POLLEN AND PHYSICOCHEMICAL ANALYSIS OF HONEY PRODUCED BY *Apis cerana indica* OF NAGPUR, MAHARASHTRA (INDIA)

K. J. Cherian*, M. Bhowal and S. D. Godghate

Department of Botany, Hislop College, Nagpur, Maharashtra (INDIA)

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ABSTRACT

The present paper reports pollen and physicochemical analysis on nine honey samples. Honey (capped and uncapped) and pollen load were directly collected from the domesticated bee hives over a period of eleven months from February to December 09. Nineteen morpho types of pollen were identified and categorized into, predominant, major and minor pollen. Majority of the honey sample were unifloral and only one sample was multifloral. The predominant pollen found in honey sample were *Brassica compestris, Azadiracta indica, Psidium gaujava*, *Alternanthera sp., Albizia lebbeck,* The Physicochemical profile and honey analysis helped in the characterization of honey with regards to their botanical origin.

Key Words : Pollen load, Physicochemical analysis, Capped honey, *Apis cerana indica*, Uncapped honey,

INTRODUCTION

There is a significant reduction in the number of domesticated bee colony and beekeepers in Nagpur district and its adjacent areas in Vidarbha region of Maharashtra state India, which in turn resulted into a reduction in honey production from the region. It plays an important role in the adulteration of honey in the market. Increase in the domesticated bee colonies especially among the farmers will definitely improve the income generated by the farmers directly by selling of honey and indirectly by increase in the crop production of bee forage plants. The resource of food i.e. pollen and nectar may be the prime factor for the growth of bee colony. Pollen is an important food resource for honey bees[4]. The bee collects the pollen and nectar from different flowers of their choice and availability. The collected pollen and honey are used as food for the bees and the brood, the surplus pollen and honey are stored in the hives for future use.Melittopalynogy is concerned with the identification of pollen in honey. Evaluation of plants for their utility as sources of bee forage provides the information needed to assess the potential for Beekeeping in an area[12,15]. Melittopalynological studies are thus helpful in bee management and in promoting the beekeeping development. Significant work has been reported by Sen,et al[6, 13-17] Composition of honey is affected by contributions of the plants, climate, environmental conditions and ability of the Beekeeper[15,22] The diversity of the physical and chemical properties of honey like, color, flavor, moisture and content of protein and sugars etc depends on the nectar and pollen of the original plants[3].It has also been observed that the composition of the minor constituents of natural honeys varies with location, nectar sources and different climatic condition. Some physicochemical properties of honey that can be easily determined have been found to be helpful for comparison and can help to distinguish natural honey from artificial honey. The physicochemical properties provide

*Author for correspondence

the parameter for characterization and classification of honey.

MATERIAL AND METHODS

Nagpur has hot climatic conditions with tropical dry deciduous forest due to its geographical location i.e. central part of India. For the present study fully grown strong Apiary **(Fig. 1)** of *Apis cerana* indica **(Fig. 2)** was setup in the Police Line takli area i.e. North West part of Nagpur.

Fig. 1 : An apiary

Fig. 2 : *Apis cerana* indica

Even though Nagpur is a city, this area has very good vegetation with a large water tank and several small water bodies. Nine honey samples were collected with the help of centrifugal extractor **(Fig. 3)**. And at the same time, the stored pollen load **(Fig. 4)** was also collected with the help of forceps according to their color for pollen analysis. Pollen load were also collected from worker bees directly by trapping few workers at the time of entry into the apiary along with pollens.

Fig 3 : Extractor

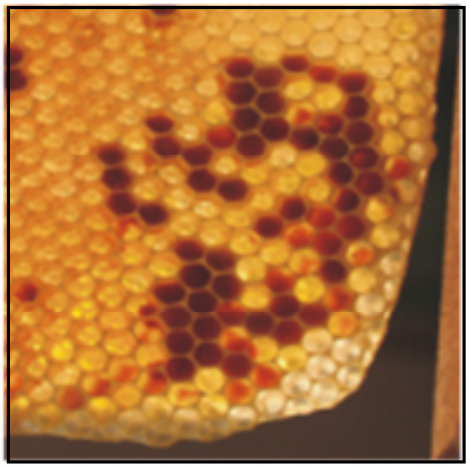

Fig. 4 : Pollen load stored in hive

Melittopalynological analysis

10 gm of extracted honey capped and uncapped (**Fig. 5** and **Fig. 6**) was dissolved in 25 ml distilled water and centrifuged. The recovered sediment was treated with 5 ml of Glacial Acetic Acid and the mixture was subjected to Acetolysis[8].Three Pollen slides were prepared from each sample. The recovered pollen types were identified with the help of reference slides prepared from the local flora and relevant literature. As far as possible the pollen types were identified to generic and specific levels. In some cases however only family identification was

possible. A few types which could not be identified even to family level were placed under the category, "Unknown".

Fig. 5 : Capped honey

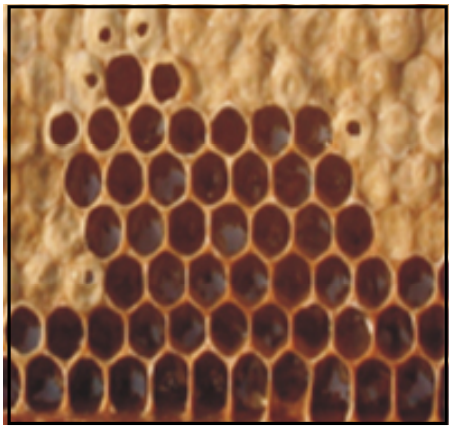

Fig. 6 : Uncapped honey

The frequency classes and frequencies of the pollen types of each sample were determined in accordance with[10]. Pollen spectra of the honey samples were constructed based on the Frequencies of the pollen types. Discrete pollen loads were observed neatly stacked one above the other in the pollen storing chamber of the combs. A total 14 pollen load were directly taken out with fine needle and forceps from pollen storing chambers, during the month of February to December 2009 on basis of their color. Each pollen load was dispersed in 5 ml of glacial acetic acid. After centrifuging the acid was decanted and the sediment was subjected to Acetolysis technique of Erdtman. One slide was prepared for each pollen load, the pollen loads were designated as unifloral (exclusively with pollen grains of one taxa, and multifloral or mixed (with pollen grains of two or more than two taxa)[18].

Physicochemical Analysis

The water content of the sample was determined by the Digital Refractometer IHC, Bogdanov, (2002).The measurement of pH and determination of free acidity were performed at 20 ±0.1°c on stirred solution (obtained after dissolving 10 gm of sample in the 75 ml distilled water by potentiometric titration with a 0.1M NaoH solution until pH reach 8.3.[7]

Sugars were determined using an HPLC (chromatographic method given by AOAC, (1990).HMF and proline concentration were determined according to the IHC method.

RESULTS AND DISCUSSION

Physicochemical properties of honey (Table 1)

Moisture – Moisture depends on the botanical origin of the sample, the degree of ripeness, processing techniques and storage conditions Instituto zooprofilalattica[9]. In the present study moisture content in the sample ranges from 19.1-23.1.The caped honey having moisture percentage ranging from 19.1-19.8 and uncapped or immature honey ranging between 21.3-23.1.The uncapped honey **(Fig. 5)**, sample was found to be higher in moisture content (>21%) than the maximum allowable content for honey determined by the International honey commission.

pH – In the entire honey sample 'pH value fell within the normal range i.e. 3.5 to 3.6. The pH is of great importance during honey extraction and storage due to influence on texture, stability[20].

Free acidity – Free acidity of all the sample fell within the permitted ranges proposed by IHC (2002), with none one of them more than 50 meq/ kg. The free acidity of honey samples in this study ranged from 15 to 47 respectively **(Table 1)** high free acidity values may indicate the fermentation of honey sugar by yeasts.

Sugars – Honey consists of mostly glucose and fructose. The actual proportion of fructose to glucose, in any particular honey, depends largely on the source of the nectar[2].The glucose level in

uncapped honey doesn't show much variation in different seasons. While, in caped honey or mature honey, it shows the percentage of glucose in March and December is lower while October and February are higher it mean that the high temperature and low temperature period show low

Table 1 : Physicochemical properties of honey

Parameter Sample	Moisture %	pH	Free Acidity	Clucose	Fructose	HMF	HMF After Stronge	Proline
Feb	19.20%	3.6	15 meq/kg	24.70%	35.30%	3.66mg/kg	502.99 mg/kg	210.7 mg/kg
Mar.	19.30%	3.7	47 meq/Kg	16.47%	35.82%	13.5mg/kg	287.60 mg/kg	300.1mg/kg
Apr.cap	19.10%	3.7	35 meq/kg	23.10%	34.82%	12.6mg/kg	228.35 mg/kg	391.7 mg/kg
Apr.uncap	22.10%	3.7	35 meq/kg	23.59%	32.61%	9.05 mg/kg	180.3 mg/kg	423.4 mg/kg
Oct.cap	19.40%	3.6	30 meq/kg	24.50%	30.12%	2.55mg/kg	52.04 mg/kg	131.1 mg/kg
Oct.uncap	21.30%	3.5	31 meq/kg	23.68%	37.19%	2.10mg/kg	–	153.4mg/kg
Nov.	19.80%	3.9	45 meq/kg	23.80%	32.32%	2.6mg/kg	302.7 mg/kg	162.9 mg/kg
Dec.cap	19.40%	3.6	29 meq/kg	16.76%	29.41%	1.01mg/kg	3.885 mg/kg	194.7mg/kg
Dec.uncap	23.10%	3.6	29 meq/kg	23.89%	36.53%	1.12mg/kg	3.986 mg/kg	192.5mg/kg

glucose level. All the samples contained more fructose than glucose **(Table 1)**; this indicates that Nagpur honeys would be less prone to granulation. The fructose level in honey for both capped and uncapped are higher than that of glucose. In comparison the capped honey shows low fructose proportion than the uncapped honey. Honey with high fructose to glucose ratios would remain liquid for longer periods[1-2]. The fructose/ glucose ratios may have an impact on honey flavor, since fructose is much sweeter than glucose[11].

HMF – The Hydroxymethyl furfural concentration is an important indicator of the freshness of honey .It is one of the chief products of carbohydrate degradation in food known as non-enzymatic browning. In nine samples it was observed that the HMF values ranges from 1.01 to 13.5 mg/kg, this values was found to be within the range allowed by IHC, 2002 in fresh honey sample. According to the IHC the maximum content of HMF permitted is 40mg/kg, while 80mg/kg for honeys produce in tropical countries. In April 2010 the sample were evaluated for the HMF, striking results were obtained for most of the samples where the values ranged between 180 to 502.9 mg/kg. Four of the sample had values within the ranged because the storage period of that sample was of six months till April 2010, and values range between 3.88 to 52.04 mg/kg. Other sample that had storage period more than six months it was observed that the HMF values were very high. The HMF formed slowly during storage and very quickly when heated.

Proline – Proline comes mainly from the salivate secretions of honey bee during the conversion of nectar into honey[5]. The concentrations of proline ranged between 202 and 680mg/kg. Some authors reported that high values of proline are typical for honeydew honeys. Proline content is a criterion of honey ripeness and in some cases, also of sugar adulteration. According to IHC proline content equals to or higher than 180.0mg/kg , the average values of proline mass fraction in analyzed honey sample ranged from 131.1 to 423.4 mg/kg **(Table 1)** i.e. within the range

Pollen analysis in honey

Pollen grains of 15 plant species belonging to 10 families were identified in 9 samples of honey from Nagpur region **(Table 2)**, the complete palynograph of the Unifloral and multifloral honey is given in **Plate 1**. Pollen spectra of honey revealed a variety of not only necteriferous but also necterless sources available to bees. The

Table 2 : Results of pollen analysis of *Apis cerana* honey

Samples	Date of collection	Type of honey	Predominant pollen (>45%)	Pollen types secondary pollen (16-45%)	Important minor pollen (3-15%)	Minor pollen (<3%)
Feb.	7/2/2009	Unifloral	*Brassica compestris L.* 71.40%	*Tridax procumbens L.* 28.50%	–	–
Mar.	3/3/2009	Unifloral	*Azadirachta indica L.* 57.14%	*Psidium guajava.L* 28.57%	*Prosophis julifera* (Sw.)DC 14.28%	–
Apr.cap	25/4/09	Unifloral	*Psidium guajava.L.* 53.24%	*Delonix regia(Bojer ex Hook.) Raf* 32.46%	*Bombax ceiba L* 5.19% *Poaceae type* 6.493%	*Cassia fistula* L.2.59%
Apr. uncap	25/4/09	Unifloral	*Psidium guajava.L* 100 %	–	–	–
Oct. cap	2/10/2009	Unifloral	*Alternanthera sp.* 47.50%	*Parthenium hysterophorus L.* 27.5% *Ocimum sanctum L.* 25%	–	–
Oct. uncap	2/10/2009	Unifloral	*Alternanthera sp.*100%	–	–	–
Nov.	13/11/09	Mutifloral	–	*Alternanthera sp.*37.7% *Parthenium . hysterophorus L.* 22.2%	–	–
Dec.cap	24/12/09	Unifloral	*Albizia lebbeck L.* 58.90%	*Poaceae* 23.07% *Chrysanthemum sp.*17.94%	–	–
Dec.uncap	24/12/09	Unifloral	*Albizia lebbeck L.*100%	–	–	–

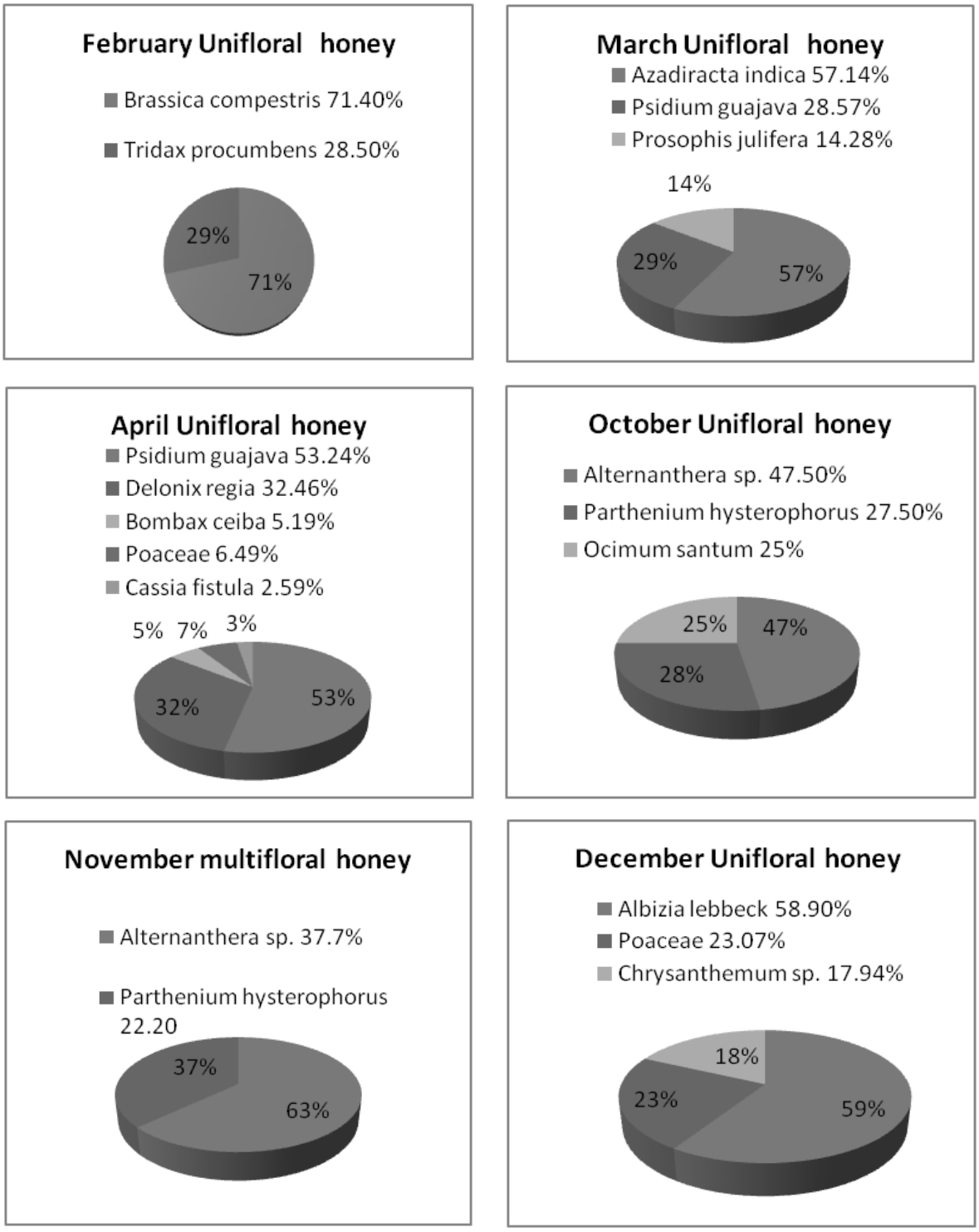

Plate 1 : Composite palynograph of Nagpur honey

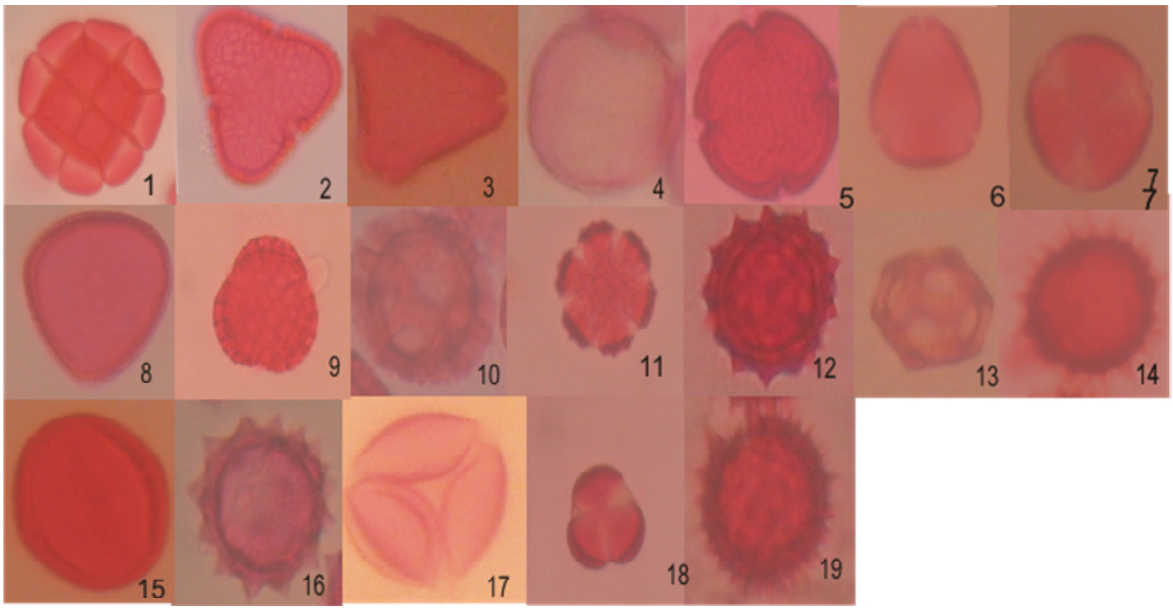

Plate 2 : Light microscopic photographs of pollen grains found in honey samples and pollen loads

1. *Albizia lebbeck L.* 2. *Bombax ceiba L.* 3. P*sidium guajava L.* 4. *Poaceae* 5. *Melia azadirachta L, Cassia fistula L* 7. *Prosophis juifera (Sw) DC* 8. unknown-2 9. *Delonix regia* (bojer ex Hook) *Raf* 10. *Asteraceae* 11. *Ocimum sanctum L.* 12. *Parthenium hysterophorus L.* 13. *Altermanthera sp.* 14. *Thespesia populnea* 15. *Azadiracta indica* 16. *Chrysanthemum sp.* 17. *Melintonia hortensis L.* 18. *Brassica camperstris L.* 19. *Tridax procumbens L.*

amount and diversity of pollen present in honey usually related to vegetation, climate and geographical location of bee hives. The pollen composition of the honey studied revealed important information on the flora of that region. Out of 9 samples 8 samples were unifloral and 1 sample was multifloral. In multifloral condition the pollen of *Parthenium hystophorus* L. and *Alternanthera sp*. represent the secondary pollen type. In unifloral honey *Brassica comprestris L., Azardiracta indica L*., *Psidium guajava L*., *Alternanthera sp*., *Albizia lebbeck* L. Are the Predominant pollen with the combination of the Secondary pollen that included, *Tridax procumbens* L., *Psidium guajava L*, *Poaceae*, *Chrysanthemum sp.* Important minor pollens were Prosopis julifera (Sw)DC., Bombax ceiba L., Poaceae, and *Cassia fistula L*. (**Plate 2**). It was observed that the pollen grain present in uncapped sample was very less or negligible and only one type of pollen was present. It may be possible that the bees added the pollen grain before capping to lower down the moisture content of the honey because pollen is very hygroscopic in nature or might be possible that the pollen had accidently fallen in it . Parthenium hysterophorus L. is the major secondary pollen found in honey as well as Pollen load, Suryanarayana et al., (1992), in their study at Muzaffarpur (Bihar) India reported that Parthenium hysterophorus L.provided forage for a major part of the year and other member served as forage sources only for few months The taxon endemic to North America and West Indies is a troublesome weed which was reported for the first time in Pune (Maharashtra) but is now widely spread,[1]. The pollen grain of the species is allergic and cause skin eruptions this indicates that pollen of this taxon are not toxic to the bees who visited them for the collection of nectar and pollen. It is rather curious that there is no dearth period for the plants during winter[6]. Parthenium is available throughout the year but bees collected during the month of October to November as clearly seen in the pollen study.

Pollen load

From the analysis of 14 pollen load from a single

Table 3 : Color and botanical source of pollen load of *Apis cerana indica*

Sample	Color	No. of pollen types in sample	Frequency of pollen types
Feb.	Yellow	2	*Brassica comprestis L.* 92 %, *Tridax procumbens L.* 8 %
Mar.	Red	5	*Albizia lebbeck L.* 7% , *Bombax ceiba L.* 45%, *Psidium guajava L.* 18.5 %, *Poaceae* 16%, *Melia azadirachta L.*13%
	Yellow	4	*Cassia fistula L.* 24.7 %, *Prosophis julifera* (Sw.)DC 13.04%, *Psidium guajava L.*30% , *Poaceae* 8.6%
Apr.	Yellow	1	*Typha angusta 100%*
	Orange	3	*Delonix regia* (Bojer ex Hook.) *Raf* 80 %, *Asteraceae* 10.9%, *Cassia fistula.*7.27%
	Brown	4	*Delonix regia* (Bojer ex Hook.) *Raf* 31.25 %, *Psidium guajava L.* 37.96 %, *Albizia lebbeck L.* 3.75 %.
Oct.	Brown	2	*Ocimum sanctum* 42 %, *parthenium hysterophorus* L.58 %.
	White	1	*Parthenium hysterophorus L.* 100 %.
	Brown	1	*Ocimum sanctum* 100 %.
Nov.	Light yellow	2	*Parthenium hysterophorus L.* 38.7 %. *Alternanthera* sp. 61.3 %
Dec.	Orange	2	*Thespesia populnea L.* 40 %, *Poaceae* 53.3 %.
	White	4	*Thespesia populnea* L.*4%,Alternanthera sp.13.3%., Poaceae 26.6%,*
	Yellow	3	*Poaceae* 73.17%, *Chrysanthemum sp.*20.73 % , *Albizia lebbeck L.* 4.87 %
	Grey	3	*Albizia lebbeck L.56 % , Poaceae 28.16 %* *Mellingtonia hortensis L.* 14.8 %

apiary in the different months showed that 11 pollen loads were Unifloral and 3 were Multifloral. Out of the 11 unifloral load 4 pollen loads were 100% Unifloral. A detailed account of number of pollen loads collected from each of the combs and their pollen content is given in **Table 3**. The analysis of pollen load showed that the taxa *Bombax ceiba L*, *Typha angusta*, *Delonix regia* (Bojer exHook) *Raf.*, *Parthenium hysterophorus L.*, *Ocimum sanctum L*, *Alternanthera sp, Poaceae, Albizia lebbeck L.,Brassica* comprestris *L.,Asteraceae species* represent the major pollen sources and *Melia azardiracta L.*, *cassia fistula L*, prosophis julifera (Sw)DC., *psidium guajava L.,Thesphesia populea L.*, *Mellintonia hortensis L.*, *Tridax procumbens L.* are secondary major pollen source for *A.cerana* **(Fig. 2)** in Nagpur region.

CONCLUSION

From **(Table 2** and **Table 3)** it is seen that the predominant pollen types more than 45 % in the honey was represented by *Brassica compestris L.*, *Azadiracta indica L.*, *Psidium guavaja L.*, *Alternanthera sp. Albizia lebbeck.L.*where as the minor pollen types was cassia fistula which is less than 3%.It is observed that the same genera are represented in the pollen loads.

While the divergence is that of *Melia azadiracta L.*, *Mellingtonia hortensis L.*, Thespesia

populnea L., and *Asteraceae* type which is present in the pollen load but is absent in the honey sample this might be because they are used as food material for the growth of brood and hence this pollen was not taken into the super chamber. The pollen grain of *Azadiracta indica L.*, was present in caped honey but was not found in the brood chamber which might be because that this pollen was used for honey storage, helps in reducing the moisture, or may be inhibitor for the microbial or bacterial growth.

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