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RESEARCH ARTICLE

Melittopalynological Investigation of winter honeys collected from *Apis dorsata* hives of Mul tahsil of Chandrapur District of Maharashtra State (India)

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ABSTRACT

The paper incorporates a qualitative and quantitative analysis of pollen contents in four squeezed honey samples collected from forest area of Mul tahsil of Chandrapur district. *Lathyrus sativus* represents the predominant pollen type (46.16%) in one sample is designated as *Lathyrus* honey. The other significant pollen types recorded include *Cajanus cajan, Celosia argentea, Prosopis juliflora, Hyptis suaveolens, Capparis grandis, Cleome gynadra, Capsicum annuum, Dodonea viscosa.* The pollen counts ranged from 6,000 to 1376,000. The data reflects the floral situation of the place where particular honey was produced and the identification of geographical origin based on the presence of a combination of pollen types of that particular area

Keywords : Pollen, Honey, *Apis dorsata,* Mul tahsil.

INTRODUCTION

Melittopalynology is an applied branch of palynology dealing with the study of pollen grains in honey samples and its application in Apiculture. Plant produces nectar and pollen both of which are avidly sought after by the bees to provide nutrition to the colony. Melittopalynology is concerned with the identification of pollen in honeys. Evaluation of plants for their utility as sources of bee forage provides the information needed to assess the potential for bee keeping in an area. Melittopalynological studies are thus helpful in bee management and in promoting the beekeeping development. Laboratory studies using Melittopaloynological methods have been made to evaluate sources of pollen and nectar for honey bees in different parts of the country namely Maharashtra (Bhusari et al., 2005; Phadke, 1962; Kumar and Jagtap, 1988), Andra Pradesh (Ramanujam and Khatija, 1991, Kalpana and Ramanujam, 1991, Moses, 1987), Karnataka (Yoganarasimhan, 1982; Agashe and Ranjaswami, 1997;

Sheshagri, 1985; Bhargava *et al.*, 2009), Lucknow (Suryanarayana, 1976) and Indian honeys (Sen and Banarjee, 1956; Nair, 1964; Seethalakshmi, 1993). Present investigation incorporates a quanlitative and quantitative pollen analysis of four honey sample from forest area of Mul tahsil of Chandrapur District. In order to identify the chief bee foraging plants recognize the uni and multifloral honeys and identify areas suitable for bee-keeping industry in this area. It is further investigated that a study of this nature would also highlight the geographical source of the honey samples.

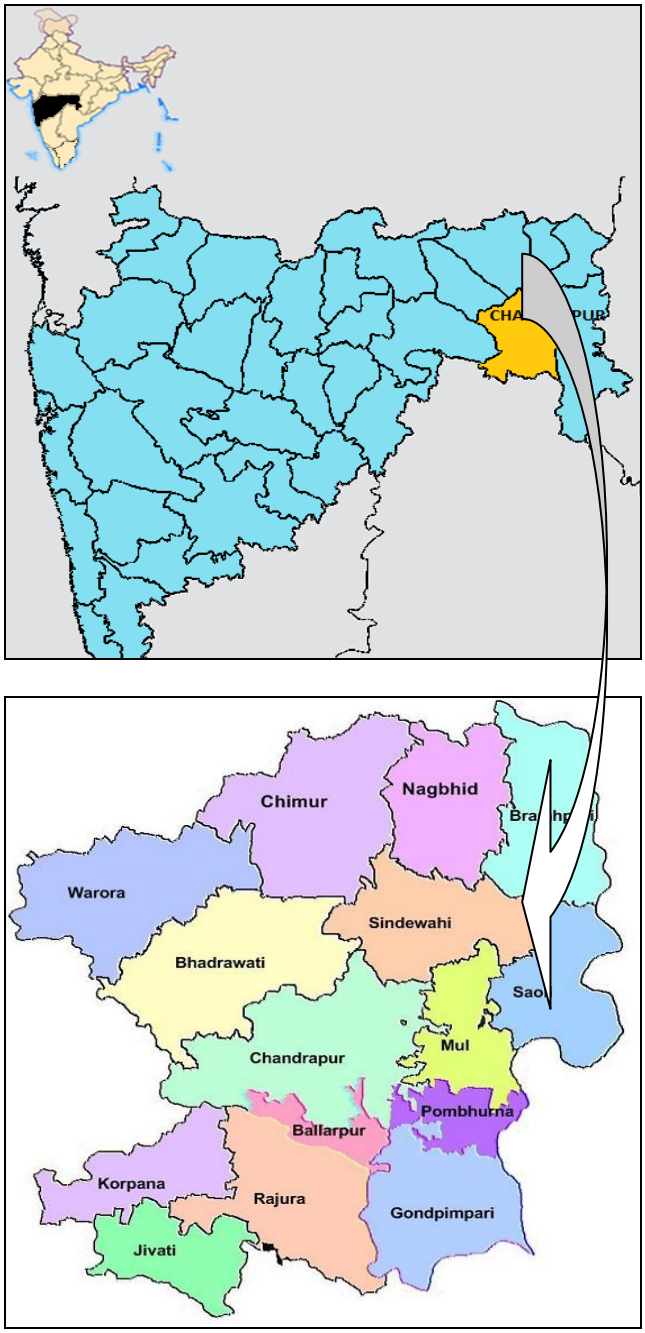


Fig. 1: Map of Maharashtra Showing Chandrapur District & Mul Tahasil

MATERIALS AND METHODS

Four honey samples viz., CHN-MUL-Raj, CHN-MUL-Don, CHN-MUL-Bha, CHN-MUL-Chi were collected during the period February 2012 to February 2013 from Rajuli, Dongargaon, Bhansuli, Chikhali respectively. All the samples represent squeezed honey collected from the natural *Apis dorsata* hives (Map).

The squeezing (pressing) of the honey combs was carried out under personal supervision and only honey bearing portion of the comb was used for this purpose.

1 ml of the honey sample was dissolved in 10 ml of distilled water & centrifuged. The sediment obtained was treated with 5 ml glacial acetic acid. The acetic acid was decanted and the material was subjected to acetolysis (Erdtman, 1960) for analysing the pollen content in honeys qualitatively & quantitatively, three pollen slides were prepared for each sample. The recorded pollen types were identified with the help of reference slides collection & relevant literatures for quantification of pollen types recorded, a total of 300 pollen grains were counted at random from the three palynoslides prepared for each sample. Based on their frequencies, the pollen types encountered were placed under the pollen frequency classes recommended by the international commission for bee Botany Louveaux *et al;*(1978) viz., predominant pollen type (>45%), secondary pollen type (16-45%), important minor pollen types (3-15%),and minor pollen types (<3%). Non-melliferous (anemophilous) pollen types were excluded while determine the frequencies of melliferous pollen types (International Commission for Bee Botany Louveaux *et al. ;*1978). The absolute pollen counts of each sample was determined in accordance with the method recommended by Suryanarayana *et al.* (1981). Unacetolysed samples of honey were examined for the study of honeydew elements (fungal spores, hyphal shreads and algal filaments).

RESULTS AND DISCUSSION

Of the 4 honey samples collected from Mul tahasil, *Lathyrus sativus* (46.16%) represented the predominant pollen type in one sample (CHN-MUL-Raj) while 3 are multifloral (CHN-MUL-Don), (CHN-MUL-Bha), (CHN-MUL-Chi). The other significant pollen types recorded includes (secondary to minor pollen) *Cajanus cajan, Celosia argentea, Prosopis juliflora, Capparis grandis, Cleome gynandra, Capsicum annuum, Dodonea viscosa*.

Table 1: Pollen frequency class and frequencies (%) in *Apis dorsata* honey.

Sample No.	Date of Collection	Type of Honey	Absolute pollen counts (APC) / g	HDE/P	Pollen Type
CHN-MUL-Raj	04-02-2012	Unifloral	293,000	0.02	P –Lathyrus sativus(46.16) S - Cajanus cajan(17.32) I – Celosia argentea (6.5), Prosopis juliflora(5.83), Coriandrum sativus(4.5), Capparis grandis(4.33), Hyptis suaveolens(3.83), Tridax procumbens(3.16) M–Ci, Bl(each2.83), Do(2), All(1.5), La(0.5) NMP –Holoptelea intergrifolia(1.2)
CHN-MUL-Bha	16-02-2012	Multifloral	1376,000	0.01	P –Nil S - Cajanus cajan(20.66), Lathyrus sativus(29.83), Hyptis suaveolens(23), Celosia argentea(19.83) I – Prosopis juliflora(5.16), Capsicum annuum(3.33) M –Nil NMP –Nil
CHN-MUL-Don	14-02-2013	Multifloral	6,000	0.02	P –Nil S - Lathyrus sativus(28), Celosia argentea(16.16), Cajanus cajan(15.83) I – Capsicum annuum(12), Capparis grandis(7.16), Cleome gyandra(5.5), Tridax procumbens(3.66), Pisidium guajava(3.16) M –Hy(2.5), De, Bl(each 2.16), Leu(0.83), Cart(0.5) NMP –Nil
CHN-MUL-Chi	28-02-2013	Mutlifloral	300,000	0.01	P –Nil S - Celosia argentea(24.16), Cajanus cajan(21), Hyptis suaveolens(20.5), Lathyrus sativus(20.33) I – Capsicum annuum(4.5), Dodonea viscosa(3.16) Capparis grandis(3.14) M –Bl(1.83), Cart(0.88), Tri(0.86) NMP – Sorghum Vulgare(8.17) Amaranthus/ Achyranthus (0.94)

Table 2 – showing pollen morphology of Melliferous taxa

Sr. No.	Pollen types	Pollen Size, Shape and symmetry	Aperture pattern	pollen wall (Sporoderm) Structure and sculpture
01	***Allium cepa*** Linn.	14-28× 32-48µm, ellipsoidal, Bilaterally symmetrical	Monosulcate, sulcus tenuimarginate	Exine 1.5 µm thick, subtectate, surface faintly reticulate
02	***Blumea*** sp.	21-24 µm, Amb spheroidal, isopolar, Radially symmetrical	Tricolprate, colpi long	Exine 3 µm thick, surface echinate, spines 5-6 µm long, 4 spines in the inter apertural region interspinal area psilate
03	***Cajanus cajan*** (Linn.) millsp.	35-37 µm Amb rounded triangular ; 32-34× 35-39 µm, oblate spheroidal; radially symmetrical	Tricolporate, colpi long, ends tapering, tips acute, ora circular	Exine 3.1 µm thick, sub tectate, surface reticulate, heterobrochate, meshes smaller near the apertural regions and larger elsewhere, lumina hexa to pentagonal, psilate, muri simplibaculate
04	***Capparis grandis*** Linn.	10-12 µm , Amb spheroidal; 14-16 ×9-12 µm prolate to subprolate; Radially symmetrical	Tricolporate, colpi linear to narrowly elliptic, ends tapering, tips acute, ora faint lalongate	Exine 1 µm thick, tectate, surface faintly granular to almost psilate
05	***Capsicum annuum*** Linn.	29-34 µm, Amb spheroidal; 29-35× 26-30 µm, subprolate; radially symmetrical	Tricolporate , colpi constricted at oral region, ends tapering, tips acute, ora prominently lalongate	Exine 1.5 µm thick, tectate, surface faintly granular to almost psilate
06	***Carthamus tinctorius*** Linn.	59-65 µm, Amb spheroidal: 58-62× 66-73 µm, subprolate, radially symmetrical	Tricolporate , colpi with tapering ends, ora lalongate	Exine (spinoid processes included) about 8 µm thick at poles, 10 µm at equator tectate, tectum prominently columellate, columella simple or branched, sharply undulating with supratectal solid, pointed, robust sinule like processes

Table 2: Continued…

Sr. No.	*Pollen types*	Pollen Size, Shape and symmetry	Aperture pattern	pollen wall (Sporoderm) Structure and sculpture
07	***Cleome* gynadra** Linn	19-21 µm, Amb spheroidal, 18-22 ×14-16 µm, prolate spheroidal; radially symmetrical	Tricolporate, colpi with tapering ends, ora faint, lalongate	Exine 1 µm thick, sub-tectate, surface finely reticulate, homobrochate, lumina polygonal, smooth, muri simplibaculate
08	***Celosia argentea*** Linn	30-35 µm spheroidal radially symmetrical	Pantoporate, pore No. 15-20, circular. Diam; 4-5 µm, pore membrance flecked with granules, interporal distance 8-11 µm	Exine 2 µm thick, tectate, interporal space coarsely granular
09	***Coriandrum sativum*** Linn.	23-28 µm, Amb seenonly occasionally , rounded triangular; 35-28× 15-16 µm perprolate constricated of the equator, Radially symmetrical	Tricolporate, colpi long, narrow, ora lalongate to circular	Exine 1.5-2 µm thick at poles and 2.5 – 3.5 µm thick at equator, subtectate, surface finely reticulate
10	***Dodonea viscosa***(Linn). *Jacq.*	29-32 µm, Amb subtriangular to rounded with slightly projecting obtuse angles: 30-33 × 26-29 µm prolate spheroidal, Radially symmetrical	Tricolporate, colpi long and narrow, almost reaching the poles, ora lalongate with Plate Fig.heavy endexinous thickening on the polar sides.	Exine 2.5 µm thick, subtectate, surface faintly microreticulate
11	***Hyptis suaveolens*** (Linn.) Poit.	35-39 µm, Amb spheroidal; 32-35× 36-39 µm, oblate spheroidal ; Radially symmetrical	Hexacolpate, colpi long, tips acute	Exine 2.5 µm thick, subtectate, surface reticulate (at places retipilate), reticulum homobrochate, lumina polygonal to circular with few free pila heads, muri simplibaculate.
12	***Lagascea mollis*** Cav.	38-42 µm, Amb spheroidal to rounded triangular; 33-35 × 39-43 µm, oblate spheroidal ; Radially symmetrical	Tricolporate, colpi linear, tips acute, ora lalongate	Exine 5 µm thick tectate, surface echinate, spines 6.5 µm long, base 2.3 µm broad
13	***Lathyrus sativus*** Linn.	42 × 31.5 µm, prolate to perprolate , Radially symmetrical	Tricolporate, colpi long, ends tapering, ora circular to slightly lalongate	Exine 1.5 µm thick, subtectate, surface reticulate.
14	***Prosopis juliflora*** (Sw.) DC	36-39 µm, Amb rounded triangular; 38-42× 30-35 µm, prolate to subprolate; Radially symmetrical	Tricolllporate, occasionally syncolpate, colpi tapering towards poles, tips acute, ora lalongate	Exine 3.2 µm thick, tectate surface faintly reticulate
15	***Psidium guajava*** Linn.	24-25 µm, Amb subtriangular; 13-16× 26-28 µm, oblate; Radially symmetrical	Tricolporate, syncolpate, parasyncolpate, ora lalongate	Exine 1.5 µm thick , tectate surface granular to pailate
16	***Tridax procumbens*** Linn.	31-38 µm, Amb rounded triangular to squarish; 30-35x 32-38 µm, oblate spheroidal; Radially symmetrical	Tri to tetra colporate, colpi linear, sharply tapering, ora faint, circular	Exine 5 µm (without spines) thick, tectate, surface echinate, spines 6 µm long, 2.5 µm in diam, at base

Table 3: showing pollen morphology of Non-melliferous taxa

Sr. No.	*Pollen types*	Pollen Size, Shape and symmetry	Aperture pattern	pollen wall (Sporoderm) Structure and sculpture
1	*Amaranthus/Achyranthes sp.*	19-36 µm, spheroidal; Radially symmetrical	Pantoporate, pores, 25-35 in number, circular, 2-3 in diam, interporal distance 3-5 µm	Exine 1.5 µm thick, tectate, interporal space finely granular
2	*Holoptelea integrifolia (Roxb.) Planch*	26-28 µm, Amb spheroidal to slightly angular; Radially symmetrical	Tetra to hexaporate, generally hexaporate pores circular with distinct margins, 2-3 µm in diam	Exine 1.5 µm thick, subtectate, surface faintly microreticulate
3	*Sorghum vulagare Pers.*	51-55 µm, spheroidal; Radially symmetrical	Monoporate, pore circular provided with annulus, pore diam with annulus 4.1 µm without annulus 3.3 µm	Exine 1 µm thick, tectate , surface faintly granular to almost psilate

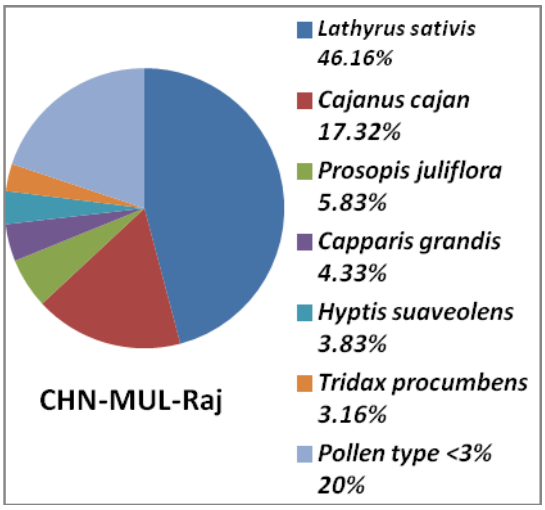


Fig. 1.1: Palynograph of Rajoli

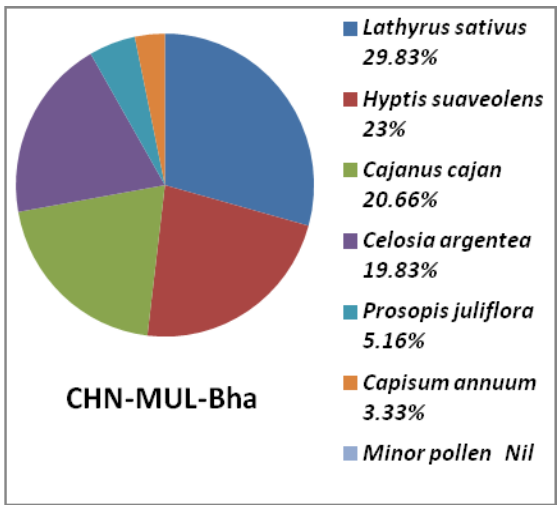


Fig. 1.2: Palynograph of Bhansuli

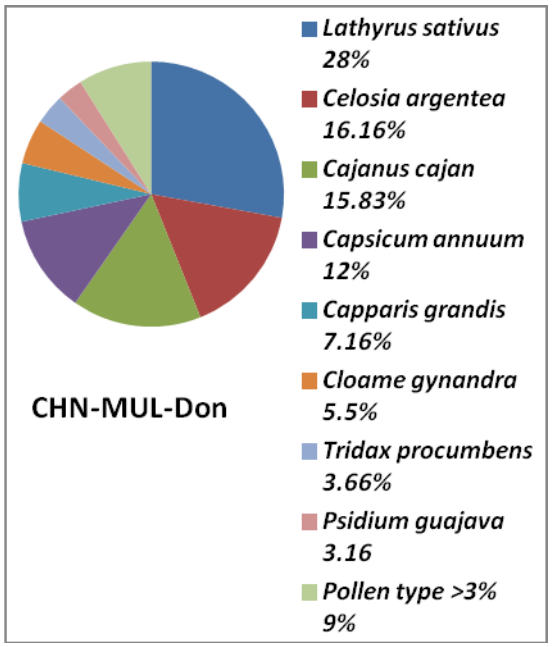


Fig. 1.3: Palynograph of Dongargaon

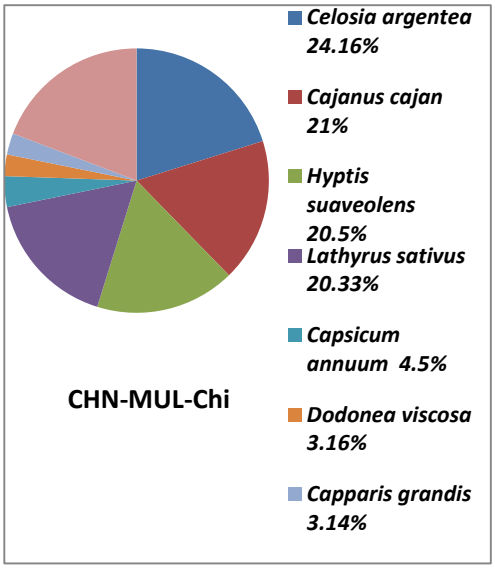


Fig.1.4: Palynograph of Chikhali

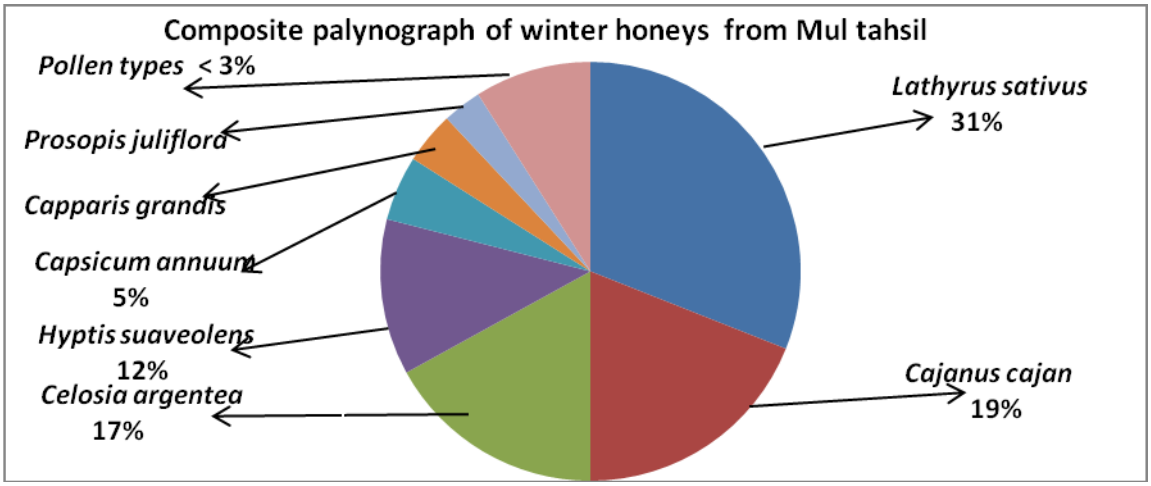
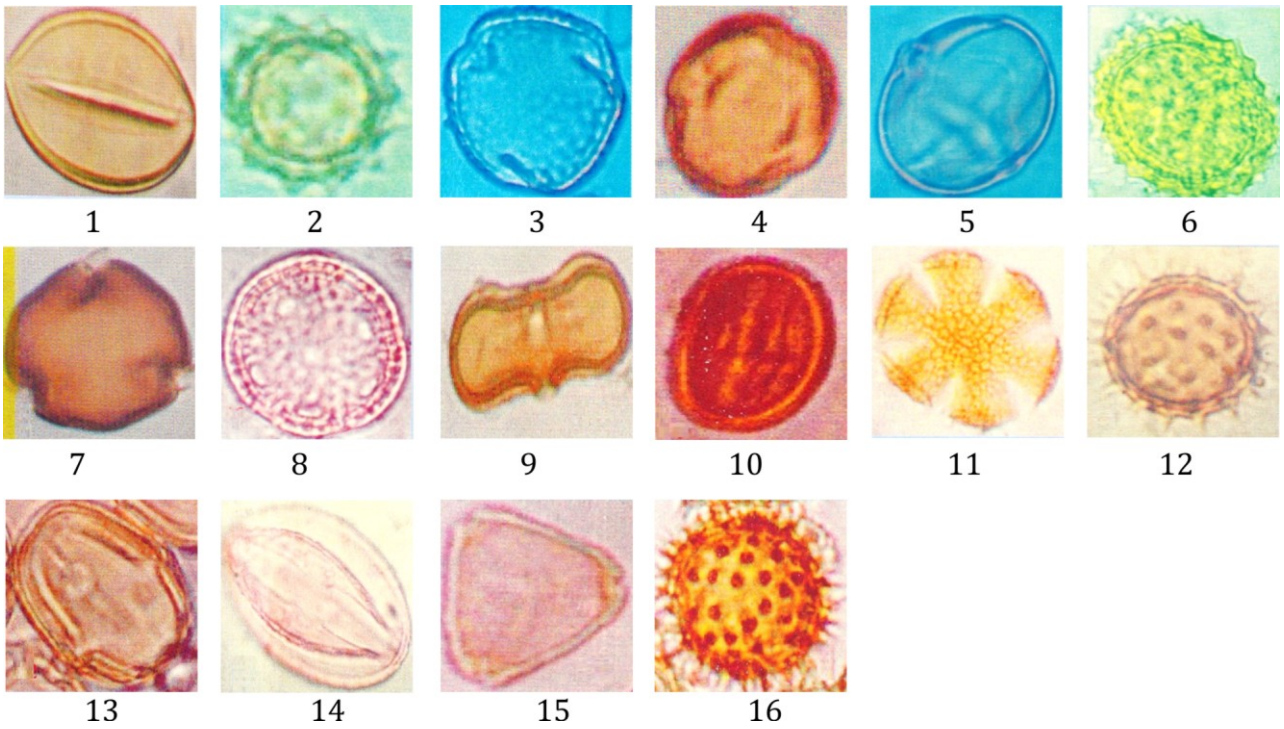


Fig 1.5: Composite Palynograph of winter honeys from Mul Tahsil

Fig. 1: Pie charts showing pollen spectra of Apis dorsata honeys samples



Fig, 2: Microscopic photograph of pollen grains found in honey sample

1) *Allium cepa* 2) *Blumea* sp. 3) *Cajanus cajan* 4) *Capparis grandis*
5) *Capsicum annuum* 6) *Carthamus tinctorius* 7) *Cleome gynadra* 8) *Celosia argentea*
9) *Coriandrum sativum* 10) *Dodonaea viscosa* 11) *Hyptis suaveolens* 12) *Lagascea mollis*
13) *Lathyrus sativus* 14) *Prosopis juliflora* 15) *Psidium guajava* 16)*Tridax procumbens*

All together 22 pollen types (19 of melliferous and 3 of non-melliferous taxa) referable to 9 families have been recorded from these samples (Photoplate). The sample (CHN-MUL-Raj) showed the maximum number of pollen type (14) and the sample (CHN-MUL-Bha), the minimum number (6) & had no minor pollen types. In the sample (CHN-MUL-Chi) however the pollen of *Sorghum vulgare* were found to be good number (8.17%). The absolute pollen counts ranged from 6,000/g to 1376,000/g and the HDE/P ratio ranged from 0.01 to 0.02 and represented by fungal spores (Table 1).

The details of the pollen analysis of the 4 honey samples (melliferous/non-melliferous) are represented in table 2. Similarly individual palynograph (Pollen spectra) of each honey sample and composite palynograph was also given to show the pollen contents of the samples of Mul tahsil. The distinguishing morphological features of the pollen types encountered in the present study are given below.

The bee plants of Mul tahsil are referable to 3 categories:

1. **Crop plants**: *Cajanus cajan, Lathyrus sativus, Cariandrum sativus, Capsicum annuum* and *Sorghum vulgare.*

2. **Arborescent taxa/shrub:** *Pisidium guajava, Dodonea viscosa, Capparis grandis, Prosopis juliflora.*

3. **Herbaceous weeds:** *Celosia argentea, Hyptis suaveolens, Carthamous tincterius, Blumea sp., Tridax procumbens*

Of these three categories the crop plants are mostly preferred by the bees of this tahsil. The crop plants *Lathyrus sativus* and *Cajanus cajan* and *Capsicum annuum* cultivated extensively during winter constitute the chief bee forage plants. In this tahsil during winter season of the *Cajanus cajan* & *Lathyrus sativus* represents most preferred nectar sources for the honeybees. Our observation indicates that *Lathyrus sativus* and *Cajanus cajan* represent abundant nectar and pollen sources to *Apis dorsata*.

The region selected for the present study has good potential for sustaining bee keeping ventures because of the diversity of nectar and pollen taxa. Since *Cajanus cajan, Lathyrus sativus* are major sources of forage for honey bees therefore efforts should be made to increase, their cultivation. The other plant encountered in these honey samples are the member of families like Fabaceae, Asteraceace, Lamiaceace, Capparidaceace, Solanaceace in this area.

To improve the bee-keeping industry a proper understanding and mutualism between bees and available plant taxa in the region and in a particular season is necessary. The identified taxa were not only the economic crops but also play an important role in the development of bee-keeping in this region.

This data reflects the floral situation of the place where particular honey was produced and the identification of geographical origin based on the presence of a combination of pollen types of that particular area.

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