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Pollen analysis of summer honeys collected from forest area of Chandrapur tahsil of Chandrapur district, (Maharashtra state)

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Abstract---The paper incorporates a qualitative and quantitative analysis of pollen contents in five squeezed honey samples of *Apis dorsata* hives collected from forest area of Chandrapur tahsil of Chandrapur district. *Terminalia sp.* represents the predominant pollen type in five samples ranged from (48% to 61.66%) are designate as Termanalia honey. The other significant pollen types recorded include *Blumeasp*, *Sapindencea marginatus*, *Lagiscea mollusc*, *Delonix regia*, *Bombax ceiba*, *Albizia lebbek*, *Pongamia pinnata*, *Carthamus tinctorius*, *Brassica sp.*, *Capsicum annum* and *Astericanthalongifolia*. The pollen counts ranged from 9000/g to 399000/g. 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*, forest area, chandrapur 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 beekeeping in an area. Melittopalynological studies are thus helpful in bee management and in promoting the beekeeping development.

Laboratory studies using Melittopalynological methods have been made to evaluate sources of pollen and nectar for honey bees in different parts of the country namely Maharashtra (Laxmikant Borkar and Devendra Mate, 2017-18, Bhusari *et al.*, 2005; Phadke, 1962; Kumar and Jagtap, 1988), Andhra Pradesh (Ramanujam and Khatija, 1991, Kalpana and Ramanujam, 1991, Moses, 1987, Karnataka (Yoganarasimhan, 1982; Agashe and Ranjaswami, 1997; Sheshagiri, 1985; Bhargava *et al.*, 2009), Lucknow (Suryanarayana, 1976) and Indian honeys (Sen and Banarjee, 1956; Nair, 1964; Seethalakshmi, 1993). Present investigation incorporates a qualitative and quantitative pollen analysis of five honey samples from forest area of Bramhapur 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 & Chandrapur Tahsil

Materials and Methods

Five honey samples viz., CHN-CHN-LOH, CHN-CHN-AJA, CHN-CHN-NAN, CHN-CHN-URJ, CHN-CHN-CHI were collected during the period 16 March 2012 to 03 May 2013 from Lohara, Ajaypur, Nandgaon Pole, Urjanagar and Chichpalli 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 literature for quantification of pollen types recorded, a total of 300 pollen grains were counted at random from the three palynoslides prepared for each samples. 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 threads and algal filaments).

Results and Discussion

Of the 5 honey samples collected from Chandrapur tahsil, *Terminalia sp.* ranged from (48% to 61.66%) represent the predominant pollen type in five sample (CHN-CHN-LOH, CHN-CHN-AJA, CHN-CHN-NAN, CHN-CHN-URJ, CHN-CHN-CHI). i.e. unifloral. The other significant pollen types recorded includes (upto important minor) *Blumea sp.*, *Sapindusemarginatus*, *Lagiscea mollusc*, *Delonix regia*, *Bombax ceiba*, *Albizia lebbbeck*, *Pongamia pinnata*, *Carthamus tinctorius*, *Brassica sp.*, *Capsicum annum* and *Asteracanthalongifolia*.

Table 1
Pollen frequency class & frequencies(%) in *Apis dorsata* summer honey

Sample No.	Date of Collection	Type of Honey	Absolute pollen counts (APC) / g	HDE/P	Pollen Type
CHN-CHN-LOH	16-03-2012	Unifloral	241,000/g	0.01	P – <i>Terminalia sp.</i> (50) S - Nil I - <i>Sapindusemarginatus</i> (9.66) <i>Pisidium guajava</i> (6.83) <i>Blumea sp.</i> (5.83) <i>Bombax ceiba</i> (5.16) <i>Asteracanthalongifolia</i> (4.66) <i>Brassica sp.</i> (3.83) <i>Delonix regia</i> (3) M – Pr(2.66), Pon(2), Ma, Alb, Caps(each 1.83), Ci, Leu(each 1), Car(0.83), La(0.66), Cor(0.33), Alb(1.83), Mo(0.5) NMP – <i>Typha angustata</i> (6.8)

CHN- CHN- AJA	19-03-2012	Unifloral	241,000/g	0.01	P – Terminalia sp.57.25) S - Nil I - Sapindusemarginatus(7.83) Pisidiumguajava(7.51) Delonixregia(3.5) Bombaxceiba(3.33) M – Ast.1(2.87), Ci(2), Br(1.83), May(1.5), Ma(1.33), Ru, All(each 1), Bl(0.83), Car, Leu,Cel(each 0.66), Alb, Cu(each 0.5) NMP –Nil
CHN- CHN- NAN	10-04-2012	Unifloral	173,000/g	0.02	P – Terminalia sp.(50) S - Nil I - Sapindusemarginatus(10.13) Pisidiumguajava(10) Asteracanthalongifolia(5.5) Blumea sp.(3.83) M –Pon, Caps(each 2.66), Mel(2.5), Bo(2.83), Do(1.6), All(1.5), Pr(1.33), Ca(1.16), Car(1), Cas, Ma(each 0.66), Ci(0.5), De(0.3) NMP – Sorghum vulgare(0.83)
CHN- CHN- URJ	26-04-2012	Unifloral	252,000/g	0.02	P – Terminalia sp.(61.66) S - Nil I - Capsicum annuum(9.66) Pisidiumguajava(8.16) Sapindusemarginatus(7.16) Bombaxceiba(5) M – Pon(2.5), Ast.1(2.33), Ci(1.83), Pr(1.16), Mel , Bl , Ma(each 0.66) NMP – Typhaangustata(6.91) Sorghum vulgare(0.81)
CHN- CHN- CHI	03-05-2013	Unifloral	399,000/g	0.01	P – Terminalia sp.(48) S - Nil I - Blumea sp.(10.66) Sapindusemarginatus(10) Pisidiumguajava(8.66) Lagasceamollis, Carthamustinctorius(each 3) Albizialebeck(2) M –Cl(2.33), Ma(2), Bo(1.66), De(1.33), Sy(0.83), Car(0.66), Pon(0.5) NMP – Typhaangustata(7)

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
1	Albizialebeck	16-celled polyad with central eight cells of two superimposed groups of 4 cells each and peripheral 8 cell (4+4+8 condition), spheroidal or biconvex disc, 72-74 μm in diam., each cell of the polyad 17-19m in diam, Amb of individual cell squarish; radially symmetrical	Individual pollen triporate , pores faint	Exine 1.6 μm thick, tectate, surface psilate to faintly granular
2	Allium cepa Linn.	14-28 $\times$ 32-48 μm , ellipsoidal, Bilaterally symmetrical	Monosulcate, sulcus tenuimarginate	Exine 1.5 μm thick, subtectate, surface faintly reticulate
3	Asteracanthalongifolia(Linn.) Nees.	56-59 μm , Amb spheroidal or quadrangular; 50-55 $\times$ 52-59 μm , oblate spheroidal; Radially symmetrical	Tetracolporate, colpi long, ends tapering, tips acute, colpi alternating with 4 streak like pseudocolpi, ora more or less circular.	Exine 3.3 μm thick, subtectate , surface reticulate, homobrochate, lumina polygonal and psilate.
4	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
5	Bombaxceiba Linn	51 μm (49.5 $\times$ 52.5) μm , peroblate, isopolar, Radially symmetrical	Tricolprate, col. length 12 (10.5-13.5) μm	Exine thick 3 μm , coarsely reticulate, mesh 4.1 μm (3-4.5 μm) in the major part except at the angles showing medium reticulations 1-8 μm (1.5 -3 μm), greater number of baculae are found in the lumen.

				Murisimplibaculate, faint LO pattern.
6	<i>Brassica</i> sp.(Linn) Koch	30-33 μm , Amb rounded triangular to almost spheroidal; 27-31 $\times$ 24-27 μm , prolate spheroidal; radially symmetrical	Tricolpate , colpal ends tapering, tips acute	Exine 2.5 μm thick, sub tectate, surface reticulate, heterobrochate, meshes narrow at mesocolpial regions giving a striate look ,lumina polygonal.
7	<i>Capparis</i> grandis	10-12 μm , Amb spheroidal; 14-16 $\times$ 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
8	<i>Capsicum</i> annumLinn.	29-34 μm , Amb spheroidal; 29-35 $\times$ 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
9	<i>Careya</i> arboreaRoxb.	52.1 $\times$ 40.1 μm (48-54 $\times$ 37.5 -43.5) μm , subprolate, isopolar, radially symmetrical	Hexacolpate, syncolpate with crassimarginatecolpi, col. Length 43.5 (42-46.5) μm	Exine thick , 3 μm , undulating, considerable thick at the poles sexine-nexine not differentiated medium reticulate, more coarse at the poles. Mesh 1.5-3 μm , clear LO pattern
10	<i>Carthamus</i> tinctorius	59-65 μm , Amb spheroidal: 58-62 $\times$ 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 supracteal solid, pointed, robust sinule like processess
11	<i>Casaria</i> elliptica	29-37 μm , Amb spheroidal ; 28-36 $\times$ 27-33 μm subprolate radially	Tricolporate , colpi with tapering ends, ora lalongate	Exine 1.5 μm thick, tectate, surface psilate

		symmetrical		
12	<i>Celosia argentea</i>	30-35 μm spheroidal radially symmetrical	Pantoporate, pore No. 15-20, circular. Diam; 4-5 μm , pore membrane flecked with granules, interpore distance 8-11 μm	Exine 2 μm thick, tectate, interpore space coarsely granular
13	<i>Citrus</i> sp.	27-29 μm , Ambispherical, 26-30 $\times$ 25-27 μm , prolate spheroidal radially symmetrical	Tetracolporate, colpi linear, tips acute, oral alongate	Exine 2 μm thick subtectate, surface Reticulate. Heterobrochate, meshes smaller near the apertural regions and larger elsewhere, lumina hexa to pentagonal or irregular, psilate, murisimpli to locally duplibaculate
14	<i>Clerodendrum</i> sp.	43-48 μm , Ambispherical, 41-44 $\times$ 37-40 μm , prolate spheroidal; Radially symmetrical	Tricolporate, colpi fairly long, tips acute, colpal margins broken	Exine 1.5 μm thick (excluding spinules), tectate surface spinulate, spinules 0.6-1 μm long, interspinular space finely granular
15	<i>Coriandrum sativum</i>	23-28 μm , Ambispherical, occasionally rounded triangular; 35-28 $\times$ 15-16 μm per prolate constricted of the equator, Radially symmetrical	Tricolporate, colpi long, narrow, oral alongate to circular	Exine 1.5-2 μm thick at poles and 2.5 - 3.5 μm thick at equator, subtectate, surface finely reticulate
16	<i>Cucurbitaceae</i> type	50-63 μm , subprolate, isopolar, Radially symmetrical	Tricolporate, col. Length 48.7 (48-49.5) μm	Exine thick 4.5 μm , sexine - nexine not discernible, rather coarsely reticulate, mesh 3.4 (3 - 4.5) μm , baculae distinct, clear LO pattern
17	<i>Delonix regia</i>	59.62 μm , Amb more or less spheroidal to subtriangular; 53-56 $\times$ 57-60 μm , oblate to	Tricolporate, colpi long with blunt ends, oral faint, more or less rounded	Exine 5.2 μm thick, subtectate, surface coarsely reticulate. Heterobrochate,

		suboblate; Radially symmetrical		meshes smaller near the apertural regions & larger elsewhere, lumina poly to hexagonal with a number of free bacules, muri thick, sinuous, simpli to locally duplibaculate
18	<i>Dodonea viscosa</i>	29-32 μm , Ambsubtriangular to rounded with slightly projecting obtuse angles: 30-33 $\times$ 26-29 μm prolate spheroidal, Radially symmetrical	Tricolporate, colpi long and narrow, almost reaching the poles, oralalongate with Plate Fig. heavy endexinous thickening on the polar sides.	Exine 2.5 μm thick, subtectate, surface faintly microreticulate
19	<i>Lagascea mollis</i>	38-42 μm , Amb spheroidal to rounded triangular; 33-35 $\times$ 39-43 μm , oblate spheroidal; Radially symmetrical	Tricolporate, colpi linear, tips acute, oralalongate	Exine 5 μm thick tectate, surface echinate, spines 6.5 μm long, base 2.3 μm broad
20	<i>Leucanea leucocephala</i>	52-59 μm , Amb spheroidal : 47-49 $\times$ 51-58 μm , suboblate: Radially symmetrical	Tricolporate colpi long, tips acute, oralalongate	Exine 4 μm thick, subtectate surface microreticulate, homobrochate
21	<i>Mangifera indica</i> Linn.	27-31 μm , Ambsubtriangular; 29-32 $\times$ 26-28 μm , subprolate; Radially symmetrical	Tricolporate colpi long, tips acute orally prominently lanlongate	Exine 2.5 μm thick, subtectate, surface striatoreticulae, striations more or less parallel in equatorial view, lumen generally elongated in polar direction, murisimplibaculate
22	<i>Maytenus marginata</i> Wild.	Oblate, 45-49 μm , Amb, rounded triangular to almost spheroidal, isopolar, Radially symmetrical	Tricolporate, colpi length 9.4 μm , (9-10.5) μm , oralalongate	Exine thick 3 μm , sexine thicker than nexine, reticulate size of mesh 2.4 (1.5-3) μm , distinct LO pattern.
23	<i>Melia azadirachta</i>	47.2 $\times$ 36.7 μm , 46.5-48 $\times$ 36.37.5 μm , subprolate, isopolar, Radially symmetrical	Tetracolporate, col. Length 36 μm , orally 6.5 μm , lanlongate, pantoporate, pores, circular 6.9 μm ,	Exine thick 3 μm , sexine- nexine not clear, psilate

24	Momordicacharantia	68-76 μm , Amb spheroidal; 67-72 $\times$ 64-65 μm , prolate spheroidal; radiallysymmetrical	Tricolporate , colpi narrow with tapering ends, ora faint , lalongate	Exine 4 μm thick, subtectate, surface reticulate, lumina irregularly polygonal psilate
25	PisidiumguajavaLinn.	24-25 μm , Ambsubtriangular; 13-16 $\times$ 26-28 μm , oblate; Radially symmetrical	Tricolporate, syncolpate, parasyncolpate, oralalongate	Exine 1.5 μm thick , tectate surface granular to pailate
26	Pongamiapinnata(Linn) Pierre.	29-31 μm , Ambsubtriangular: 27-31 $\times$ 25-28 μm , subprolate; Radially symmetrical	Tricolporate, colpi linear to narrowly elliptic tips acute, oralalongate	Exine 1.5 μm thick, subtectate, surface granular to locally faintly microreticulate
27	Prosopisjuliflora(Sw.) DC	36-39 μm , Amb rounded triangular; 38-42 $\times$ 30-35 μm , prolate to subprolate; Radially symmetrical	Tricolporate, occasionally syncolpate, colpi tapering towards poles, tips acute, oralalongate	Exine 3.2 μm thick, tectate surface faintly reticulate
28	Rungiarepens(Linn.) Nees.	40-44 $\times$ 25-26 μm , oblong; Bilaterally symmetrical	Diporate, pores circular, 2.5 μm , in diam, margin of the pores densely beset with small processes	Exine 3 μm thick at poles, 4.6 μm at equaltor, subtectate, tectum undulating, distinct rounded to irregular areolae (2-4 μm) linearly aligned in the vicinity of apertures, rest of the wall microreticulate
29	Sapindusemarginatusvahi.	24-26 μm , Amb triangular, sides straight or even slightly concave; 18-20 $\times$ 26-29 μm , oblate (occasionally suboblate); Radially symmetrical	Tricolporate, colpi narrowly elliptic long, tips acute, oralOlongate	Exine 2 μm thick on mesocolpia, 1-1.5 μm thick near apertures, surface psilate
30	Syzygiumcumini	16-18 μm , Amb triangular to rarely quadrangular, sides slightly concave; 10.5-12 $\times$ 17-20 μm , oblate; Radially symmetrical	Tricolporate, rarely tetra colporate, syncolpate, parasyncolpate, oralalongate	Exine 1.25 μm thick, tectae, surface granular to smooth
31	Terminaliasp.	19-22 μm , Amb spheroidal; 21-24	Tricolporate, colpi alternating with	Exine 1.5 μm thick, tectae, surface

		x20-22 μm , subprolate; Radially symmetrical	pseudocolpocolpi linear, tips acute pseudocolpi almost equal the size of colpi, ora more of less circular	psilate to locally finely granular
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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
01	Sorghum vulgare 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, faintly granular to almost psilate
02	Typha angustata Bory. et Chaub	28-35 μm , ellipsoidal, triangular or spheroidal; Radially symmetrical	Monoporate pore more or less circular 4-5 μm in diam, margin wavy, pore membrane densely granular	Exine 2.5 μm thick, sub surface reticulate in places reticulum homobrochate, polygonal to circular, murisimplibaculate

Fig. 1.1: Palynograph of Lohara

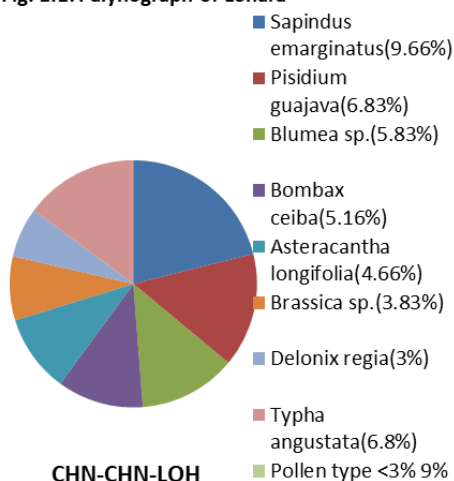


Fig. 1.2 Palynograph of Ajaypur

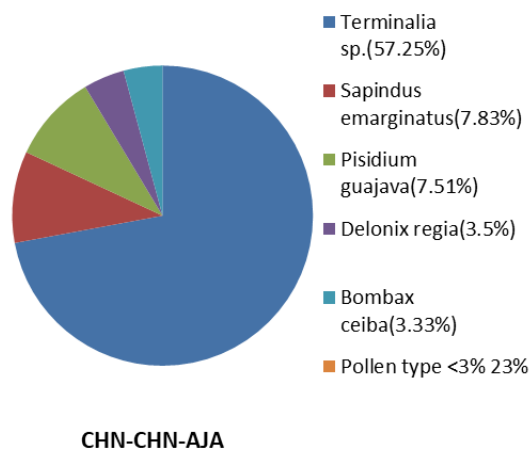
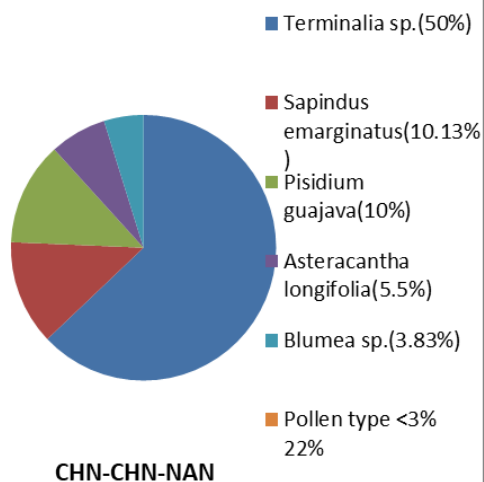
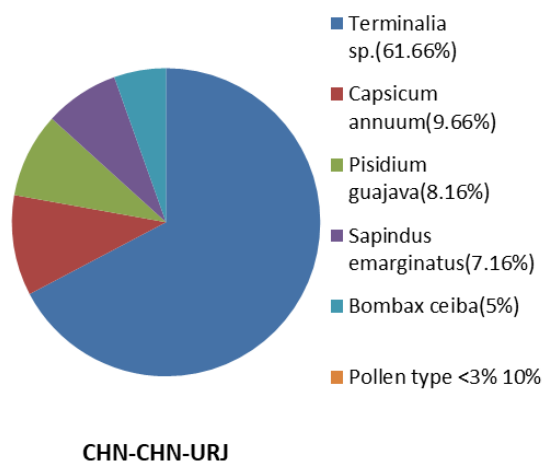
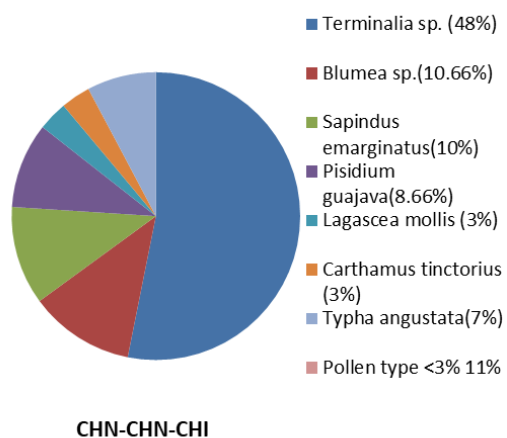


Fig. 1.3 Palynograph of Nandgaon Pole**Fig. 1.4 Palynograph of Urjanagar****Fig. 1.5 Palynograph of Chichpalli****Fig 1.6. Composite palynograph of summer honeys from Chandrapurtehsil**

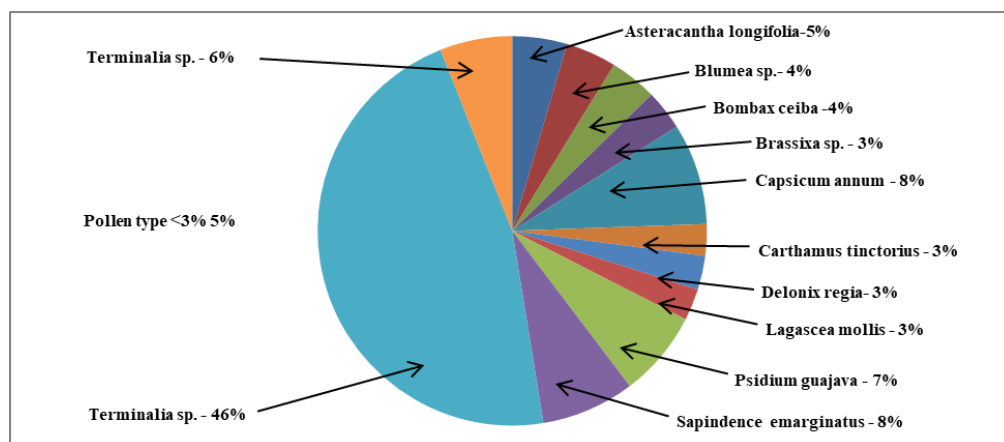
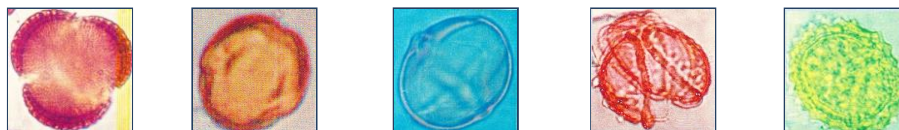


Plate Microscopic photograph of pollen grains found in honey sample



Albizia lebbeck *Allium cepa* *Asteracantha Blumea* sp. *Bombax ceiba*
longifolia



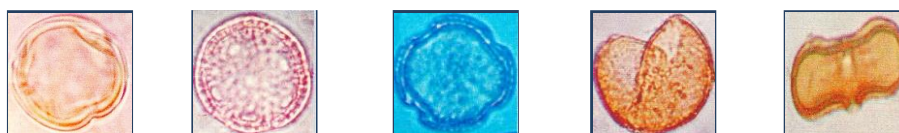
Brassica sp.

Capparis grandis

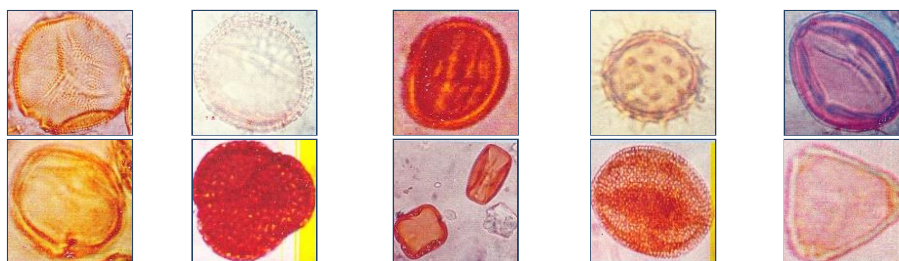
Capsicum annum

Careya arborea

Carthamus tinctorius



Casearia elliptica *Celosia argentea* *Citrus* sp. *Clerodendrum* sp. *Coriandrum sativum*



Cucurbitaceae type *Delonix regia* *Dodonea viscosa* *Lagascea mollis* *Leucanea leucocephala*

Mangifera indica *Maytenus emarginata* *Melia azadirachta* *Momordica charantia* *Pisidium guajava*



Pongamia pinnata *Prosopis juliflora* *Rungia repens* *Sapindus emarginatus* *Syzygium cumini*

All together 33 pollen types (31 of melliferous and 2 of non-melliferous taxa) referable to 25 families have been recorded from these samples (Photoplate). The sample (CHN-CHN-LOH) showed the maximum number of pollen type (21) and the sample (CHN-CHN-URJ), the minimum number (14). In the sample (CHN-CHN-LOH), (CHN-CHN-URJ), (CHN-CHN-CHI) however the pollen of *Typha angustata* were found to be good number (6.8 to 7%). The absolute pollen counts ranged from 9000/g to 399,000/g and the HDE/P ratio ranged from 0.01 to 0.03 and represented by fungal spores (Table 1). The details of the pollen analysis of the 5 honey samples (melliferous/non-melliferous) are represented in table 1. Similarly individual palynograph (Pollen spectra) of each honey sample

and composite palynograph was also given to show the pollen contents of the samples of Chandrapurtehsil (Fig. 1.1-1.6). The distinguishing morphological features of the pollen types encountered in the present study are given below. The bee plants of Chandrapurtahsil are referable to 3 categories:

- **Crop plants:**, *Allium cepa*, *Brassica* sp., *Capsicum annuum*, *Carthamustinctorius*, *Coriandrumsativum*, *Momordicacharantia* and *Sorghum vulgare*.
- **Arborescent taxa/shrub:** *Azadirachta indica*, *Bombaxceiba*, *Citrus* sp., *Casearia elliptica*, *Delonix regia*, *Leucaneaaleucocephala*, *Albizia libbeck*, *Mangifera indica*, *Melica azadirachta*, *Pongamia pinnata*, *Psidium guajava*, *Sapindenceemarginatus*, *Syzygium cumini*, *Terminalia* sp., *Capparis grandis*, *Mayetusemarginatus*, *Prosopis juliflora*, *Rungea repens*.
- **Herbaceous weeds:** *Asteracanthalongifolia*, *Sapindusemarginatus*, *Celosia argentea*, *Blumea* sp.

Of these three categories the arborescent plants *Terminalia* sp. constitute the chief bee forage plants in this tahsil during summer season. Besides the other arborescent plants *Delonix regia*, *Pongamia pinnata*, *Azadirachta indica* represents most preferred nectar sources for the honeybees. Our observation indicate that *Terminalia* sp. 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 *Terminalia* sp. are member of Combretaceae is major sources of forage for honey bees therefore efforts should be made to increase its cultivation. The other plant encountered in these honey samples are the member of families like Acanthaceae, Anacardiaceae, Mimosaceae, Caesalpiniaceae, Celastraceae, Myrtaceae, Samydaceae, Menispermaceae, Liliaceae, Capparidaceae, Amaranthaceae, Cleomaceae, Solanaceae, Papilionaceae and Sapindaceae 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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