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Studies on identification of monofloral/multifloral honey by pollen morphology

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Abstract
The pollen analysis of fourteen honey samples collected from different locations of northern hill region of Chhattisgarh revealed that all samples of honey were identified as unifloral except Ajirma (KVK) honey. The major pollen source found in honey was *Brassica campestris* with the pollen ranging from 48 to 61%, *Guizotia abyssinica* with 50-61% pollen while 60% of pollen belonging to *Litchi chinensis* was pre dominant pollen type among 14 samples of honey. The pollen content of *Moringa oleifera* (18-35.5%); *Cajanus cajan* (16%); *Zea mays* (17-32.71%); *Eucalyptus obliqua* (11-33%); *Guizotia abyssinica* (27-38.6%), *Litchi chinensis* (40.2%) comes under the category of secondary pollen type. The morphology of pollen grains of 19 plant species were studied and 8 shapes of pollens were recognized out of which round shape was found in *Guizotia abyssinica*, *Brassica campestris*, *Mangifera indica*, *Cajanus cajan* and Amaranthus. The triangular shape was found in *Eucalyptus obliqua*, *Litchi chinensis*, *Callistomon* spp., *Psidium guajava*, *Bombax cieba* and *Syzyzium cumini*. The oval shape pollen was observed in *Zea mays*. Prolate spheroid was found in *Moringa oleifera*. Dyads shape was found in *Coriandrum sativum*. The oblate spheroid shape was found in *Lantana camara* and *Mimosa pudica*. The oblate and sub oblate shape was seen in *Fagopyrum esculentum* and *Sesamum indicum*. Identification and quantification of the pollen grains in honey samples are helpful in determining the floral nectar & pollen source used by bees to produce honey. The quantity and quality of pollen grains present in honey are of significance because of the nutritional qualities and play important role in determining the commercial value of honey in the international market.

Keywords: Unifloral, multifloral, honey, pollen, morphology, Chhattisgarh

Introduction
Honey is a by-product of nectariferous floral plant and the upper aero-digestive tract of the honey bee, which is concentrated through a regurgitation and dehydration process inside the bee hive. Honey has a very complex chemical composition that varies depending on the botanical source. Honey contains traces of pollen, which predominantly transferred from the stigma of a flower by foraging of bees along with some wind and a possible addition of pollen from the bee bread during extraction of honey (Jusbin, 1996) [11]. The pollen involuntarily collected by bees at nectar collection time is an important indicator of its botanical and especially geographical origin (Barth 1989) [2]. Pollen is very important for the bee's diet and it is necessary for their growth, development and reproduction (Dietz, 1978) [8]. If honey is unifloral, it is named after their floral source such as litchi, mango, mustard, niger, saffron, coriander, thyme honeys and so on. The unifloral honey can be characterized by the physicochemical, microscopical and organoleptical characteristics (Soria *et al.*, 2004) [27]. Beekeeping entirely depends on the type of flowering plants available in any given area (Shubharani *et al*., 2013) [23].

India is an important honey producer, in the financial year 2022-23 about 79,929.17 metric tonnes natural honey worth of Rs. 1,622.77 Crore (US $203.07 Million) have been exported to the world as reported by Agricultural and Processed Food Products Export Development Authority (APEDA), Government of India. The major states of honey production in India are Punjab, Haryana, Rajasthan, Himachal Pradesh, Uttar Pradesh, Bihar, Tamilnadu, West Bengal and Maharashtra. In the year 2022-23, major export countries of this product are USA, Saudi Arab, United Arab Emirates, Bangladesh and Canada.

Identification and quantification of the pollen grains in honey samples will help in determining the floral nectar source used by bees to produce honey.

The quantity and quality of pollen grains present in honey is significant because of its nutritional qualities (Cocan *et al.*, 2005) [7] and play important role in determining the commercial value of honey in the international market (Bryant, 2001) [5]. The authenticity of good quality honey is normally assessed based on its physical, botanical and chemical properties and this can be achieved by means of pollen and physical/chemical analysis of honey (Ohe *et al.*, 2004) [17].

Pfister (1895) [19] was the first to describe the pollen contents of various Swiss, French and European honeys. He determined the possibility of the geographical origin of honey from the pollen present in a particular honey sample. Fehlmann (1911) [10] and Louveaux (1965) analyzed the pollen types found in various samples of honey. Parker (1923) [18] conducted a study of bees and their honey types in the United States. However, the foundation of melissopalynological research in Europe was laid down by the work of Zander (1951) [28] who has published in five volumes. In these publications he has elucidated description, drawings and photographs of the pollen grains present in various types of European honey. There are several methods available for the determination of botanical and geographical origins of honey (Anklam, 1998) [1]. The traditional approach consists of the microscopic examination of pollen present in honey (Barth, 2004; Sodre *et al.*, 2007) [3, 25].

Melissopalynology deals with the qualitative as well as quantitative study of pollen grains present in a particular honey sample. With the help of such studies the source of floral nectar utilized by honey bees for the honey production can be determined (Lieux, 1975, 1978; Moar, 1985; Louveaux *et al.*, 1970; Sawyer, 1988) [12-13, 15, 16, 21]. The melissopalynological studies also helps in identifying the geographical origin of the source as well as the assessment of phytogeography of a particular region. However, present investigation deals with the pollen identification & quantification or melissopalynological analysis of the honey samples collected from various villages of Surguja district of Chhattisgarh. It is pertinent to note that there is no detailed study on the honey types and pollen morphology from northern hill zone of Chhattisgarh. Hence, the present studies examine the pollen content in honey samples obtained from northern hill zone of Chhattisgarh state. The pollen identification and quantification in honey will indicate the source of honey thereby classifying it into monofloral or multifloral honey.

Methods and Materials

Fourteen samples of honey produced by honeybees were collected from November to April, directly from the apiary of RMD CARS Ambikapur, KVK Ambikapur and adjoining 11 villages of Northern Hill Zone of Chhattisgarh during 2017. The pollen analyses of honey were performed at the Department of Entomology, RMD CARS Ambikapur. The origins of each honey sample were confirmed by melissopalynology**.** Honey samples were classified according to their botanical origin using the method described by Von der Ohe *et al.* (2004) [17]. Ten g of honey was dissolved in 20 ml of warm distilled water. The solution was centrifuged for 10 min at 3000 rpm. The supernatant liquid was decanted and then 10 ml of distilled water was again added in the sediments and centrifuged for 5 min at 3000 rpm. The sediments was put on a haemocytometer and spread out. The pollen grains were counted in binocular light-microscope with SP40/0.65 magnifications (LABOVISION U/L EGLASS).

Calculations of pollen count in honey

The average number of pollen counted over the haemocytometer is for the volume 1 mm square × 0.1 mm depth. For this, calculation of the pollens present in 1 mm was done, which is equivalent to their absolute number present in 10 g of honey. Number of pollens counted is X

Total number of pollen present per ml = X × ml

The International Commission for Bee Botany (Louveax *et al.* 1978) suggested four frequency classes as indicated below.

Classification of pollen frequency

S. No.	Pollen frequency class	Percentage of pollen
1	Predominant pollen	> 45%
2	Secondary pollen	16-45%
3	Important minor pollen	3-15%
4	Minor pollen	< 3

The honey samples were classified as "unifloral", having a predominant pollen type and samples containing more than one species were classified as "multifloral".

Results and Discussion

The pollen analysis of fourteen honey samples collected from different locations of northern hill region of Chhattisgarh (Table 1 and Fig 1) revealed that all samples of honey were unifloral except Ajirma (KVK) which was multifloral type honey. The honey samples namely Bhitthikala, Rampur Lodhima, Ranpur, Mendrakala, Mudesa, Aamgaon, Sukhari and Ajirma (RMD-I) were having pollen of *Brassica campestris* with the pollen ranging from 48 to 61%. The honey sample of Mangari, Sitapur, Devgarh and Lamgaon were having pollen of *Guizotia abyssinica* with the pollen ranging from 50 to 61% while sample Ajirma (RMD-II) contained 60% of pollen belonging to *Litchi chinensis* and was pre dominant pollen type among 14 samples of honey during the year 2017. The pollen content of *Moringa oleifera* ranged between 18-35.5% in honey collected from Bhitthikala, Rampur Lodhima, Mendrakala, Ajirma (RMD-I) and Ajirma (RMD-II); *Cajanus cajan* (16%) in Rampur Lodhima; *Zea mays* (17-32.71%) in Ranpur, Lamgaon and Ajirma (KVK); *Eucalyptus obliqua* (11-33%) in Ranpur, Sitapur and Devgarh; *Guizotia abyssinica* (27-38.6%) in Mudesa, Aamgaon and Sukhari similarly, *Litchi chinensis* (40.2%) in Ajirma (KVK) comes under the category of secondary pollen type. The samples of *Cajanus cajan* with the range 8 to 14% in Bhitthikala, Ranpur, Mendrakala, Mudesa, Aamgaon and Sukhari; *Guizotia abyssinica* (7.75%) and *Psidium guajava* (5%) in Rampur Lodhima; *Moringa oleifera* (7.5%) in Ranpur; *Sesamum indicum* (14%) in Devgarh; *Brassica campestris* (11%) in Lamgaon; *Bombax ceiba* (8.8%), *Mangifera indica* (11.5%), *Eucalyptus obliqua* (3.6%) in Ajirma (KVK); *Litchi chinensis* (9.4%) in Ajirma (RMD-I); *Coriandrum sativum* (5%) in Ajirma (RMD-II); *Zea mays* (3.1-11%) in Mangari, Sitapur, Devgarh, Aamgaon, Ajirma (RMD-I) and Ajirma (RMD-II) were categorized under the important minor pollen type. Whereas, *Psidium guajava* (2.5%), *Coriandrum sativum* (1%), *Guizotia abyssinica* (1.5%), *Zea mays* (2.5%), *Brassica campestris* (3%), *Sesamum indicum* (2%), *Fagopyrum esculentum* (0.4%), *Eucalyptus obliqua* (2.5%), *Moringa oleifera* (2%), *Lantana camara* (0.5%), *Mimosa pudica*. (0.4%), *Moringa oleifera* (2.4%), *Callistemon* spp.

(1.69%), *Bombax ceiba* (1%) and *Callistemon* spp. (2%) were identified as minor pollen type with less than 3% pollen content. The varieties of pollen grains in honey collected from the northern hill region belong to 13 families. The morpho-taxonomical characteristics of various pollen grains found in the honey samples collected during 2017 are annotated (in Table 2) and the percentage of each type of pollen/10 g honey. The morphology of pollen grains of 19 plant species were studied and 8 shapes of pollens were recognized out of which round shape was found in *Guizotia abyssinica*, *Brassica campestris*, *Mangifera indica*, *Cajanus cajan* and Amaranthus with the size of 34-36, 32-35, 20-22, 61-63 and 22-25 µm, respectively. The triangular shape was found in *Eucalyptus obliqua*, *Litchi chinensis*, *Callistemon* spp, *Psidium guajava*, *Bombax ceiba* and *Syzygium cumini* with size of 20-23, 12-15, 25-28, 37-40, 60-63 and 29-31 µm, respectively. The oval shape pollen was seen in *Zea mays* having size of 62- 65 µm. Prolate spheroid was found in *Moringa oleifera* with the size of 20-23 µm. Dyads shape was found in *Coriandrum sativum* with 19-21 µm size. The oblate spheroid shape was found in *Lantana camara* and *Mimosa pudica* with the size of 15-18µm and 10-13µm, respectively. The oblate and sub oblate shape was seen in *Fagopyrum esculentum* and *Sesamum indicum* with the size of 22-25 µm and 58-60 µm, respectively.

The morphology of 19 plant species were studied out of which 6 species of plant had triangular pollen shape followed by 5 plant species having round shape pollen. The present study included 14 honey samples from Northern hills of Chhattisgarh, out of which 13 samples were found to be unifloral and 1 sample was multifloral. *Brassica campestris*, *Guizotia abyssinica* and *Litchi chinensis* were predominant pollen types while *Moringa oleifera*, *Cajanus cajan*, *Zea mays*, *Eucalyptus obliqua* and *Mangifera indica* were secondary pollen types. Similar study was done by Ramnath and Venkataramegowda (2012) [20] who found uniflorate pollens in *Mangifera indica*, *Syzygium cumini* and *Eucalyptus obliqua*. Similarly, Bhargav *et al.*, (2009) [4] found that pollens belonging to family Asteraceae, Poaceae, Fabaceae, Myrtaceae were uniflorate while Chaturvedi (1989) [6] studied 12 spring honey samples out of which Brassica and Rosaceae were uniflorate pollen types. Shubharani and Sivaram (2012) [22] studied that unifloral honey samples, *Sapindus laurifolia*, *Areca catechu*, *Acacia* sp., *Mangifera indica*, *Terminalia bellerica*, *Syzygium* sp., *Pongamia pinnata* and *Eucalyptus* sp. were predominant pollen types. Song *et al.* (2012) [26] determined the floral sources based on melissopalynological studies on the Chinese honeys. They observed that in the unifloral honeys *Ziziphus jujuba*, *Robinia pseudoacacia*, *Vitex negundo* var. *heterophylla*, *Sophora japonica*, *Ailanthus altissima*, Asteraceae type and Fabaceae type were the predominant pollen. Ebenezer and Olugbenga (2010) [9] result indicated that *Prinari kerstigii*, *Lannea* sp., *Syzigium* sp., Poaceae, *Elaeis guineensis*, *Entanda abyssinca* and *Butyrospermum paradoxum* were dominated pollen. While Sivaram *et al.*, (2012) [24] recorded 15 honey samples out of which only one *i.e.*, coffee sample was uniflorate and remaining 14 samples belonging to the family Fabaceae, Asteraceae and Myrtaceae which were found to be multiflorate.

Table 1: Pollen analysis of honey collected from different villages in the northern hills of Chhattisgarh during the year 2017

S. No.	Honey Sample	Date of Collection	Absolute Pollen Count	Type of Pollen				Type of Honey
				Predominant Pollen (> 45%)	Secondary Pollen (16-45%)	Important Minor Pollen (3-15%)	Minor Pollen (< 3%)	
1	Bhitthikala	02/02/2017	47000	*Brassica campestris* (48.35%)	*Moringa oleifera* (35.5%)	*Cajanus cajan* (14.47%)	*Psidium guajava* (1.04%) Amranthaceae (0.64)	U
2	Rampur Lodhima	01/02/2017	30000	*Brassica campestris* (52.25%)	*Cajanus cajan* (16%) *Moringa oleifera* (17.07%)	*Guizotia abyssinica* (7.75%) *Psidium guajava* (5.03%)	*Coriandrum sativum* (1%) Amranthaceae (0.90%)	U
3	Ranpur	19/01/2017	15000	*Brassica campestris* (55.4%)	*Zea mays* (16.6%) *Eucalyptus oblique* (11%)	*Cajanus cajan* (8%) *Moringa oleifera* (7.52%)	*Guizotia abyssinica* (1.48%)	U
4	Mendrakala	21/01/2017	36000	*Brassica campestris* (49.31%)	*Moringa oleifera* (34.69%)	*Cajanus cajan* (13.5%)	*Zea mays* (1.06%) *Guizotia abyssinica* (1.44)	U
5	Mangari	28/11/2016	78000	*Guizotia abyssinica* (52%)	*Eucalyptus oblique* (36%)	*Zea mays* (9%)	*Brassica campestris* (3%)	U
6	Sitapur	28/11/2016	50000	*Guizotia abyssinica* (57.7%)	*Eucalyptus oblique* (33.01%)	*Zea mays* (7.3%)	*Sesamum indicum* (0.69%), *Brassica campestris* (1.3%)	U
7	Devgarh	28/11/2016	38000	*Guizotia abyssinica* (50%)	*Eucalyptus oblique* (32.5%)	*Sesamum indicum* (14%) *Zea mays* (3.1%)	*Fagopyrum esculentum* (0.4%)	U
8	Lamgaon	28/11/2016	57000	*Guizotia abyssinica* (61%)	*Zea mays* (24.5%)	*Brassica campestris* (11%)	*Sesamum indicum* (1.32%) *Eucalyptus obliqua* (2.18%)	U
9	Mudesa	19/01/2017	28500	*Brassica campestris* (51.2%)	*Guizotia abyssinica* (36.6%)	*Cajanus cajan* (10.2%)	*Moringa oleifera* (2%)	U
10	Aamgaon	24/01/2017	9000	*Brassica campestris* (55.6%)	*Guizotia abyssinica* (26.94%)	*Cajanus cajan* (9%) *Zea mays* (7.5%)	*Lantana camara* (0.5%) *Mimosa pudica*. (0.4%)	U
11	Sukhari	24/01/2017	59000	*Brassica campestris* (49%)	*Guizotia abyssinica* (38.6%)	*Cajanus cajan* (10%)	*Moringa oleifera* (2.4%)	U
12	Ajirma (KVK)	02/06/2017	130000	-	*Zea mays* (32.71%) *Litchi chinensis* (40.2%)	*Bombax ceiba* (8.8%) *Mangifera indica* (11.5%) *Eucalyptus oblique* (3.6%)	*Moringa oleifera* (1%) *Callistemon* spp. (1.69%) *Coriandrum sativum* (0.5%)	M
13	Ajirma (RMD-I)	12/02/2017	50000	*Brassica campestris* (61.03%)	*Moringa oleifera* (22.5%)	*Litchi chinensis* (9.4%) *Zea mays* (4%)	*Psidium guajava* (1.47%) *Fagopyrum esculentum* (1.6%)	U
14	Ajirma (RMD -II)	10/04/2017	32000	*Litchi chinensis* (60.88%)	*Mangifera indica* (21%)	*Coriandrum sativum* (5.4%) *Zea mays* (6.37%) *Syzygium cumini* (4.03%)	*Bombax ceiba* (0.87%) *Callistemon* sp. (1.13%) *Allium* sp. (0.32%)	U

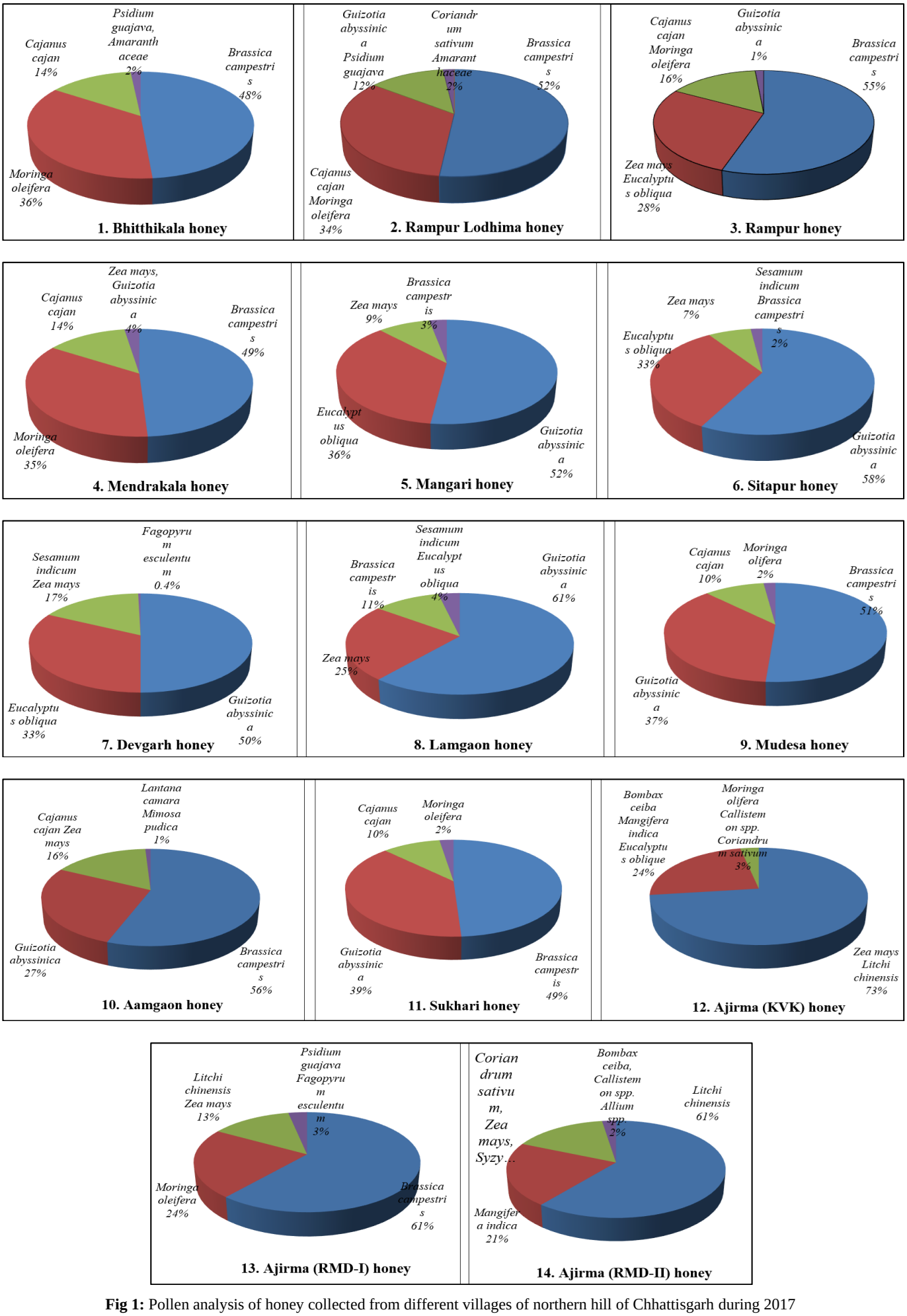
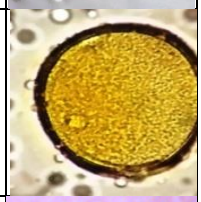
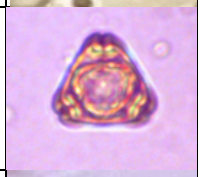
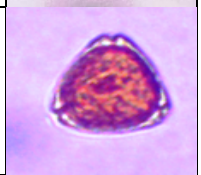
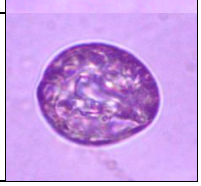
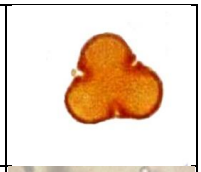
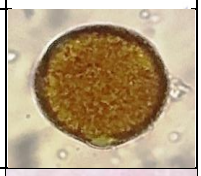
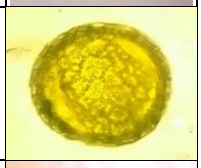
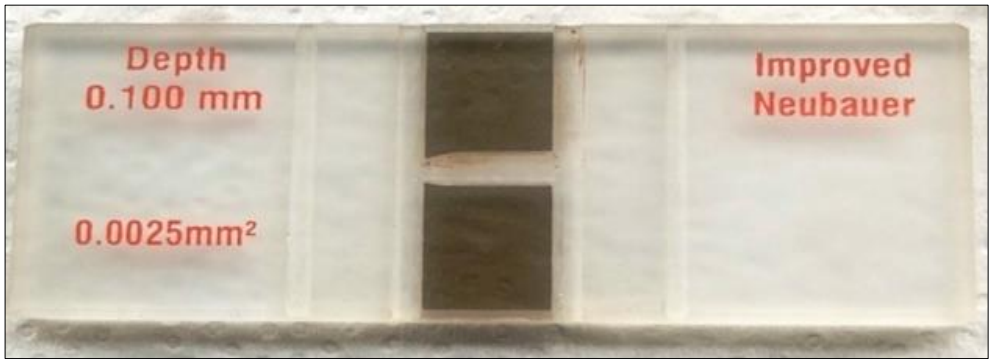


Fig 1: Pollen analysis of honey collected from different villages of northern hill of Chhattisgarh during 2017

Table 2: Morphological attributes of the pollen grains identified under the honey investigation during 2017

S. No.	Name of Fauna		Family	Pollen Morphology		Type and Number of Germinal Aperture	Figure of Pollen
	Botanical	Common		Shape	Size (µm)		
1	*Guizotia abyssinica* L	Niger	Compositae / Asteraceae	Round	34-36	Polyzonoheterocolporate	
2	*Brassica campestris* L.	Mustard	Brassicaceae	Round triangular	32-35	Triporate	
3	*Moringa oleifera*	Drumstick	Moringaceae	Prolate -spheroid	20-23	Periporate, tricolporate, radial symmetry	
4	*Zea mays*	Maize	Poaceae	Oval	62-65	Monoporate	
5	*Eucalyptus obliqua* L' Herit	Eucalyptus	Myrtaceae	Triangular	20-23	Triporate, obsure pattern, bilateral symmetry,	
6	*Litchi chinensis*	Litchi	Sapindaceae	Triangular	12-15	Tricolporate	
7	*Mangifera indica* L.	Mango	Anacardiaceae	Round	20-22	Tricolporate	
8	*Coriandrum sativum* L.	Coriender	Apiaceae	Dyads,	19-21	Bicolporate, bilateral symmetry,	
9	*Callistemon* spp.	Bottle brush	Myrtaceae	Triangular	25-28	Tricolporate	
10	*Psidium guajava* L.	Guava	Myrtaceae	Triangular	37-40	Triporate	
11	*Lantana camara*	Lantana	Verbenaceae	Oblate-spheroid	15-18	Monoporate	

12	*Bombax ceiba* L.	Semal	Malvaceae Bombacaceae	Triangular	60-63	Triporate	
13	*Cajanus cajan* (L) Millsp	Arhar	Fabaceae	Round	61-63	Monoporate	
14	*Mimosa pudica* L.	Laajwanti	Fabaceae	Oblate-spheroid	10-13	Tetrad, tetragonal, psilate radial symmetry	
15	*Fagopyrum esculentum* Moench	Tau/ Buckwheat	Polygonaceae	Oblate	22-25	Bicolporate	
16	*Sesamum indicum* L.	Sesame	Pedaliaceae	Suboblate	58-61	Polyforate	
17	*Syzygium cumini* L.	Jamun	Myrtaceae	Sub-triangular	29-31	Parasyncoplate, obscure pattern, Bilateral symmetry	
18	Amaranthaceae	Bathua	Amaranthaceae	Round /spheroid	22-25	Polyforate, radially symmetry	
19	*Allium* sp.	Onion	Amryllidaceae	Sub-oblate	21-23	Monolate	



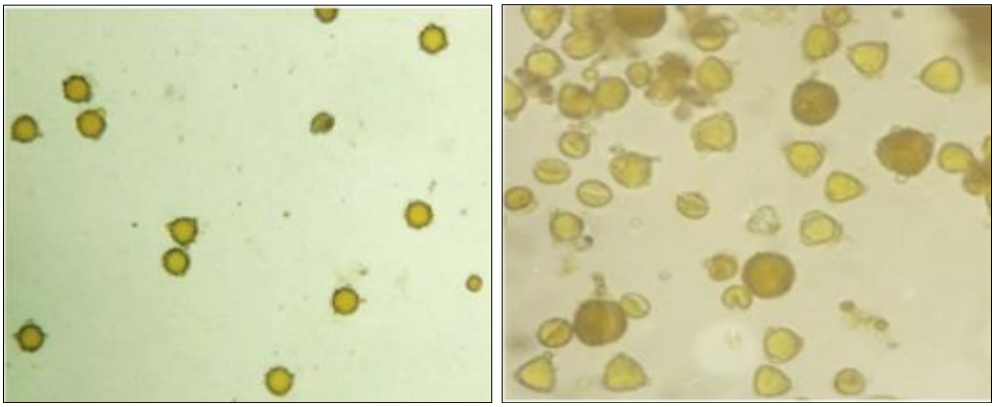


Fig 4: View of pollens extracted from honey sample

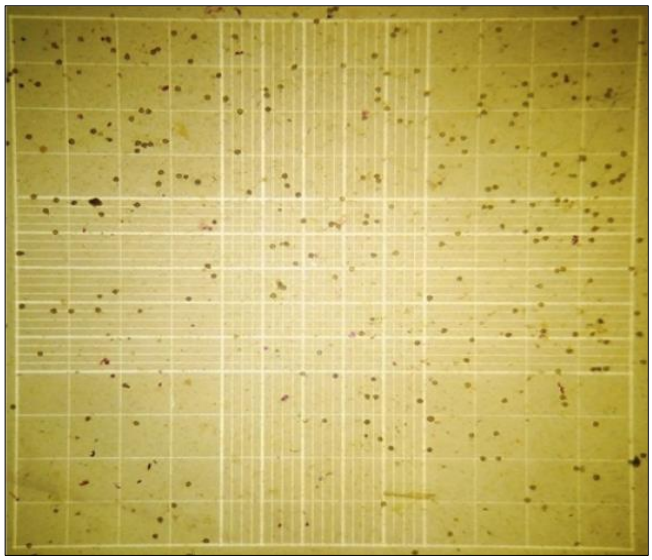


Fig 5: View of pollen grains over Haemocytometer

Conclusion

The region selected for the present study has good potential for sustaining beekeeping ventures because of the diversity of nectar and pollen taxa. Since the crop plant of *Guizotia abyssinica, Brassica campestris, Litchi chinensis, Eucalyptus obliqua, Moringa oleifera, Cajanus cajan* and *Zea mays* are major sources of bee forages, efforts should be made to increase their cultivation in these localities. The predominant plants which were most preferred by honey bees have greater contribution of both pollen and nectar which plays an important role in better growth and development of honey bees.

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