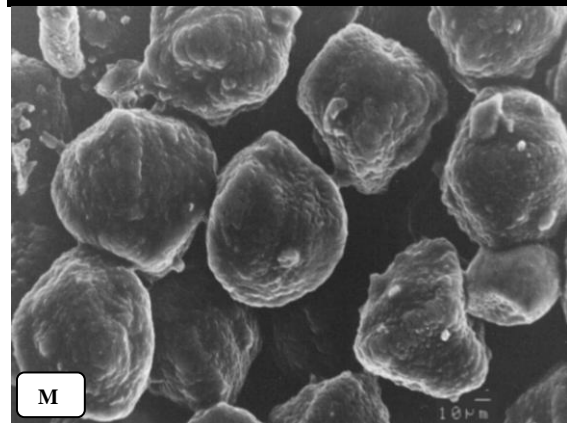
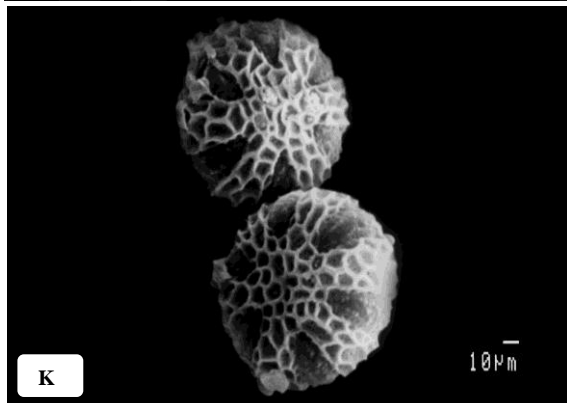
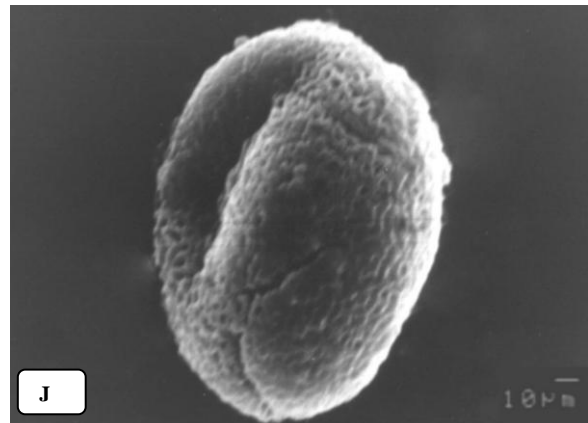
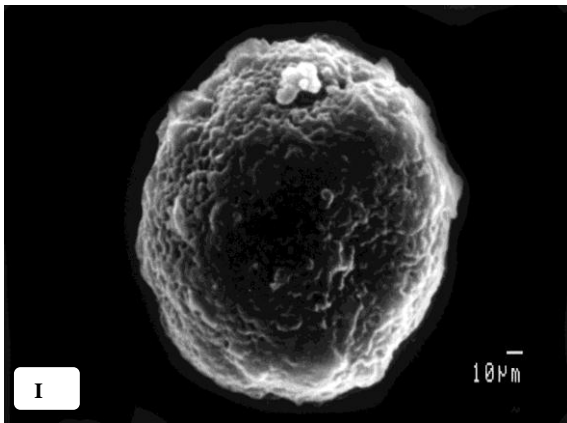
Table 1. Showing distribution of major, medium and minor honey plants in Himachal Pradesh

Plant species	Family	Common name	Vernacular Name	Honey potentiality	Flowering period	Distribution
1	2	3		4	5	6
<i>Foeniculum vulgare</i> Mill.	Apiaceae	Fennel	Meethi Saunf	N ³ P ³	AUG-SEPT	Throughout
<i>Taraxacum officinale</i> Wigg.	Asteraceae	Dandelion	Kadavi Sunaehari	N ¹ P ¹	MAR-NOV	Throughout
<i>Jacaranda mimosifolia</i> D.Don	Bignoniaceae	Blue Jacaranda or Brazilian Rosewood	Neeli gulmohur	N ³ P ³	APRIL-JUNE	Valley, low and mid hills
<i>Sechium edule</i> SW.	Cucurbitaceae	Chayote	Lonku	N ² P ²	JULY-NOV	Throughout
<i>Emblica officinalis</i> Gaetrn.	Euphorbiaceae	Indian gooseberry	Amla	N ² P ²	MAR-MAY	Valley, low and mid hills
<i>Ocimum sanctum</i> Linn.	Lamiaceae	Holy Basil	Tulsi	N ² P ³	JUNE-SEPT	Valley, low and mid hills
<i>Lagerstroemia indica</i> L.	Lytheraceae	Pride of India	Saoni	N ² P ²	JULY-SEPT	Valley, low and mid hills
<i>Cedrela toona</i> Roxb. ex Rottl.& Willd	Meliaceae	Indian Mahagany tree	Tunni	N ¹ P ²	MAR-JUNE	Valley, low and mid hills
<i>Eucalyptus tereticornis</i> Sm.	Myrtaceae	Eucalyptus	Safeda	N ¹ P ¹	MAY-JUNE	Valley and low hills
<i>Moringa oleifera</i> Lam.	Moringaceae	Drumstick tree	Sonani	N ¹ P ¹	JAN-MAR	Low and mid hills

Table 2. Showing pollen morphology of honey plants

Plant Name and Sub Family Name	Pollen unit	Shape	Shape Class (100 P/E)	Size (Length x Breadth)	Aperture	Polarity	Symmetry	Ornamentation
<i>Foeniculum vulgare</i> Mill. (Apiaceae)	Monad	Elongated/Elliptical,	Per-prolate	Small grains, 18.1µm x 8.52µm	3-Colporate	Isopolar	Radial	Striate-reticulate.
<i>Taraxacum officinale</i> Weber (Asteraceae)	Monad	Circular	Spheroidal	Medium grains, 32.1µm x 30.9µm	3-Colporate	Isopolar	Radial	Echinate and Perforate
<i>Jacaranda mimosifolia</i> D. Don (Bignoniaceae)	Monad	Circular /Oval	Sub-prolate	Large grains 48.8µm x 43.2µm,	3-Colporate	Isopolar	Radial	Psilate
<i>Sechium edule</i> SW. (Cucurbitaceae)	Monad	Circular	Sub-oblate	Large grains, 53.5µm x 49.5µm	9-Colpate	Isopolar	Radial	Echinate/Gemmate
<i>Emblica officinalis</i> Gaetrn. (Euphorbiaceae)	Monad	Circular	Prolate-spheroidal to Sub-prolate	Small grains 16.4µm x 14.9µm	3-4-Zonocolporate	Isopolar	Radial	Finely reticulate
<i>Ocimum sanctum</i> L. (Lamiaceae)	Monad	Circular /Oval	Sub-oblate	Medium sized grains, 46.2µm x 44.5µm	6-Colpate	Isopolar	Radial	Reticulate
<i>Lagerstroemia indica</i> Linn. (Lythraceae)	Monad	circular /Oval	Prolate-spheroidal	Medium grains, 32.9µm x 28.6 µm	3-4 Zonocolporate	Isopolar	Radial	Scrabate, Ganulate
<i>Cedrela toona</i> Roxb. ex. Rottl. & Willd. (Meliaceae)	Monad	circular	Oblate-spheroidal	Small grains, 17.6µm x 16.4µm	3-Zonocolporate	Isopolar	Radial	Psilate
<i>Eucalyptus tereticornis</i> Sm. (Myrtaceae)	Monad	Triangular	Oblate-spheroidal	Small grains, 19.2µm x 16.6µm	3-Colporate	Isopolar	Radial	Psilate on edges and Rugulate in centre
<i>Moringa oleifera</i> Lamk. (Moringaceae)	Monad	Circular/Oval	Prolate-spheroidal	Small grains, 20.5µm x 17µm	3-Zonocolporate	Isopolar	Radial	Psilate





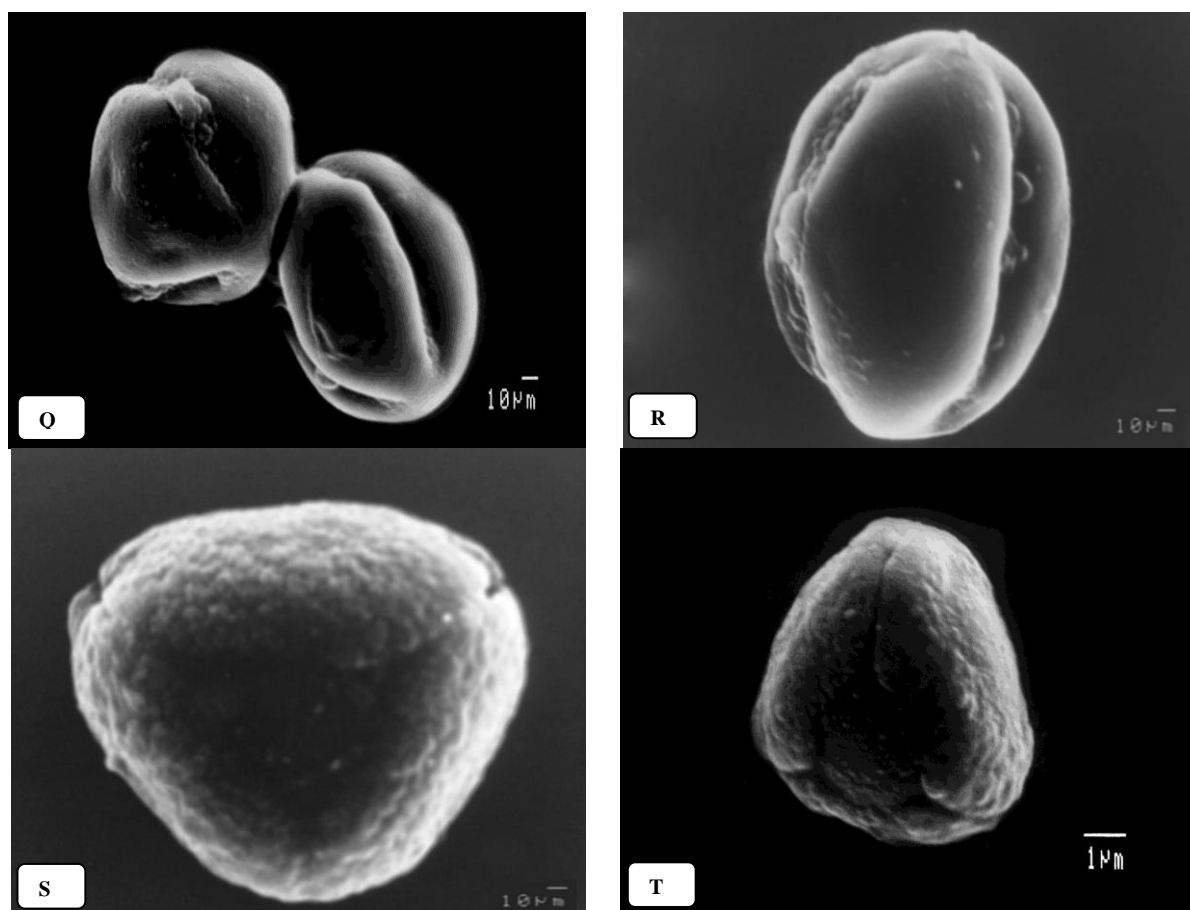


Fig. 1. Showing photomicrographs of pollen grains through Scanning Electron Microscopy

A-B *Foeniculum vulgare*, EV ($X_{3,300}$), EV ($X_{3,300}$) C-D *Taraxacum officinale* EV ($X_{2,200}$), EV ($X_{2,200}$) E-F *Jacaranda mimosifolia* PV& EV ($X_{6,50}$), EV ($X_{1,600}$) G.-H *Sechium edule*, PV ($X_{1,100}$), EV ($X_{1,500}$) I-J *Emblica officinalis* EV ($X_{3,000}$), EV ($X_{3,300}$) K-L *Ocimum sanctum*, PV ($X_{1,300}$), EV ($X_{1,600}$) M-N *Lagerstroemia indica* PV& EV ($X_{1,100}$), EV ($X_{2,200}$) O-P *Cedrela toona* PV& EV ($X_{1,700}$), EV ($X_{3,300}$) Q-R *Moringa oleifera*, PV& EV ($X_{1,600}$), EV ($X_{2,500}$) S-T *Eucalyptus tereticornis*, EV ($X_{3,500}$), EV ($X_{4,000}$)

Various pollen characters were studied including pollen unit/aggregation, shape, shape class (100 P/E) size, aperture, polarity, symmetry, surface pattern/exine ornamentation. All pollen observed belonging to different plant families had solitary grains (Monads). There was not much variation in shape among different pollens under study. The shape of pollen grains was observed elongated/elliptical in *Foeniculum vulgare*, triangular in *Eucalyptus tereticornis*, circular in *Taraxacum officinale*, *Sechium edule*, *Emblica officinalis*, *Cedrela toona*, circular/oval in *Jacaranda mimosifolia*, *Ocimum sanctum*, *Lagerstroemia indica* and *Moringa oleifera*. However, the shape class varies considerably among different plant families. The shape class of pollens was arrived at using ratio of the polar axis and equatorial diameter measurements (PA/ED) X 100. The pollens observed were sub-oblate (75-88) in *Sechium edule* and *Ocimum sanctum*, oblate-spheroidal (88-99) in *Cedrela toona* and *Eucalyptus tereticornis*, spheroidal

(100) in *Taraxacum officinale*, prolate-spheroidal (101-114) in *Lagerstroemia indica* and *Moringa oleifera*, prolate-spheroidal (101-114) to sub-prolate (114-133) in *Emblica officinalis*, sub-prolate (114-133) in *Jacaranda mimosifolia* and per-prolate (< 200) in *Foeniculum vulgare*. The size of pollen grains studied ranged from $18.1\mu\text{m} \times 8.52\mu\text{m}$ to $53.5\mu\text{m} \times 49.5\mu\text{m}$ among the members different families.

Most of pollens studied were tricolporate i.e having both colpi and pori in same aperture except *Cedrela toona* and *Moringa oleifera* grains were 3-zonocolporate (colpi and pori on equator), *Emblica officinalis* and *Lagerstroemia indica* grains were 3-4-zonocolporate, *Ocimum sanctum* grains were hexacolpate (with six colpa) and *Sechium edule* grains were 9-colpate (with nine colpa). Pollen grains having tricolporate aperture are more advanced while, polyad and colpate type are considered as of primitive status [26,27,28].

Exine ornamentation include striate-reticulate (radial projections are either parallel or form reticular pattern) in *Foeniculum vulgare*; echinate and perforate (surface with pointed sculpturing elements (echini) and having small holes or depressions less than 1µm in diameter) in *Taraxacum officinale*, completely smooth surface or with pits with diameter <1 µm (psilate) in *Jacaranda mimosifolia*, *Cedrela toona*, *Moringa oleifera* and psilate on edges/regulate (elongated sculpturing elements greater than 1µm long and irregularly distributed) in centre in *Eucalyptus tereticornis*; echinate/gemmate (pointed sculpturing elements (echini)/sculpturing elements (gemma) higher than 1µm and approximately the same width as height) in *Sechium edule*, finely reticulate and reticulate (sculpturing elements forming net- like pattern) in *Emblica officinalis* and *Ocimum sanctum*; scrabate/granulate (any sculptural element less than 1µm in diameter) in *Lagerstroemia indica*. Sarkar et al. [29] found similar observation for *M. oleifera*. Studies on *Emblica officinalis*, *Lagerstroemia indica*, *Toona ciliata*, *Moringa* sp. have also shown similar results [30].

Similar studies on pollen morphology have been conducted in various parts of India [31,32,30,29, 33,34] and Abroad [35,36,37,38,39,40,41].

4. CONCLUSION

On observation of characteristics of pollen grains belonging to 10 different families, it is concluded that they reflect a great variety of morphological characters, such as shape and size variation, aperture condition, and exine patterns. These variations can be utilized in taxonomic identification of different bee floral resources. Moreover, these studies are helpful in recognizing the pollen in honey and can utilized in apiculture industry as richness of nectar and pollen resources and duration of time for which bee forage is available around an apiary is quite important for the success of beekeeping in an area.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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