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MELITTOPALYNOLOGICAL INVESTIGATION OF WINTER HONEYS COLLECTED FROM *APIS DORSATA* HIVES OF NAGBHID TAHSIL OF CHANDRAPUR DISTRICT OF MAHARASHTRA STATE (INDIA)

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ABSTRACT

The paper incorporates a qualitative and quantitative study of pollen contents in four squeezed honey samples collected from forest area of Nagbhid tahsil of Chandrapur district. *Cajanus cajan* (50.91%) and *Capparis grandis* (45.50%) represents the predominant pollen type in 2 sample are designated as *Cajanus* honey and *Capparis* honey. The other significant pollen types recorded include *Cajanus cajan,* Tridax procumbens, *Capparis grandis, Celosia argentea, Cloame gyanandra, Capsicum annuum. Blum e a sp.* The pollen counts ranged from 5,000 to 460,000. 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,* Nagbhid 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 Melittopaloynological methods have been made to evaluate sources of pollen and nectar for honey bees in different parts of the country namely Maharashtra (Borkar Lamikant & Mate Devendra 2014;2016,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).

Investigations incorporate a quanlitative and quantitative pollen analysis of four honey sample from forest area of Nagbhid tahsil of Chandrapur district (Text fig. 1). 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.

MATERIALS AND METHODS

Four honey samples viz., CHN-NAG-Saw, CHN-NAG-Aaw, CHN-NAG-Gir, CHN-NAG-Yen were collected during the period Nov 2011 to Dec 2012 from Sawargaon, Aawalgaon, Girgaon, Yenoli. all the samples represent squeezed honey collected from the natural *Apis dorsata* hives.

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

One 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 Acelolysis (Erdman, 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 slide collection & relevant literature for quantification of pollen types recorded, a total of 300 pollen grains were counted at random from the three palyno slides 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 (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 (ICBB 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 sample collected from Nagbhid tahsil (CHN-NAG-Raj) ***Capparis grandis*** (45.50%) represented the predominant pollen type in one sample (CHN-NAG-Saw), ***Cajanus cajan*** (50.15%) represented the predominant pollen type in second sample (CHN-NAG-Aaw), while 2 are multifloral(CHN-NAG-Gir), (CHN-NAG-Yen). The other significant pollen types recorded includes (secondary to minor pollen) Cajanus cajan, Tridax procumbens, Capparis grandis, Cloame gynandra, Celosia argentea, Capsicum annuum, Blumea sp.

All together 30pollen types (27 of melliferous and 3 of non-melliferous taxa) referable to 21families have been recorded from these samples (Photoplates). The sample Aawalgaon (CHN-NAG-Aaw) and Girgaon(CHN-NAG-Gir) shows Maximum number of pollen type each (17) and the sample (CHN-NAG-Saw) the minimum number (12) .The absolute pollen counts ranged from 5,000/g to 460,000/g and the HDE/P ratio ranged from 0.01 to 0.04 (Table 1).

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-NAG-Saw	15-11-2011	Unifloral	445,000	0.01	P –Capparis grandis (45.5) S - Nil I - Cloame gunandra (10.33) Blumea sp.(10.33) Capsicum annuum (10) Lathtrus sativus (4.23) Cajanus cajan (5.83) Celosia argentea (6.16) Citrus sp. (4.83) M –Si (2.33), Bau (2.5), Par (1.5) NMP – Holopteled integrifoloa (0.75)
CHN-NAG-Aaw	11-11-2012	Unifloral	460,000	0.02	P – Cajanus cajan (50.91) S - Nil I - Capparis grandis (12.33) Celosia argentea (8.16) Cloame gunandra (3.5) Hyptis suavedens (4.16) Lathtrus sativus (4.5) Blumea sp. (7.5) Dodonia viscisa (3) M –Tri(1.83), Ps(0.33), Ve (0.16), Ti (2.16), Par (0.5) Cart (2.66) NMP – Sorghum Vulgare (0.18)

					Typha angustata(0.14) Holopteled integrifoloa(0.20)
CHN-NAG-Gir	13-12-2012	Multifloral	78,000	0.02	P –Nil S - Cajanus cajan(20.16) I – Tridax precumbens(12.33) Bidens pilosa(3.66) Hyptis suaveolens(7.83) Celosia argentea(8.5) Ocimum basilicum(35) Vernomia cineria(3.16) Blumea sp.(6.16) Cloame gunandra(8.5) Capparis grandis(4.5) Capsicum annuum(7.16) M –He(1.5), Sp(1.6), Cart (2.16), Mo (2.66), Ps (1.33) NMP – Sorghum Vulgare (0.75)
CHN-NAG-Yen	23-12-2012	Mutlifloral	5,000	0.04	P –Nil S - Cajanus cajan (31.33) Capsicum annuum (16.83) Capparis grandis (16.16) I - Citrus sp. (7.66) Pisidium guajava (3.33) Leucaena leucocephala (4) Blumea sp. (3.16) Coriandrum sativum (8.66) M –Cl (1.83), Pr (2.66), Hy (0.5), Ju(1.33) Bou (1.16) NMP – Holopteled integrifoloa (0.25)

The details of the pollen analysis of the 4 honey sample (melliferous / non-melliferous) are represented in table 2. The distinguishing morphological feature of the pollen types encountered in the present study are given below.

Table 2: Showing pollen morphology of Melliferous/Non- Melliferous taxa.

Sr. No.	Pollen Type	Size, Shape & Symmetry	Aperture Pattern	Pollen Wall (sporoderm) structure & sculpture
01	**Bauhinia variegata** L.	36.63- 46.62 µm spheroidal-prolate spheroidal radially symmetrical	Triangular equatorial outline elliptic, Tricolpor poidate colpi 36.39 × 46 µm colpi long narrow toward the ends	Exine thick 3.33- 4.99 µm sculpturing striate
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	**Bidena pilosa** Linn.	25-29 µm Amb spheroidal; 23-25× 27-30 µm, sub-oblate; Radially symmetrical	Tricolprate, colpi long, ends tapering, tips acute, ora lalongate	Exine 1.5 µm thick,tectate, surface echinate, spines 6.8 µm long, base 2µm broad
04	**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
05	**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
06	**Capparis grandis**	10-12 µm , Amb spheroidal; 14-16	Tricolporate, colpi	Exine 1 µm thick, tectate, surface faintly

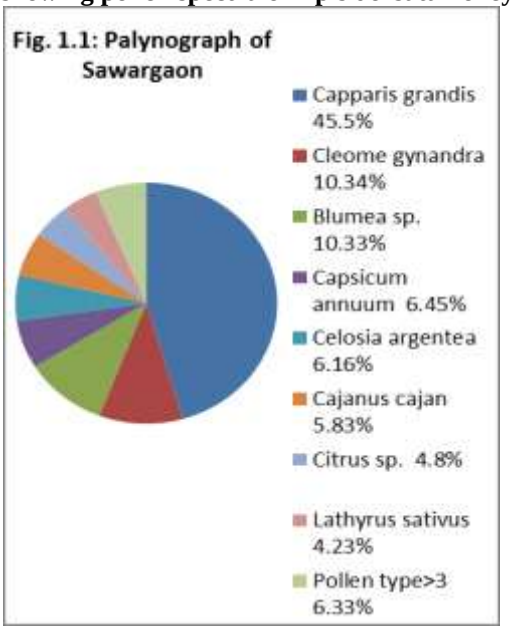
	Linn.	×9-12 µm prolate to subprolate; Radially symmetrical	linear to narrowly elliptic, ends tapering, tips acute, ora faint lalongate	granular to almost psilate
07	**Capsicum annuum** Linn.	29-34 µm, Amb spheroidal; 29-35× 26-30 µm, subprolate; radially symmetrical	Tricolporate, colpi constricted at oral region, ends tapering, tips acute, ora prominently lalongate	Exine 1.5 µm thick, tectate, surface faintly granular to almost psilate
08	**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 processess
09	**Cloame 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
10	**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
11	**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
12	**Dodonaea 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
13	***Helianthus annuus*** Linn.	40-44 µm, Amb spheroidal, 37-39× 40-42 µm, oblate spheroidal; Radially symmetrical	Tricolporate, colpal ends tapering, ora lalongate	Exine 3 µm thick (without spines), tectate, surface densely echinate, spines 7-8 µm long, base 2.4 µm wide, tip pointed.
14	**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.
15	***Justicia procumbens*** Linn.	24-28× 16-18 µm, oblong; Bilaterally symmetrical	Dicolporate, colpi faint, narrow, streak like, ora lalongate	Exine 1 µm thick at poles, 2.5 µm thick at equator, tectum undulating, circular to irregular areoies (2-4 µm) aligned linearly are seen on either side of the colpi, rest of the wall finely reticulate
16	**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.
17	**Leucaena leucocephala** (Lam.) de Wit	52-59 µm, Amb spheroidal : 47-49×51-58 µm, sub oblate: Radially symmetical	Tricolporate colpilong, tips acute, ora lalongate	Exine 4 µm thick, subtectate surface microreticulate, homobrochate
18	***Momordica charantia*** Linn.	68-76 µm, Amb spheroidal; 67-72× 64 -65 µm, prolate spheroidal;	Tricolporate, colpi narrow with tapering	Exine 4 µm thick, subtectate, surface reticulate, lumina irregularly polygonal

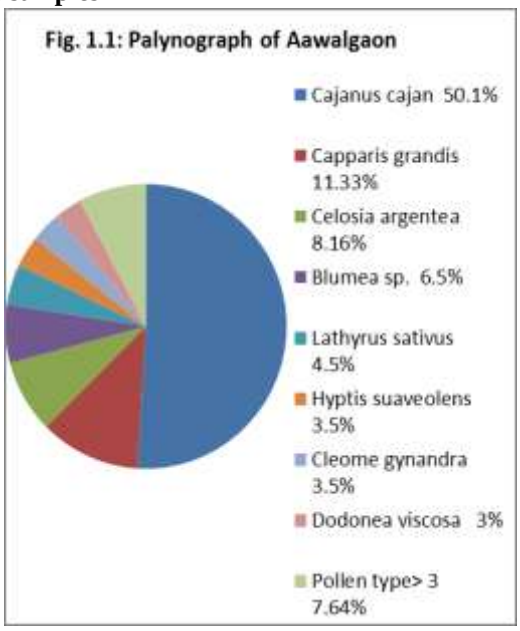
		radiallysymmetrical	ends, ora faint, lalongate	psilate
19	***Ocimum basilicum*** Linn.	41-45 µm, Amb spheroidal; 41-46× 36-39 µm subprolate; Radially symmetrical	Hexacolpate, colpi broad with somewhat blunt ends, colpal membrane densely granular	Exine 4 µm thick, subtectate, surface prominently reticulate, homobrochate, lumina polygonal, beset with many free piloid elements, muri simpli baculate
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	**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
22	**Parthenium hysterophorus** Linn.	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.
23	***Sida glutinosa*** cav	Large size 70.5 µm × 71.2 µm spheroidal radially symmetrical	Isopolar pentaporate pore 4.5 µm, interpolar distance 15-7 µm	Exine thick 4.5 µm, Sexine-nexine not clear, echinate, basal cushions well defined, spine height 4.5 µm cushion 3 µm thick, interspinal area to oveolate colunellar fused in the basal cushion, clear LO pattern
24	***Sphaeranthus indicus*** Linn.	28-33 µm, Amb spheroidal; 26-29x 30-34 µm, suboblate; Radially symmetrical	Tricolporate, colpilinear, tips acute ora lalongate	Exine (without spines) 3 µm thick, tectate, surface echinate, spines 4-5 µm long , 3 µm broad at the base
25	***Tinospora cordifolia*** (Wild) Miers ex hk. f. & Thoms.	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
26	**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
27	***Vernonia cinerea*** (Linn.) cess	35-38 µm, Amb spheroidal; 34-37 x 31-35 µm, prolate spheroidal; Radially symmetrical	Tricolporate, colpi fine and inconspicuous due to heavy sculpturing, ora more or less circular	Exine 6 µm thick, tectae, surface echinolophate (echinofenestrate), spines of different sizes, upto 3 µm long, fenestral lumina prominent, hexa to pentagonal sometimes irregular, 5-12 µm in dial psilate.

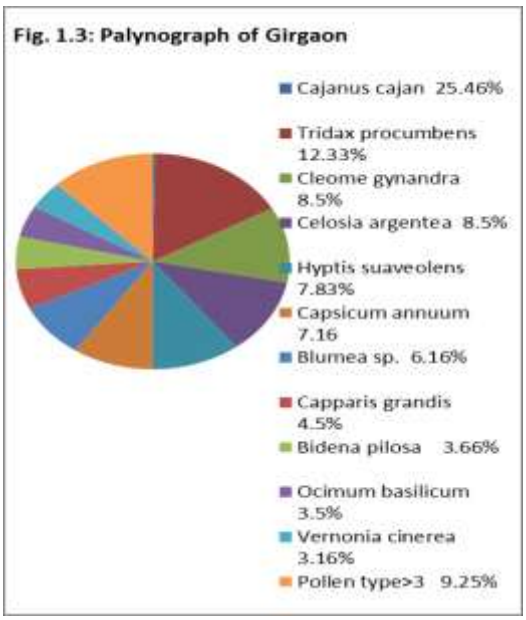
Non-melliferous taxa

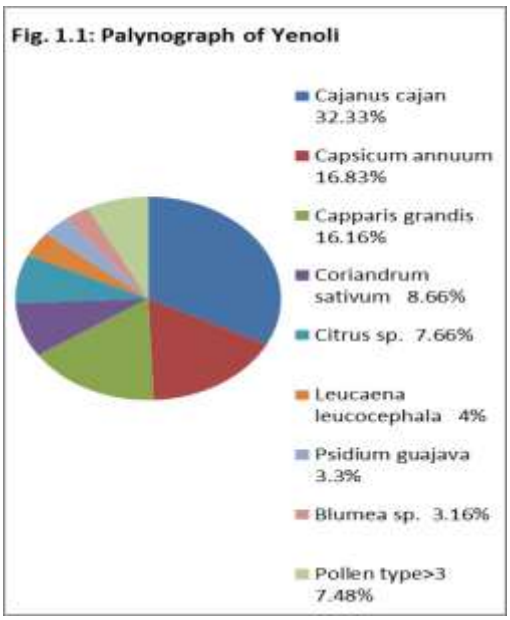
1	**Holoptelea integrifolia** (Roxb.) Planch	26-28 µm, Amb spheroidal to slightly angular; Radially symmetrical	Tetra to hexaporate, generally hexaporate pores circular with distinct margins, 2-3 µm in diam	Exine 1.5 µm thick, subtectate, surface faintly microreticulate
2	**Sorghum vulagare** Pers.	51-55 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
3	***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

Pie charts showing pollen spectra of Apis dorsata honeys samples









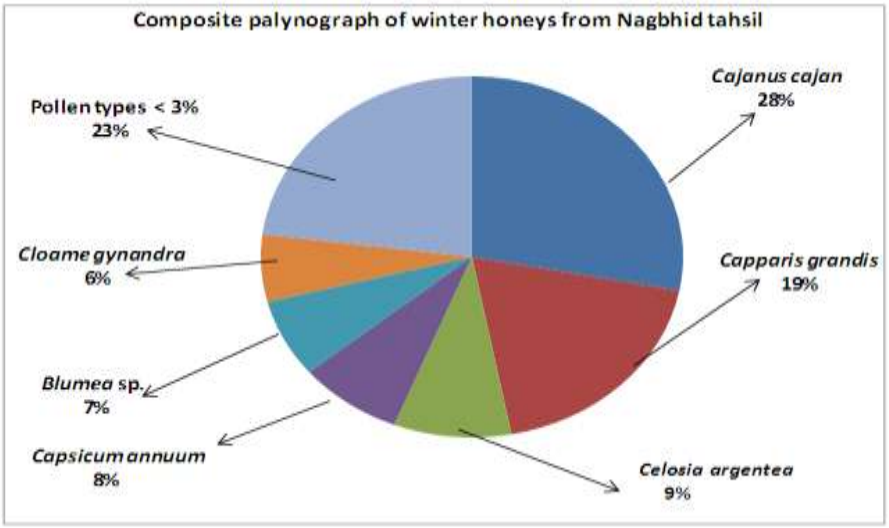
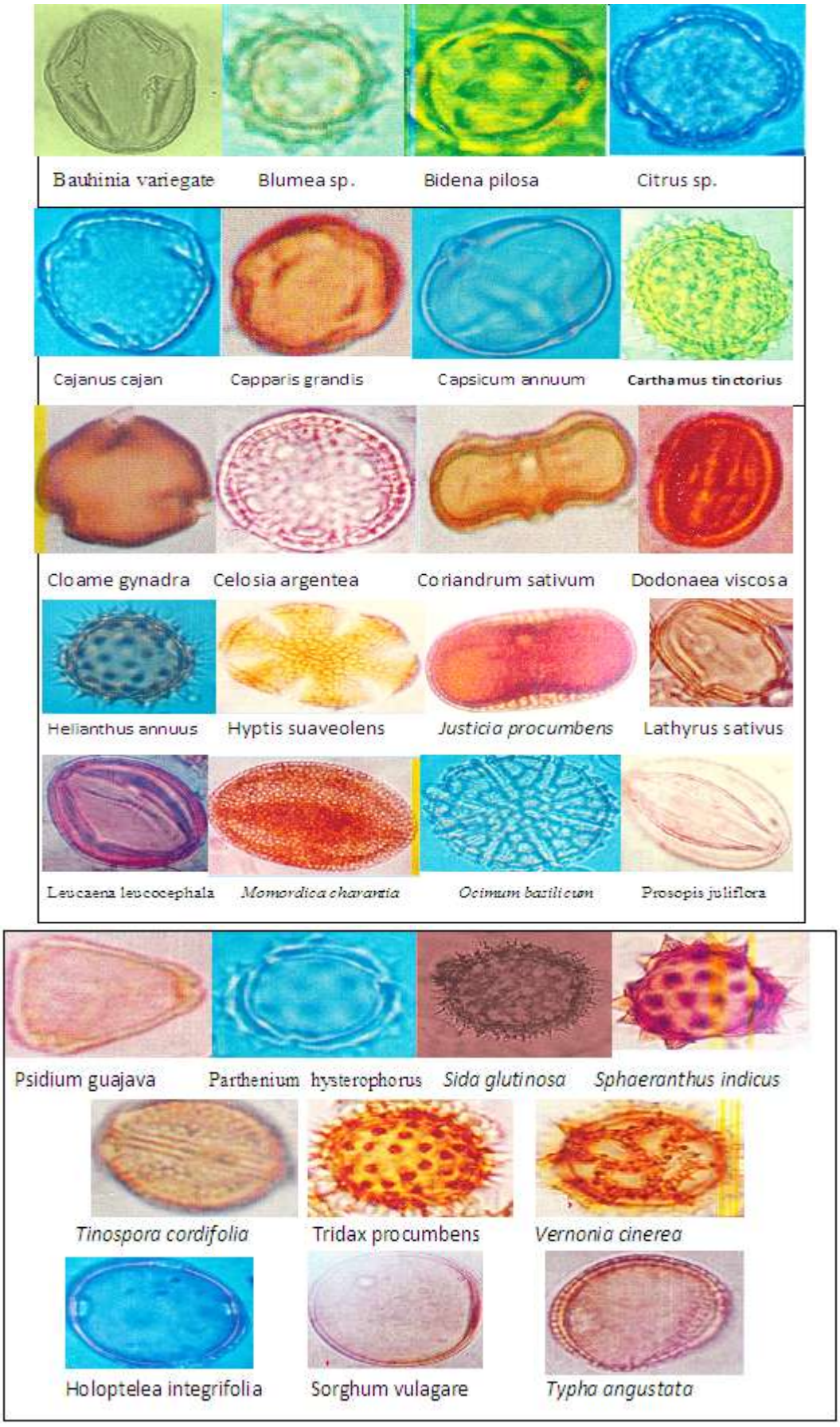


Photo Plate: Microscopic photograph of pollen grains found in honey sample



The bee plants of Nagbhid 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 It is the crop plants. which are mostly preferred by the bees of this tahsil. The crop plants *Lathyrus sativus* and *Cajanus cajan, Cariandrum sativus* and *Capsicum annuum* cultivated extensively during winter constitute the chief bee plants. Of this tehsil during winter seasons of the *Cajanus cajan* & *Lathyrus sativus* represents most preferred nectar sources for the honeybees. Our observation indicate that *Lathyrus sativus* and *Cajanus cajan* representabundant nectar and pollen sources to *Apis dorsata*.

The region selected for the present study has good potential for sustaining beekeeping ventures because of the diversity of nectar and pollen taxa. Since *Cajanus cajan, Lathyrus sativus* are major sources of forage for honey bees efforts should be made to increase. Their cultivation under social forestry like *Prosopis juliflora*. In the family like *Fabaceae, Asteraceace, Lamiaceace, Capparidaceace, Solanaceace* in these areas.

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

These 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.

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