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Melissopalynological studies of Amadobh region, Bilaspur district, Chhattisgarh

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Abstract
Melissopalynology manages the investigation of pollen grains exhibit in a specific honey sample. The nature of honey relies on the recurrence of pollen grains introduce in a specific honey test. The present study has been led on the winter season (January - February), summer season (April-May) and rainy season (August-September) of honey gathered from Amadobh area of Bilaspur region, Chhattisgarh, India., total 58 types of pollen grains belonging 58 genera and 28 families have been found. The summer season honey has been portrayed as unifloral in view of the presence of *Butea monosperma* (Lam.) Taub. pollen grain with 53.3% as dominant plant species. Whereas other two seasons samples were reported as multifloral honey. Color, pH, Moisture %, Ash %, Total solids and sugars were analyzed as physicochemical characterization of the collected honey samples. Fiehe's test, Lugol's response and Lund's response was performed to uncover the contaminated and adulteration of honey samples. The color of the honey is varied from light brown, golden yellow to golden brown. The range of pH is 4.01-4.6, moisture % is 16-19, ash % is 0.001-0.16, total solid % is 80-82, fructose is 36.28-39.06g/100g, and glucose is 29.16-33.85g/100g. All of the outcomes fall inside the international standard point of confinement. This study is valuable for examination of immaculateness of honey.

Keywords: melissopalynology, honey sample, unifloral, multifloral, Bilaspur Chhattisgarh

1. Introduction
Melissopalynology is the branch of palynology, which deals with the study of pollen grains present in a particular honey sample. It incorporates the subjective and additionally quantitative examination of pollen grains. The melissopalynological study provides correct information of the floral components of honey for identifying the authentic names of taxa used by honey bees in the formation of honey. Analysis of pollen grains, present in a honey sample, is also useful in the determination of the geographical as well as botanical origin of a particular honey type.

Pfister (1895) was the first individual to portray the pollen substance of different Swiss, French and European honeys and attempted to show the likelihood of deciding the geographical origin of honey in view of pollen sorts.

Fehlman 1911; Maurizio 1951; Maurizio and Louveaux 1965; Lieux 1969 distributed their work on spectra found in various examples of Swiss honey. Betts (1923, 1925) made portrayals of 15 various types of pollen sources, which he found in English honey and recommended that blossoms from herbarium examples could be utilized as source of pollen to make comparative samples. The differing qualities of the physical and chemical properties of honey like, color, flavor, moisture, proteins and sugars, and so on rely on upon honey and the pollen of the original plants (Barth, 1989). It has also been observed by Cherian *et al*. 2011, that the composition of the minor constituents of natural honeys varies with location, nectar sources and different climatic condition. Honey contains a mixture of different carbohydrates, including fructose (27.3-44.3 %), glucose (22.0-40.8 %), maltose (2.7-16.0%), sucrose (1.5-3.0%), high sugars (0.1-8.5%), proteins, amino acids, vitamins and minerals. (Belitz *et al*. 2004). As per National Honey board (2005), honey contains other disaccharides which make up more than 7 percent of its organization.

The average pH of honey is 3.9 (with a typical range of 3.4 to 6.1). Honey also contains Protein 0.266%, Nitrogen 0.043% and its Isoelectric Point is 4.3. Honey is classified by the U.S. Division of Agriculture into seven colour classifications: water white, additional white, white, additional light golden, light golden, golden and dull golden. Honey solidifies in light of the fact that it is a supersaturated solution. This supersaturated state happens on the grounds that there is such a great amount of sugar in the nectar (over 70%) in respect to the water content (often under 20%). Glucose tends to precipitate out of solution and the solution changes to the more stable saturated state. National honey board (2005) determined the average amount of ash 0.2g/100g.

In India numerous researcher have studied melissopalynological analysis of honey to decide the nature of honey gathered from a specific locality.

Chaubal and Deodikar (1965) have detailed melissopalynological information from Mahabaleshwar and Western Ghats respectively. They announced significant nectar yielding plants from the researched area. Chaturvedi (1983) examined pollen in Kumaon district in autumn season. Mattu et.al. (1989) made pollen investigation of thirteen honey samples in autumn from different regions of Kumaon locale. Chaturvedi & Temsunungla (2004, 2009) reported unifloral and multifloral nature of honey samples collected from various villages of Nagaland state in North- east, India. The present study manages the melissopalynological investigations in Amadobh region of Bilaspur region, Chhattisgarh in Central India

2. Material & Methods

2.1 Collection of honey sample

Honey sample for the present study have been collected from the Amadobh region of Bilaspur district, Chhattisgarh during winter season (January - February), summer season (April-May) and rainy season (August-September).

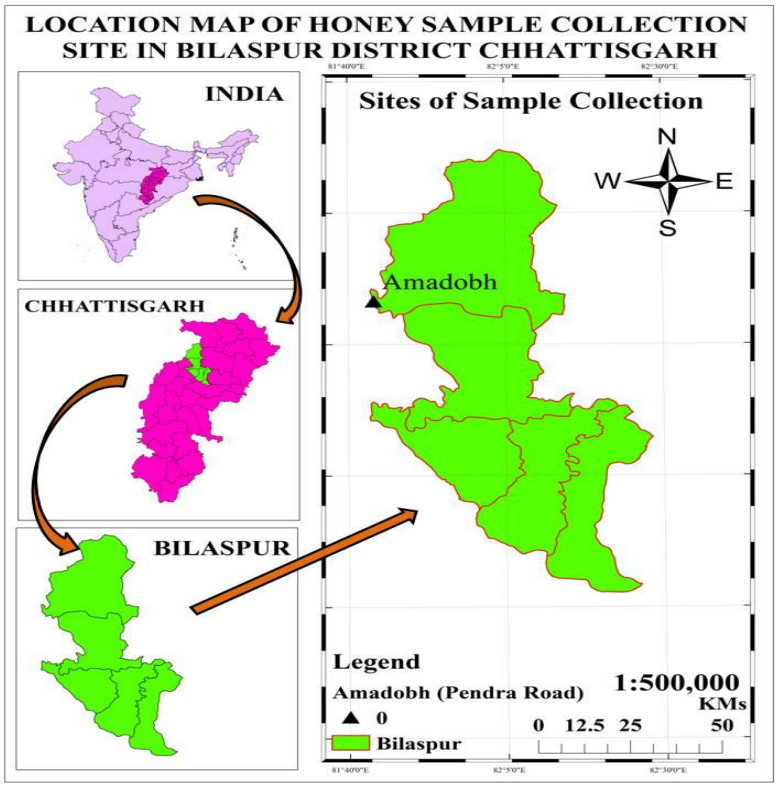

Fig 1: Site of honey sample collection Amadobh.

2.2 Preparation of slide

10 g honey was taken in a pointed centrifuge tube and it was dissolved it 20 ml lukewarm distilled water. The diluted honey was centrifuged for 10 minutes at 2500 rpm in a Weiber Centrifuge. The supernatant was decanted and the residue was transferred to another tube. Both tubes were filled with water (to balance the centrifuge) and the mixture was centrifuged again for 5 minutes at 2500 rpm. The supernatant mixture was decanted and the sediments were collected into a conical tube and treated with an acetolysis mixture (Acetyl chloride: Conc. Sulphuric acid = 9:1 V/V) for approximately 30 minutes at room temperature. After treatment with the acetolysis mixture, the sediments were rinsed with distilled water and centrifuged for 5 minutes at 2500 rpm (as suggested by Song *et al.* (2012)). The supernatant liquid was decanted and 30% glycerin was mixed with the residue. With the help of dropper residue was transferred and spread over the glass slides (76 mm X 26 mm). Now the cover slip (22 mm X 60 mm) was placed carefully, on the slide, so as to avoid air bubbles. A waterproof marker was used to draw 1 X 1 mm square on the slide. During the whole procedure, great care was taken to prevent contamination from foreign pollen grains. The morpho-taxonomical examination of pollen grains was carried out under the digital microscope Leica, DM- 2000. Pollen grains were counted and identified with the help of reference slides available in the Department of Botany, GGV, Bilaspur. As far as possible the pollen grains were identified up to family, genus and species level as suggested by Maurizio & Louveaux (1965), Sawyer (1988), Ricciardelli d' Albore (1997,1998). For the identification of unifloral or multifloral honey the well established formula has been applied. According to this formula if a honey sample contains more than 45% of pollen grains of a particular species, the honey is called "Unifloral" honey but if none of the pollen types present in the honey sample reaches 45%, the honey sample known as "Multifloral" honey.

2.3 Physicochemical test

2.3.1 Ash content (%)

2 gm of each sample was put in a crucible and dried in an oven at 105ºC for three hours to prevent loss by foaming. After cooling it was ashes in muffle furnace at 600ºC overnight (for 6 hours). It was then cooled and weighed into a constant weight (Association of Official Analytical Chemists, 1990).

2.3.2 Moisture content (%)

The moisture was assessed by measuring 10.0 g of each sample and put in a flat dish and dried in the oven at 105 0C for three hours. It was then covered, cooled in desiccators and weighed. The sample was re-dried for one hour in the oven, cooled and reweighed. The process was repeated at one hour during intervals until a constant weight was obtained (William, Jeffy, Barminas & Toma, 2009).

2.3.3 Total solid

The percentage total solid of each sample was determined using the following formula. Total solids (%) = 100 – Moisture content.

2.3.4 Colour
The colour of the honey sample was observed by visually.

2.3.5 Determination of pH
The pH was measured by using a digital pH meter.

2.3.6 Fiehe's test
This test was performed according to Instituto Adolfo Lutz (IAL, 2008). The following procedure was followed for conducting the test:-

- Two grams of honey was dissolved in 10 mL of water and the solution extracted with 30 mL of diethyl ether in a separating funnel. The layer formed was concentrated to 5 mL.
- Then two millilitre of freshly prepared resorcinol solution (1 g of resublimed resorcinol in 100 g mL of hydrochloric acid) was added to the preparation.
- The solution was shaken and appearance of cherry red colour indicates the presence of commercial inverted sugar. It usually takes a minute for the colour to develop. Yellow and other shades have no significance for this test.

2.3.7 Lugol's reaction
This test was performed according to Instituto Adolfo Lutz (Instituto Adolfo Lutz, 2008). Following was the procedure for conducting this test:-

- Twenty millilitre of water was added to 10 g of honey and the solution is kept in the water bath for 1 hour to get cooled to room temperature then 0.5 mL of Lugol solution was added.
- In the presence of commercial glucose or sugar syrups, the solution gets stained to blue colour and the intensity of the colour reflects the quality and quantity of dextrins or starch present in the adulterated sample.

2.3.8 Lund's reaction
This test was performed according to Instituto Adolfo Lutz (Instituto Adolfo Lutz, 2008). The following procedure was adopted for conducting this test:-

- Two grams of each sample was weighted and added to a volumetric flask (50 mL).
- Twenty millilitre of water and 5 mL of tannic acid (0.5%) was added to the mixture.
- Then water was added to increase the volume up to 40 mL.
- The mixture was then shaken and placed for 24 hour. In the presence of pure honey, a precipitate (0.6–3.0 mL) will develop.

2.3.9 Determination of glucose content
Glucose content of the honey samples is determined by enzymatic oxidation with glucose oxidase reagent (Randox Laboratories Ltd., UK) as per following procedure:- (Buba *et.al.*,2013)

- Twenty microlitres (20 µL) of the sample or standard mixed with 2.0 mL of the reagent and the reacted preparation was incubated for 10 min at 37°C.
- The absorbance of the sample (A_{sample}) and standard ($A_{standard}$) was read against a reagent blank within 60 min.
- Glucose concentration was calculated as follows:

Glucose content (mg/dL)=($A_{sample}/A_{standard}$) x Conc. of standard =($A_{sample}/A_{standard}$) x 100 (mg/dL).

2.3.10 Determination of fructose content
Fructose content was determined using the resorcinol reagent method (AOAC, 2000) and following procedure was followed:-

- A solution of the honey sample was taken and 1.0 mL resorcinol reagent was added. The preparation was mixed thoroughly and then 1.0 mL of dilute HCl was added.
- Standard solutions containing 0.2, 0.4, 0.6, 0.8 and 1.0 mg/ mL and made up to 2 mL with distilled water was also treated with 1.0 mL of the resorcinol reagent and 1.0 mL of diluted HCl as above.
- A blank solution was also prepared along with the standard solution and the solution treated in the same manner as above.
- The test solution, the standard and blank were then heated in a water bath at 80°C for about 10min.
- The solution is then removed from the water bath and was cooled by immersing it in tap water for 5 minutes and then the absorbance of both the test sample and the standard solution were read against the blank solution at 520 nm within 30 min.
- The fructose contents of the honey samples were then extrapolated from a standard curve prepared using the absorbance of the standard sample.

3. Observations
Pollen analysis was done on 3 honey samples collected from Amadobh village during the study period from 2014-2017. All honey samples were analyzed to study the content of pollen grains as qualitative and quantitative analysis. The honey samples were collected during winter (January-February), summer (April-May) and rainy (August-September) season.

Winter season
Winter sample of honey obtained from Amadobh village, Bilaspur, Chhattisgarh reveal the presence of 3, 26,160 pollen grains in 10 gm of honey sample (Table 5), belonging to 22 plant species and 16 families. Out of 22 plant species 1 species belonging to 1 family was monocotyledon and 21 species belonging to 15 families were dicotyledon (Table 5). Myrtaceae family was reported as highest species representative with 3 plant species. Asterace, Fabaceae, Malvaceae and Solanaceae had 2 plant species of each family.

Acanthaceae - *Justicia* sp., Amaranthaceae - *Amaranthus virdis* L., Apiaceae - *Coriandrum sativum* L., Asteraceae - *Chrysanthemum indicum* L., *Tridax procumbens* L., Brassicaceae - *Brassica campestris* L., Cucurbitaceae - *Coccinia grandis* (L.)Voigt., Cyperace - *Cyperus* sp., Euphorbiaceae - *Ricinus communis* L., Fabaceae - *Cicer arietinum* Linn., *Gliricida sepium* Jacq.Kunth, Malvaceae - *Bombax ceiba* L., *Helicteris isora* L.Ham., Moringaceae - *Moringa oleifera* Lam., Myrtaceae - *Callistemon citrinus* (Curtis) skeels, *Eucalyptus obliqua* L'Herit., *Psidium guajava* L., Oxalidaceae - *Oxalis corniculata* L., Polygonaceae - *Antigonon leptopus* Hook & Arn., Solanaceae - *Capsicum annuum* Linn., *Solanum xanthocarpum* Schrad & Wendl., Verbinaceae - *Vitex negundo* L.

The qualitative analysis revealed that winter honey sample of Amadobh region was "multifloral" because none of the pollen grains has been found under "Dominant" (D>45% of

toltal pollen grains) frequency class. Therefore identified plant species were classified under three frequency classes, i.e., "Secondary" (S: 16-45% of total pollen grains) which include two species, "Important minor" (IM: 3-15% of total pollen grains) which include 6 number of plant species and other 14 plant species were classified as "Minor" (M>3% of total pollen grains) (Table 4, 5). *Brassica camestris* L. (22.59%) has the maximum number of pollen grains followed by *Ricinus communis* L. (17.12%) and *Helicteris isora* L.Ham. (10.02%). However, the morphological characteristics of various pollen grains found in the honey sample collected during winter season are annotated in Table 1.

Table 1: Morphological characters of pollen grains found in winter honey sample

S. No.	Botanical	Family	Pollen morphology		Colpi
			Shape	Size in µm	
1	*Amaranthus virdis* L.	Amaranthaceae	Spheroidal	19-21	Pantoporate
2	*Antigonon leptopus* Hook & Arn.	Polygonaceae	Oval elongated	45-47	Tricolpate
3	*Bombax ceiba* L.	Malvaceae	Triangular	60-63	Triporate
4	*Brassica campestris* L.	Brassicaceae	Rounded Triangular	35-38	Triporate
5	*Callistemon citrinus* (Curtis) skeels	Myrtaceae	Triangular	15-18	Tricolporate
6	*Capsicum annuum* L.	Solanaceae	Sub-spheroid	35-38	Tricolporate
7	*Chrysanthemum indicum* L.	Asteraceae	Rounded-triangular	37-40	Tricolporate
8	*Cicer arietinum* L.	Fabaceae	Sub-prolate	22-28	Tricolporate
9	*Coriandrum sativum* L.	Apiaceae	Bilateral symmetry	18-23	Tricolporate
10	*Cyperus* sp.	Cyperaceae	Pear shaped	37-40	3-4 aperturoid
11	*Coccinia grandis* L.Voigt.	Cucurbitaceae	Prolate	64-69	Triporate
12	*Eucalyptus obliqua* L'Herit.	Myrtaceae	Triangular	30-35	Tricolporate
13	*Gliricida sepium* Jacq.Kunth	Fabaceae	Sub-oblate	28-33	Monocolporate
14	*Helicteris isora* L.Ham.	Malvaceae	Sub-prolate	18-23	Triporate
15	*Justicia* sp.	Acanthaceae	Oval	30-32	Bicolporate
16	*Moringa oleifera* Lam.	Moringaceae	Prolate- spheroidal	42-45	Tricolporate
17	*Oxalis corniculata* L.	Oxalidaceae	Oblate-spheroidal	21-23	Tricolporate
18	*Psidium guajava* L.	Myrtaceae	Triangular	37-40	Triporate
19	*Ricinus communis* L.	Euphorbiaceae	Prolate-spheroidal	37-40	Tricolporate
20	*Solanum xanthocarpum* Schrad & Wendl.	Solanaceae	Triangular rounded	28-30	Triporate
21	*Tridax procumbens* L.	Asteraceae	Sub-spheroidal	15-21	Tricolporate
22	*Vitex negundo* L.	Verbinaceae	Prolate spheroidal	15-25	Tricolpate

Summer season

During summer season honey sample was collected from Amadobh village, Bilaspur district, Chhattisgarh. In this honey sample total 1,15,560 pollen grains belonging to 26 genera and 16 families were obtained in10 gm of honey sample (Table 5). All 26 plant species belonging to 26 genera and 16 families were dicotyledonous (Table 5). Fabaceae family contained 6 plant species and considered as highest species representative family.

Amaranthaceae: *Amaranthus virdis* L., Anacardiaceae: *Buchanania lanzan* Spreng., *Mangifera indica* L., Apiaceae: *Coriandrum sativum* L., Apocynaceae: *Holarrhena antidysenterica* L. Wall., *Nerium odorum* Soland., Asteraceae: *Ageratum conyzoides* L., *Eclipta alba* L. Hassk., Combretaceae: *Terminalia arjuna* Roxb. ex DC, Euphorbiaceae: *Acalypha indica* L., Fabaceae: *Albizzia lebbeck* L.Benth., *Bauhinia purpurea* L., *Butea monosperma* (Lam.)Taub., *Pithecellobium dulce* (Roxb.)Benth, *Pongamia pinnata* L. pierre, *Putranjiva roxburghii Wall.*, Lythraceae: *Woodfordia fructiosa* L., Malvaceae: *Bombax ceiba* L., *Helicteris isora* L.Ham., Moringaceae: *Moringa oleifera* Lam., Papaveraceae: *Argemone Mexicana* L., Rosaceae: *Kalanchoe pinnata* (Lam.) Pers., Sapindaceae: *Cardiospermum halicacabum* L., *Schleichera oleosa* Lour., Sapotaceae: *Madhuca indica* J.F.Gmel, Solanaceae: *Solanum xanthocarpum* Schrad & Wendl.,

The pollen grains of *Butea monosperma* (Lam.) Taub. with 53.3% was the dominant plant species in collected honey sample. Therefore the honey sample has been categorized as "Unifloral". None of the pollen grain have been found under "Secondary" (S: 16-45% of total pollen grains) frequency class. Other identified plant species were classified in to "Important minor" (IM: 3-15% of total pollen grains) and "Minor" (M>3% of total pollen grains). The "Important minor" includes 3 plant species and other 22 species were classified as "Minor" (Table 4, 5). The plant species *Butea monosperma* (Lam.)Taub. Followed by *Magnifera indica* L. (15.3%) and *Acalypha indica* L. (13.87%). However, the morphological characteristics of various pollen grains found in the honey sample collected during summer season are annotated in Table 2.

Table 2: Morphological characters of pollen grains found in Amadobh Summer honey sample

S. No.	Botanical	Family	Pollen morphology		Colpi
			Shape	Size in µm	
1.	*Acalypha indica* L.	Euphorbiaceae	Circular	8-13	Tricolporate
2.	*Ageratum conyzoides* L.	Asteraceae	Prolate	21-26	Polypantoporate
3.	*Albizzia lebbeck* L.Benth.	Fabaceae	Perprolate	55-60	Inaperturate
4.	*Amaranthus virdis* L.	Amaranthaceae	Spheroidal	19-21	Pantoporate
5.	*Argemone Mexicana* L.	Papaveraceae	Sub-prolate	24-29	Tricolpate
6.	*Bauhinia purpurea* L.	Fabaceae	Prolate-spheroid	50-55	Tricolporate
7.	*Bombax ceiba* L.	Malvaceae	Triangular	60-63	Triporate
8.	*Buchanania lanzan* Spreng.	Anacardiaceae	Rounded triangular	34-39	Tricolporate
9.	*Butea monosperma* (Lam.)Taub.	Fabaceae	Triangular-obtuse-convex	37-40	Tricolporate
10.	*Cardiospermum halicacabum* L.	Sapindaceae	Triangular	34-37	Triporate
11.	*Coriandrum sativum* L.	Apiaceae	Bilateral symmetry	18-23	Tricolporate
12.	*Eclipta alba* L. Hassk.	Asteraceae	Spheroidal	14-18	Tricolporate
13.	*Helicteris isora* L.Ham.	Malvaceae	Sub-prolate	18-23	Triporate
14.	*Holarrhena antidysenterica* L. Wall.	Apocynaceae	Circular	12-14	Triporate
15.	*Kalanchoe pinnata* (Lam.) Pers.	Rosaceae	Triangular	43-48	Triporate
16.	*Madhuca indica* J.F.Gmel	Sapotaceae	Rounded	45-48	Bicolporate
17.	*Mangifera indica* L.	Anacardiaceae	Prolate – Trilobed	25-30	Tricolporate
18.	*Moringa oleifera* Lam.	Moringaceae	Prolate-spheroidal	42-45	Tricolporate
19.	*Nerium odorum* Soland.	Apocynaceae	Sub-prolate	25-30	Trizonocolpate
20.	*Pithecellobium dulce* (Roxb.)Benth	Fabaceae	Oval-Rectangle	25-30	Tetraporate
21.	*Pongamia pinnata* L. pierre	Fabaceae	Prolate	23-25	Tricolporate
22.	*Putranjiva roxburghii Wall.*	Fabaceae	Rounded triangular	33-38	Tricolporate
23.	*Schleichera oleosa* Lour.	Sapindaceae	Rounded triangular	19-21	Tricolporate
24.	*Solanum xanthocarpum* Schrad & Wendl.	Solanaceae	Triangular rounded	28-30	Triporate
25.	*Terminalia arjuna* Roxb. ex DC	Combretaceae	Prolate spheroidal	15-22	Tricolporate
26.	*Woodfordia fructiosa* L.	Lythraceae	Rounded	23-28	Triporate

Rainy season

Rainy honey sample obtained from Amadobh village, Bilaspur district, Chhattisgarh reveal the presence of 1,03,266 pollen grains in 10 gm of honey sample (Table 5) belonging to 22 genera and 13 families, where 2 plant species under 2 families were from monocotyledons and 20 plant species under 11 families were from dicotyledons (Table 5). Fabaceae family was reported as highest species representative with 5 plant species. Asteraceae had 3 plant species of each family.

Apocynaceae: *Holarrhena antidysenterica* L. Wall., *Thevetia peruviana* (Pers.)K.Schum., Asteraceae: *Eclipta alba* L. Hassk., *Emelia sonchifolia* L., *Tridax procumbens* L., Boraginaceae: *Heliotropium indicum* L., Commelinaceae: *Commelina diffusa* N. Burm., Cucurbitaceae: *Cucumis sativus* L., Dipterocarpaceae: *Shorea robusta* Gaertn. F.,Fruct., Euphorbiaceae: *Acalypha indica* L., *Phyllanthus amurus* Schumach. & Thonn., Fabaceae: *Acacia* sp., *Cassia alata* L., *Cassia fistula* L., *Clitoria ternatea* L., *Tamarindus indica* L. Malvaceae: *Grewia tilifolia* Vahl, *Helicteris isora* L., Myrtaceae: *Callistemon citrinus* (Curtis)Skeels, *Eucalyptus obliqua* L'Herit, Poaceae: *Sporobolus diander* Beauv., Solanaceae: *Physalis minima* L., Verbinaceae: *Clerodendrum inerme* L.Gaertn.

The result of qualitative analysis showed that rainy honey sample of amadobh area was "Multifloral". All identified plant species were classified into three frequency classes. The "Secondary" (S: 16-45% of total pollen grains) contained only one plant species. The "Important minor" (IM: 3-15% of total pollen grains) contained 9 plant species and "Minor" (M>3% of total pollen grains) contained 13 plant species. The dominant plant species were not found after the analysis of honey sample (Table 4, 5). Sporobolus diander Beauv. (17.27%) contained highest pollen grains followed by *Grewia tilifolia* Vahl (13.17%) and *Tridax procumbens* L. (10.54%). The morphological characteristics of various pollen grains found in the honey sample collected during summer season are annotated in Table 3.

Table 3: Morphological characters of pollen grains found in Rainy honey Sample

S. No	Botanical	Family	Pollen morphology		Colpi
			Shape	Size in µm	
1	*Acacia* sp.	Fabaceae	(16 celled polyad) Ovoid	50-55	Inaperturate
2	*Acalypha indica* L.	Euphorbiaceae	Circular	8-13	Tricolporate
3	*Callistemon citrinus* (Curtis)Skeels	Myrtaceae	Triangular	15-18	Tricolporate
4	*Cassia alata* L.	Fabaceae	Sub prolate	20-25	Trizonocolporate
5	*Cassia fistula* L.	Fabaceae	Prolate	28-33	Tricolporate
6	*Clerodendrum inerme* L. Gaertn.	Verbenaceae	Rounded triangular	72-77	Tricolpate
7	*Clitoria ternatea* L.	Fabaceae	Triangular	41-44	Tricolporate
8	*Commelina diffusa* N. Burm.	Commelinaceae	Elliptical	35-40	Monocolpate
9	*Cucumis sativus* L.	Cucurbitaceae	Triangular	14-18	Trizonoporate
10	*Eclipta alba* L. Hassk.	Asteraceae	Spheroidal	30-35	Tricolporate

11	*Emelia sonchifolia* L.	Asteraceae	Triangular	23-28	Triporate
12	*Eucalyptus obliqua* L'Herit	Myrtaceae	Triangular	30-35	Tricolporate
13	*Grewia tilifolia* Vahl	Malvaceae	Spheroidal to prolate	25-35	Tricolporate
14	*Helicteris isora* L.	Malvaceae	Sub prolate	18-23	Triporate
15	*Heliotropium indicum* L.	Boraginaceae	Prolate	31-35	Tricolporate
16	*Holarrhena antidysenterica* L. Wall.	Apocynaceae	Circular	12-14	Triporate
17	*Phyllanthus amurus* Schumach. & Thonn.	Euphorbiaceae	Oval elongated	45-40	Tetracolporate
18	*Physalis minima* L.	Solanaceae	Prolate	24-29	Tricolporate
19	*Shorea robusta* Gaertn. F.,Fruct.	Dipterocarpaceae	Sub-oblate	28-33	Tricolpate
20	*Sporobolus diander* Beauv.	Poaceae	Spheroidal	19-23	Monoporate
21	*Tamarindus indica* L.	Fabaceae	Oblatr-spheroidal	27-30	Tricolporate
22	*Thevetia peruviana* (Pers.)K.Schum.	Apocynaceae	Triangular	66-70	Tricolporate
23	*Tridax procumbens* L.	Asteraceae	Sub-spheroidal	15-21	Tricolporate

Table 4: Frequency Classes (According to Louveaux, 1978) and percentages of pollen grains of different plant species in honey samples

S. N.	Plant Species	Winter honey sample	Summer honey sample	Rainy honey sample
1.	*Acacia* sp.	-	-	M(0.29)
2.	*Acalypha indica* L.	-	IM(13.87)	IM(3.73)
3.	*Ageratum conyzoides* L.	-	M(0.13)	-
4.	*Albizzia lebbeck* L.Benth.	-	M(1.93)	-
5.	*Amaranthus virdis* L.	M(0.09)	M(0.37)	-
6.	*Antigonon leptopus* Hook & Arn.	M(0.13)	-	-
7.	*Argemone Mexicana* L.	-	M(0.88)	-
8.	*Bauhinia purpurea* L.	-	M(0.08)	-
9.	*Bombax ceiba* L.	M(2.71)	M(0.23)	-
10.	*Brassica campestris* L.	S(19.52)	-	-
11.	*Buchanania lanzan* Spreng.	-	M(0.04)	-
12.	*Butea monosperma* (Lam.)Taub.	-	D(53.3)	-
13.	*Callistemon citrinus* (Curtis) skeels	IM(8.51)	-	M(2.32)
14.	*Capsicum annuum* L.	M(1.67)	-	-
15.	*Cardiospermum halicacabum* L.	-	M(0.28)	-
16.	*Cassia alata* L.	-	-	IM(10.18)
17.	*Cassia fistula* L.	-	-	M(0.64)
18.	*Chrysanthemum indicum* L.	IM(6.70)	-	-
19.	*Cicer arietinum* L.	M(0.33)	-	-
20.	*Clerodendrum inerme* L.Gaertn.	-	-	IM(7.27)
21.	*Clitoria ternatea* L.	-	-	IM(8.79)
22.	*Coccinia grandis* L.Voigt.	M(2.9)	-	-
23.	*Commelina diffusa* N. Burm.	-	-	M(0.096)
24.	*Coriandrum sativum* L.	IM(6.88)	M(0.27)	-
25.	*Cucumis sativus* L.	-		IM(5.02)
26.	*Cyperus* sp.	M(0.72)		-
27.	*Eclipta alba* L. Hassk.	-	M(0.05)	M(0.69)
28.	*Emelia sonchifolia* L.	-	-	M(0.29)
29.	*Eucalyptus obliqua* L'Herit.	M(0.65)	-	M(0.68)
30.	*Gliricida sepium* Jacq.Kunth	M(0.93)	-	-
31.	*Grewia tilifolia* Vahl	-	-	IM(13.17)
32.	*Helicteris isora* L.Ham.	IM(10.02)	M(0.93)	IM(3.48)
33.	*Heliotropium indicum* L.	-	-	IM(10.04)
34.	*Holarrhena antidysenterica* L. Wall.	-	M(0.21)	M(2.62)
35.	*Justicia* sp.	M(0.07)	-	-
36.	*Kalanchoe pinnata* (Lam.) Pers.	-	M(0.05)	-
37.	*Madhuca indica* J.F.Gmel	-	M(0.19)	-
38.	*Mangifera indica* L.	-	IM(15.3)	-
39.	*Moringa oleifera* Lam.	M(0.13)	M(0.89)	-
40.	*Nerium odorum* Soland.	-	M(0.04)	-
41.	*Oxalis corniculata* L.	M(0.05)	-	-
42.	*Phyllanthus amurus* Sch. & Thonn.	--	-	M(0.24)
43.	*Physalis minima* L.	-	-	M(0.14)
44.	*Pithecellobium dulce* (Roxb.)Benth	-	M(0.26)	-
45.	*Pongamia pinnata* L. pierre	-	IM(5.27)	-
46.	*Psidium guajava* L.	M(0.05)	-	-
47.	*Putranjiva roxburghii Wall.*	-	M(0.23)	-
48.	*Ricinus communis* L.	S(17.12)	-	-
49.	*Schleichera oleosa* Lour.	-	M(2.44)	-

50.	*Shorea robusta* Gaertn. F.,Fruct.	-	-	M(0.16)
51.	*Solanum xanthocarpum* Sch. & Wdl.	M(1.14)	M(1.05)	-
52.	*Sporobolus diander* Beauv.	-	-	S(17.27)
53.	*Tamarindus indica* L.	-	-	M(1.46)
54.	*Terminalia arjuna* Roxb. ex DC	-	M(0.75)	-
55.	*Thevetia peruviana* (Pers.)K.Schum.	-	-	M(0.84)
56.	*Tridax procumbens* L.	M(0.05)	-	IM(10.54)
57.	*Vitex negundo* L.	IM(4.68)	-	-
58.	*Woodfordia fructiosa* L.	-	M(0.06)	-

D: Dominant (>45% of total pollen grains)
S: Secondary (16 -45% of total pollen grains),
IM: Important Minor (3-15% of total pollen grains)
M: Minor (< 3% of total pollen grains)

Table 5: Seasonal variation of pollen grains in honey samples.

S. No.	Amadobh	Winter	Summer	Rainy
1	Total number of pollen grains	3,26,160	1,15,560	1,03,266
2	Total number of identified plant species	22	26	23
3	Total number of genera	22	26	22
4	Total number of family	16	16	13
5	Dicot. Plant species	21	26	20
6	Dicot family	15	19	11
7	Monocot. Plant species	01	-	02
8	Monocot. family	01	-	02
9	Total number of Dominant (D>45%) plant species	-	1	-
10	Total number of Secondary (S-16-45%) plant species	02	-	01
11	Total number of Important Minor (IM- 3-15%) plant species	05	03	09
12	Total number of Minor (M>3%) plant species	15	22	19
13	Nature of sample	M	U	M

Physicochemical test

- **Color:** Rainy honey sample shows light brown color, winter honey shows golden yellow color and summer honey sample shows golden brown color (Table 6).
- **Moisture (%):** Rainy honey sample contains maximum moisture (19%) as compare to winter (18%) and summer (16%) honey sample (Table 6).
- **pH:** The pH of rainy, winter and summer honey samples were 4.12, 4.6 and 4.01 respectively (Table 6).
- **Ash (%):** The Ash (%) found in rainy sample was 0.002, in summer 0.016 and in winter 0.001 (Table 6).
- **Total solid (%):** Winter honey sample has the maximum solid content (82%) and rainy and summer honey samples have 81% and 80% solid content in each of the sample (Table 6).
- **Fiehe's test:** This test was performed to check the presence of commercial inverted sugar in honey for adulteration. The test will be positive if the colour will be occurred red (Almeida-Muradian & Matsuda, 2007). In the present study all the honey samples were found negative, which showed the freshness of these natural products. (Table 6)
- **Lugol's reaction:** This test is based on the reaction between potassium iodide and iodine in the presence of glucose, resulting in a stained solution (red-purple to blue). When the stained solution coloured blue, the reaction is considered positive (Almeida-Muradian & Matsuda, 2007). All the honey samples were found negative under this study and reveal the absence of adulteration in all samples. (Table 6)
- **Lund's reaction:** The base of this reaction is found the precipitation of natural honey's protein by tannic acid. When the precipitate ranges from 0.6 – 3.0 ml, the reaction is considered positive and indication the purity of honey (Almeida-Muradian & Matsuda, 2007)). The values of precipitated protein after Lund's reaction in all the studied samples were within the range. (Table 6)

Table 6: Physico-chemical analysis of honey samples from Amadobh region, Bilaspur district, Chhattisgarh

Parameters	Rainy honey	Summer honey	Winter honey
Color	Light brown	Golden brown	Golden yellow
Moisture	19	16	18
pH	4.12	4.01	4.6
Ash content	0.002	0.16	0.001
Total solid	81	80	82
Fiehe;s test	Negative	Negative	Negative
Lugol's test	Negative	Negative	Negative
Lund's test	1.42	2.0	1.99
Fructose	36.28	39.06	38.15
Glucose	33.45	33.85	29.16
Frutose+Glucose	69.73	72.91	67.31
Fructose/Glucose	1.08	1.15	1.30

Sugar test (Fructose and Glucose)

The result of the sugar analysis of all the honey samples of (Table 6) showed that the range of fructose content from 36.28 to 39.06 g/100g and the glucose content of the samples varied between 29.16 and 33.85 g/100g. The fructose/glucose ratio was within the range of 1.08 to 1.30. The sum of fructose and glucose (Fructose+Glucose) content were in ranged between 67.31 and 72.91. Fructose/Glucose ratio indicates the ability of crystallise. When this ratio was high, the honey sample remains liquid because of the modification of saturated glucose level by the presence of the high amount of fructose (White *et.al.* 1964).

4. Result and Discussion

The quantitative and qualitative analysis of winter honey sample of Amadobh village of Bilaspur district, Chhattisgarh reveals that out of 3 honey samples 1 honey sample was unifloral and the other 2 were multifloral. The multifloral quality of honey samples reveals that honey bees prefer wide range of flowering plants and for the collection of nectar they visit far and diverse area. On the other hand in Unifloral honey sample honey bees prefer only one plant species for the nectar collection as compare to other plant species. In this study the pollen grains of *Butea monosperma* (Lam.) Taub. with 53.3% contribution was the dominant plant species. Butea pollen grains are considered as chief nectar sources of Amadobh area.

Qualitative analysis of this study reveals a total of 58 types of pollen grains belonging to 58 genera and 28 families have been found in the honey samples of Amadobh region (plate 1 to 10). It was observed that summer honey sample has the maximum number of pollen type 26 types of pollen grains belonging to 26 genera and 16 families followed by rainy honey sample contain 23 pollen type belonging to 22 genera and 13 family. And winter honey has minimum number of pollen type 22 pollen type belonging to 22 genera and 16 family. Ige and Obsanmi (2014), also found this type of variation in the honey samples of Nigeria. 72 pollen grains belonging to 28 families were found in their study and they also concluded that the honey bees collected pollen grains from very diverse plant species for the honey production.

The quantitative analysis shows that, the winter honey sample has the maximum number of pollen grains, i.e. 3,26,160 followed by summer honey sample contain 1,15,560 pollen grains and rainy honey sample has the minimum number of pollen grains i.e. 1,03,266.

The subjective and quantitative investigation of the present study reveals that the dicotyledons pollen grains are found in greater quantities in comparison to pollen grain of monocotyledons. Pollen grains of *Cyperus* sp., *Commelina diffusa* N. Burm., and *Sporobolus diander* Beauv. have been found as monocotyledon.

The seasonal variation of pollen grains in present study uncovered that winter and summer honey sample has more number of pollen grains as contrast with rainy season and all the season demonstrates variety in dust spectra.

In winter honey sample of Amadobh town contained 2 plant species as secondary type: *Brassica campestris* L. with 22.59% pollen grains and *Ricinus communis* L. with 17.12% pollen grains, though Sporobolus diander Beauv. with 17.27% present as secondary pollen type in rainy honey sample of Amadobh town. Cherian *et.al.*, 2011 found *Brassica campestris* L. as predominant pollen grain in the honey sample of Nagpur, Maharashtra. In the whole honey samples Minor gathering (M>3%) of plants were higher in contrast with other recurrence classes. This kind of report was additionally found by Saharia, 2013. The nearness of prevailing plant species in all the honey samples uncovered that the bumble bees favor these plants for their nectar accumulation.

The color of the honey is varied from light brown, golden yellow to golden brown. Colour depends upon the botanical origin, storage temperature and duration of storage.

The range of pH is 4.01-4.6. These values fell within the prescribed acidic range according to National honey board (3.4-6.1). Kayode and Oyeyemi, 2014 also found the same range of pH value 4.00-4.65 in the honey sample of Ondo state, Nigeria. In the honey sample of Nagpur, Mharashtra, India the ph value was observed 3.5-3.6 by Cherian *et.al.* (2011).

Moisture % is found in the honey samples of Amadobh region from16% to19%. According to International standard the moisture content in honey was up to 21%. However in this study the moisture content in all the investigated honey samples were inside the range. Honey sample of rainy season have the high moisture content as contrast with winter and summer season.

The ash percentage in collected honey samples were (0.001%-0.16%) within the acceptable limit of National honey board and Codex Standard, 2001. The values of ash were so higher (0.13%-0.94%) in the Burkina Fasan honey reported by Meda *et.al.*, 2005. Sahney and Kumar (2017), found the ash percentage from 0.1257±0.008%-0.3910±0.008.

The total solid percent in all the collected honey samples were in range from 80% to 82% and found within the acceptable limits. Same type of result was found by Nayik and Nanda (2015) from 80.89±0.55% to 81.40±0.08%.

The adulteration of honey was tested by Fiehe's test, Lund's reaction and Lugol's reaction. Fiehe's test is a qualitative test and was performed to check the commercial inverted sugar in honey for adulteration. In this test all the collected honey samples were found negative, which shows the freshness of honey. In Lugol's rection also all the samples were showed the negative result, which reveal the absence of adulteration in all the honey samples. Lund's reaction was performed based on the precipitation of the natural honey's protein by the tannic acid. In this study the precipitate ranges from 1.42 to 2.0 ml, which is within the range. Same type of result was found in the study of Almeida-Muradian *et.al.*, 2013.

In this study the glucose content of all the honey samples obtained from various region of Bilaspur district varied from 29.16-33.85g/100g. And the fructose obtained in honey samples ranges from 36.28-39.06g/100g. This observation reveals that all the honey samples contain fructose more than glucose, same type of result was found by Buba *et.al.*, 2013. The sum of the glucose and fructose of the studied honey samples reveals that samples have their value 60g/100g or above, which is standardized by Codex Alimentarious Commission. The ratio of fructose and glucose is an important factor related to quality of honey. In this study fructose/glucose ratio was ranged from 1.08 to 1.30. Buba *et.al*, 2013 also observed same type of Fructose/Glucose ratio. Fructose/Glucose ratio indicates the crystallize quality

of honey. If the ratio is below than 1.0, the crystallization of honey will be faster and when its ratio is more than 1.3, honey will be slowly crystallize (Amir *et.al.*, 2010).

The variation of pollen types in the honey shows the diversity of flora in and around Amadobh area of Bilaspur district. The reason of it might be that, Amadobh area arranged in Achanakmar biosphere reserve and having greatest differing qualities of plants. The investigation of regular variety of pollen grains in honey sample demonstrated that winter and summer honey samples have more number of pollen grains per 10 gm of tests as contrast with rainy season. The analysis of this study also shows that the collected honey samples are pure and can be consumed as a food.

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