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POLLEN ANALYSIS OF SQUEEZED SUMMER HONEYS FROM FOREST AREA OF NAGBHID TAHSIL OF CHANDRAPUR DISTRICT, (MAHARASHTRA STATE).

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Abstract:

The paper incorporates a qualitative and quantitative analysis of pollen contents in two squeezed honey samples of Apis dorsata hives collected from forest area of Nagbhid tahsil of Chandrapur district. Terminalia sp. represents the secondary pollen type in two samples ranged from (41.75% to 33.66%). The other significant pollen types recorded include Psidium guajava, Capsicum annum, Sapindus emarginatus, Pogamia pinnata, Asterecantha longifolia, Mangifera indica, Alium cepa, Capparis grandis, Citrus sp., Mayetenus emarginatus . The pollen counts ranged from 173000/g to 853000/g. The data reflects the floral situation of the place were 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, Nagbhid tahsil.*

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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 Melittopaloynological 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), 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 five honey sample from forest area of Bramhapuri 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.

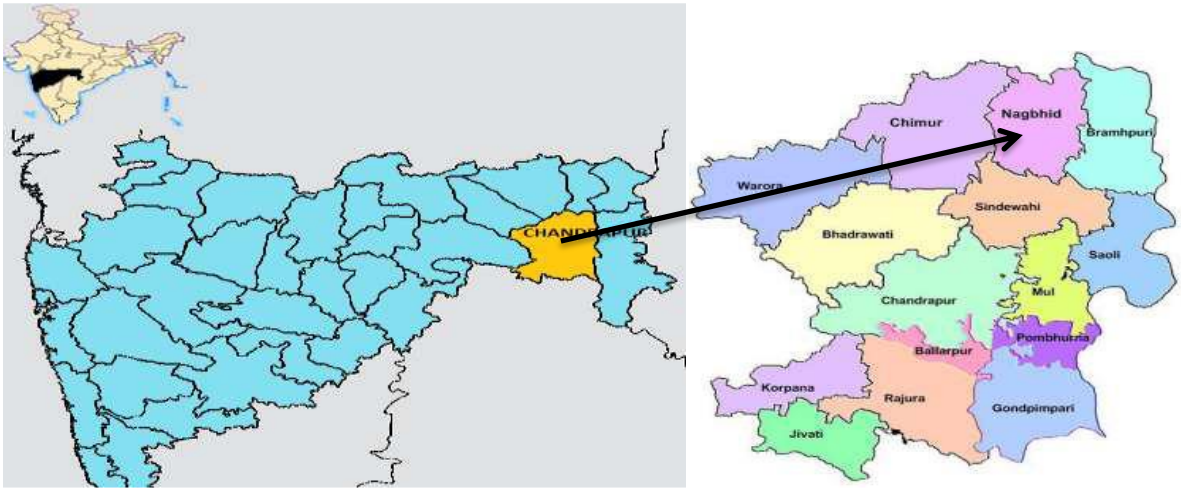


Fig. 1: Map of Maharashtra Showing Chandrapur District & Nagbhid Tahsil

MATERIALS AND METHODS:

Two honey samples viz., CHN-NAG-GIR, CHN-NAG-KAC were collected during the period 23 April 2012 and 27 April 2012 from Girgaon and Kacchepar 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 shreads and algal filaments).

RESULTS AND DISCUSSION:

Of the 2 honey samples collected from Nagbhid tahsil, secondary pollen type ranged from (41.75% to 33.66%) in both sample. i.e. Multifloral . The other significant pollen types recorded includes *Psidium guajava, Capsicum annum, Pongamia pinnata, Mayetus emarginata, Astericantha longifolia, Mangifera indica, Alium sepa, Capparis grandis, Citrus sp.*

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-NAG-GIR	23-04-2012	Multifloral	173,000/g	0.02	P –Nil S - Terminalia sp.(36.75) I – Pisidium guajava(6.66) Capsicum annuum(5.83) Pongamia pinnata(5.66) Maytenus emarginata(3) Asteracantha longifolia(3.16) M –All(2.33), Car(2), Pr(1.3), Tn(1.11), Cart(0.83), Ru(0.66), NMP –Typha angustata(7.84) Sorghum vulgare(0.18)
CHN-NAG-KAC	27-04-2012	Multifloral	853,000/g	0.02	P –Nil S - Terminalia sp.(35.66) I – Pisidium guajava(12.9) Sapindus emarginatus(11,75) Asteracantha longifolia(7.83) Pongamia pinnata(6.5) Capsicum annuum(3.66) Mangifera indica, Allium cepa (each 3.13) Capparis grandis, Citrus sp.(each 3) M – Az(2), Br(1.66), Pa(1.83), Cl(1.16), Cel(1), Bl(0.83), Car, Bo(each 0.66), Cart(0.5), Do, Bi(each 0.33) NMP – Nil

Table 2: Showing pollen morphology of Melliferous taxa

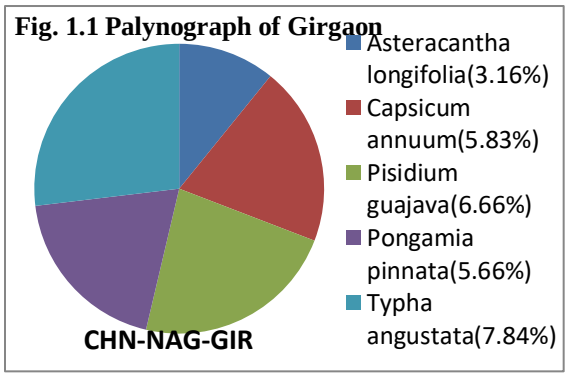
Sr. No.	Pollen types	Pollen Size, Shape and Symmetry	Aperture pattern	Pollen wall (Sporoderm) Structure and sculpture
1	**Allium cepa** Linn.	14-28× 32-48µm, ellipsoidal, Bilaterally symmetrical	Monosulcate, sulcus tenuimarginate	Exine 1.5 µm thick, subtectate, surface faintly reticulate
2	**Asteracantha longifolia** (Linn.) Nees.	56-59µm , Amb spheroidal or quadrangular; 50-55× 52-59µm, oblate spheroidal; Radially symmetrical	Tetracolporate, colpi long, ends tapering, tips acute, colpi alternating with 4 streak like pseudocolpi, ora more or less circular.	Exine 3.3µm thick, subtectate , surface reticulate, homobrochate, lumina polygonal and psilate.
3	**Azadirachta indica** A.juss	50-54µm, Amb squarish, sides convex; 47-54 38-47µm, subprolate, poles smoothly rounded; Radially symmetrical	Tetracolporate, colpi long, ends tapering, tips acute, ora lalongate	Exine 3 µm thick, tectate, surface psilate to locally granular
4	**Bidens pilosa** Linn.	25-29 µm Amb spheroidal; 23-25× 27-30 µm, sub-oblate; Radially symmetrical	Tricolporate, colpi long, ends tapering, tips acute, ora lalongate	Exine 1.5 µm thick,tectate, surface echinate, spines 6.8 µm long, base 2µm broad
5	**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
6	**Bombax ceiba** Linn	51 µm (49.5×52.5) 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. Muri simplibaculate, faint LO pattern.
7	**Brassica** sp.(Linn) Koch	30-33 µm , Amb rounded triangular to almost spheroidal; 27-31× 24-27 µm, prolate spheroidal; radially symmetrical	Tricolplate , 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.
8	**Capparis grandis**	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
9	**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
10	**Careya arborea** Roxb.	52.1× 40.1 µm (48-54× 37.5 -43.5) µm, subprolate, isopolar, radially symmetrical	Hexacolpate, syncolpate with crassimarginate colpi, 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

11	**Carthamus tinctorius**	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 processess
12	**Citrus** sp.	27-29 µm, Amb squarish, 26-30 ×25-27 µm, prolate spheroidal radially symmetrical	Tetracolporate, colpi linear, tips acute, ora lalongate	Exine 2 µm thick subtectate, surface Reticulate. Heterobrochate, meshes smaller near the apertural regions and larger elsewhere, lumina hexa to pentagonal or irregular, psilate, muri simpli to locally duplibaculate
13	**Clerodendrum sp.**	43-48 µm , Amb spheroidal, 41-44 ×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
14	**Dodonea viscosa**	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
15	**Mangifera indica** Linn.	27-31 µm, Amb subtriangular; 29-32 ×26-28 µm , subprolate; Radially symmetrical	Tricolporate colpi long, tips acute ora 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
16	**Maytenus emarginata** Wild.	Oblate, 45-49 µm, Amb, rounded triangular to almost spheroidal , isopalar, Radially symmetrical	Tricoloporate, colpi length 9.4 µm, (9-10.5) µm, ora lalongate	Exine thick 3 µm, sexine thicker than nexine, reticulate size of mesh 2.4 (1.5-3)µm, distinct LO pattern.
17	**Parthenium hysterophorus**	16.6 to 19.8 µm, Amb spheroidal , oblate spheroidal, radially symmetrical	Tricolporate colpi long, ends tapering, tips acute, ora lalongate	Exine 3 µm thick, tectate , surface echinate, spines short 2 µm, to 3 µm, , long 2 µm, in diam at base.
18	**Pisidium 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
19	**Pongamia pinnata** (Linn) Pierre.	29-31 µm, Amb subtriangular: 27-31× 25-28 µm, subprolate; Radially symmetrical	Tricolporate, colpi linear to narrowly elliptic tips acute, ora lalongate	Exine 1.5 µm thick, subtectate, surface granular to locally faintly microrecticulate
20	**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
21	**Rungia repens**(Linn.) Nees.	40-44 ×25-26 µm, oblong; Bilaterally symmetrical	Diporate, pores circular, 2.5 µm, in diam, margin of the	Exine 3 µm thick at poles, 4.6 µm at equaltor, subtectate, tectum undulating, distinct rounded to

			pores densely beset with small processes	irregular areolae (2-4 µm) linearly aligned in the vicinity of apertures, rest of the wall microreticulate
22	**Sapindus emarginatus** vahi.	24-26 µm, Amb triangular, sides straight or even slightly concave; 18-20×26-29 µm, oblate (occasionally suboblate); Radially symmetrical	Tricolporate, colpi narrowly elliptic long, tips acute, ora lOlongate	Exine 2 µm thick on mesocolpia, 1-1.5 µm thick near apertures, surface psilate
23	**Terminalia** sp.	19-22 µm, Amb spheroidal; 21-24 x20-22 µm, subprolate; Radially symmetrical	Tricolporate, colpi alternating with pseudocolpi colpi linear, tips acute pseudocolpi almost equal the size of colpi, ora more of less circular	Exine 1.5 µm thick, tectae, surface psilate to locally finely granular
24	**Tinospora cardifolia**	16-18 µm, Amb rounded triangular; 15-19 ×12-17 µm, sub-prolate; Radially symmetrical	Tricolporate, colpi linear long, often meeting at poles without forming syncolpia, operculate, operculum as long as colpus, ora not distinct	Exine 1.5 µm thick, subtectae, surface finely reticulate, lumina variously polygonal

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 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
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, subtectate, surface reticulate in places retipilate, reticulum homobrochate, lumina polygonal to circular, psilate, muri simplibaculate



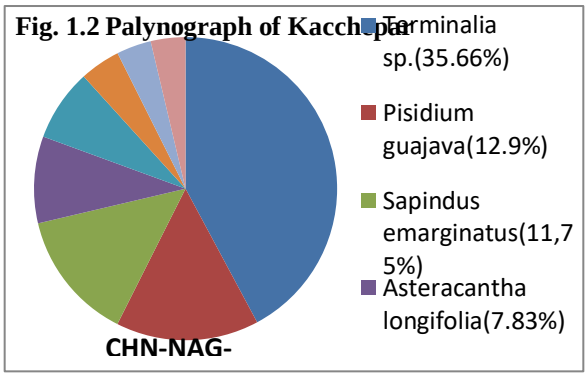


Fig 1.3: Composite palynograph of summer honeys from Sindewahi tehsil

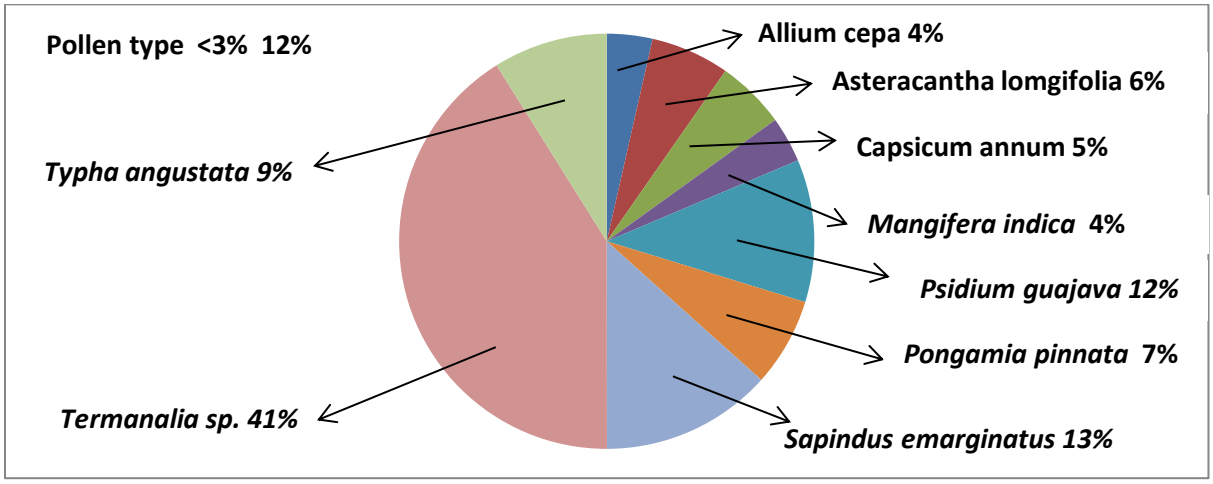
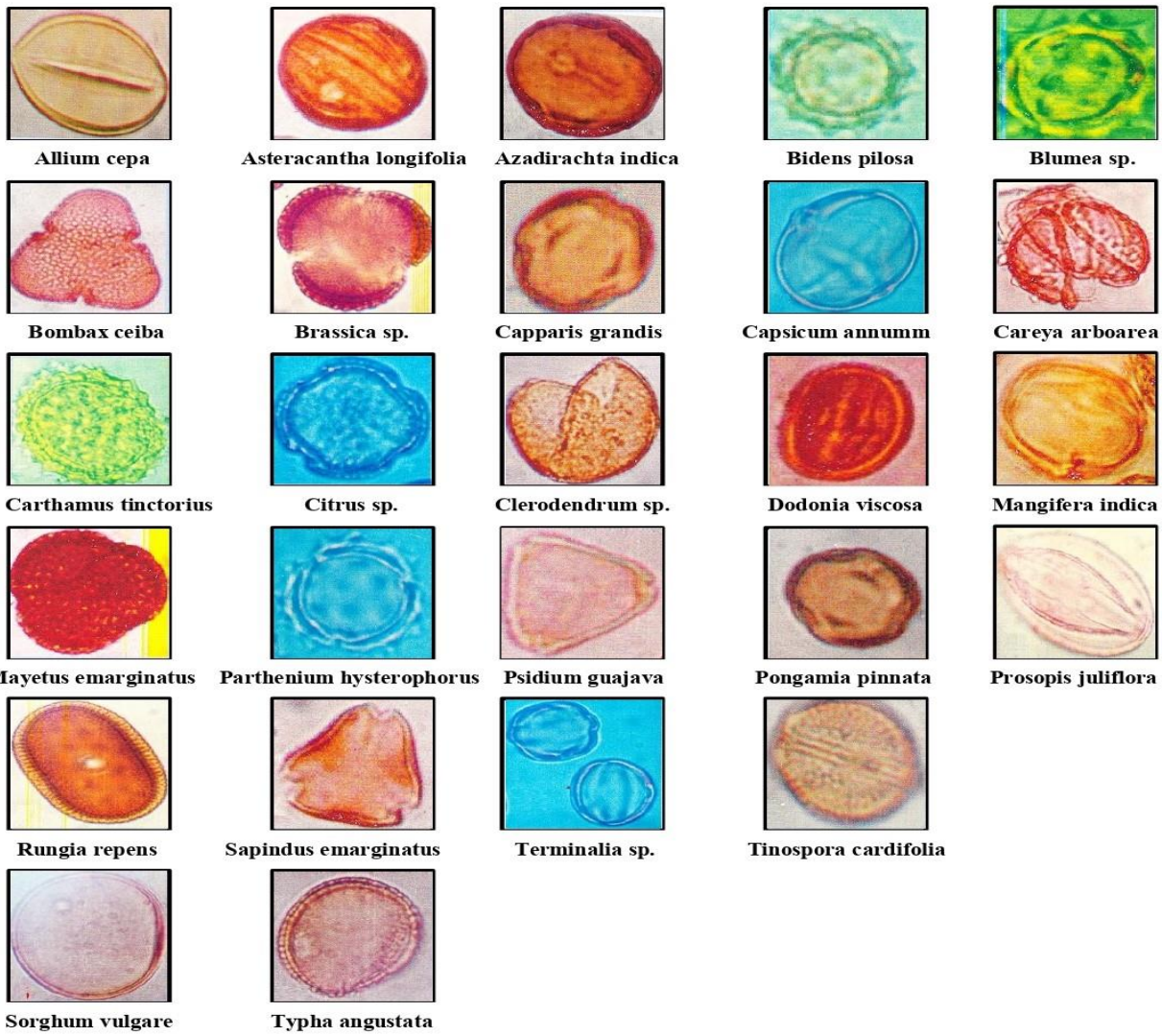


Plate Microscopic photograph of pollen grains found in honey sample



All together 26 pollen types (24 of melliferous and 2 of non-melliferous taxa) referable to 21 families have been recorded from these samples (Photoplate). The sample (CHN-NAG-KAC) showed the maximum number of pollen type (21) and the sample (CHN-NAG-GIR), the minimum number (14).

In the sample (CHN-NAG-GIR) however the pollen of *Typha angustata* were found to be good number (7.84%). The absolute pollen counts ranged from 173,000/g to 853,000/g and the HDE/P ratio is 0.2 in both the samples. (Table 1).

The details of the pollen analysis of the 2 honey samples (melliferous/non-melliferous) are represented in table1. Similarly individual palynograph (Pollen spectra) of each honey sample and composite palynograph (Fig.1.1 to 1.3) was also given to show the pollen contents of the samples of Sindewahi tahsil. The distinguishing morphological features of the pollen types encountered in the present study are given below. The bee plants of Nagbhid tahsil are referable to 3 categories:

1. Crop plants: *Allium cepa, Brassica sp., Capsicum annuum, Carthamus tinctorius and Sorghum vulgare.*

2. Arborescent taxa/shrub: *Azadirachta indica, Bombax ceiba, Citrus sp., Mangifera indica, Pongamia pinnata, Sapindus emarginatus, Terminalia sp., Capparis grandis, Rungia repens, Tinospora cardifolia, Prosopis juliflora.*

3. Herbaceous weeds: *Asteracantha longifolia, Bidens pilosa, Parthenium hysterophorus, Blumea sp.*
Of these three categories the arborersent plants *Terminalia sp.* constitutes the chief bee forage plants in this tahsil during summer season. Besides the other arborersent plants *Psidium guajava, Pongamia pinnata, Azadirachta indica represents* most preferred nectar sources for the honey bees. 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 it's cultivation. The other plant encountered in these honey samples are the member of families like Acanthaceace, Anacardeaceace, Mimoscae, Caesalpiniaceace, Celastraceace, Myrtaceace, Samydaceae, Menisphermaceace, Liliaceace, Capparidaceae, Amaranthaceae, Cleomaceae, Solanaceae, Papillionaceae 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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