SJIF Impact Factor: 5.464

WORLD JOURNAL OF ADVANCE HEALTHCARE RESEARCH

ISSN: 2457-0400
Volume: 5.
Issue: 3.
Page N. 503-512
Year: 2021

Original Article www.wjahr.com

PHYSICAL AND PALYNOLOGICAL STUDIES IN HONEY SAMPLES COLLECTED FROM UTTAR KANNADA AND SHIVAMOGGA DISTRICTS OF KARNATAKA, INDIA

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Received date: 16 April 2021 **Revised date:** 06 May 2021 **Accepted date:** 26 May 2021

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ABSTRACT

The physical and palynological studies were conducted in honey samples collected from Uttar Kannada (13^{0}55^1 02^{11} to 15^0 31^1 01^{11} N lat. and 74^{0}0^{1}35^{11} to 75^{0}10^1 23^{11}E long.) and Shivamogga (13^0 27^1 to 14^0 39^1 N lat. and 74^0 38^1 to 76^0 04^1 E long.) districts using various standard methods. Total 12 honey samples were randomly selected and analyzed for their colour, electrical conductivity and pollen grains composition. The honey colour was extra light amber (35-50 mm) to light amber (51-80 mm) and few honey samples showed light colour (18-34 mm) as per Pfund scale. The electric conductivity was ranged between 0.092 and 0.198 which was <0.8ms/cm as per Codex Standards. The pollen grains density, dominance index and pollen types belonged to 33 plant species in 23 families and didn't indicate significant variation (F=0.421; P<0.05) between honey samples. The Shannon diversity Index was ranged between 2.208 and 1.753; Fisher alpha value ranged between 4.839 and 2.831 and suggested a variation existed between the different pollen grains. Moreover, Simpson and Shannon 'J' (Equitability) indices revealed that distribution of majority of pollen grains within the honey samples collected from different places in Uttar Kannada District was 0.818 to 0.745 and 0.764 to 0.635 in the honey samples of Shivamogga District, suggested unevenness. Further, the Sorenson's (ß diversity) index ranged between 0.8235 and 0.9473 and confirmed the considerable difference existed among the corbicular pollen grains in different honey samples. The present findings provided preliminary evidences to suggest that there is a considerable specificity existed between natural honeys with specific corbicular pollen grains, which were from different plant sources amidst diversified ecosystems. Although, pollen grains composition is specific, but revealed the floral source and suggested the status of natural honey.

KEYWORDS: Honey colour, electrical conductivity, pollen analysis, Karnataka.

INTRODUCTION

Honey is a sweet substance produced by honeybees from the nectar of blossoms or from secretions of flowers of different living plants, which the honeybees collect, transforms and store in honey combs.[1] It is one of the nutritive food sources to the adult honeybees and the developing brood in their hive. The honey contain macro and micro nutrients, sugars (e.g. fructose and glucose), proteins, amino acids, phenolic compounds, vitamins, pollen grains, pigments, enzymes, essential oils and acids.[2 -11] More than 300 different honey types are recorded at various parts of the world. These honey types are known for their specific colour, flavour, carbohydrate, protein, mineral and vitamin contents and obviously, exhibit different physical and chemical properties.[12-14] Surprisingly, the floral source and pollen content also varies considerably.[15] All these constituents are known for their high nutritive value, bacterio-static, anti-inflammatory and anti-microbial properties, natural antioxidants, which are effective in reducing the risk of heart diseases, cancer, immune system deficiency, cataracts, different inflammatory processes and various health related issues in human beings.[1] Moreover, honey is useful for wound and sunburn healing effects in human beings.[4] Hence, there is a more demand for naturally occurring honey, which is consumed by many people around the world.[1, 16-17]

In India there is a wide scope for the production of different types of honey under wild and domesticated conditions[18] from *Apis dorsata*, *A. laboriosa*, *A. florea* natural colonies and *A. cerana*, *A. mellifera*, *Trigona*, *Tetragona* and *Melipona* species colonies under domesticated beekeeping activities at different apiaries.[19-20] The harvested honey is sold in the market for consumers with different local names such as Coorg honey, B.R. Hills honey, Puttur honey, Honnavar honey,

Malnad honey, Niligiris honey, Kashmir honey, Himalayan honey etc.[21] Furthermore, honey is marketed by prefixing with the plant name as Lychee (honey from *Litchi chinensis*), Clover (honey from *Trifolium hybridum*), Jamun (honey from *Syzygium cumini*) Nilgiri (honey from *Eucalyptus* spp.) and Elachi (honey from *Elettaria cardamomum*) honey from different parts of southern Karnataka.[19-23] have analyzed the unifloral, multifloral honey collectd from different geographical regions in southern Karnataka. Although, honey is marketed with different nomenclature, however published scientific information on physical property and phylonological studies are scanty in this part of the state.

Surprisingly certain honeys (Example: Unifloral honeys like Manuka, Tualang, Jujube and *Acacia*) are easily adulterated.[24] Hence, knowledge of palynological details is necessary for the apiary industry to assure quality honey production. The quality of honey is determined by conducting physico-chemical properties, elemental and phenolic acid composition, pigments, proteins, amino acids, enzymes, sugar profile, rheological, antioxidant, medicinal properties, cellular protective effects, antibacterial characteristics.[2-11, 19-22, 25-38] Because, honey is adulterated with sugar syrup, jaggary, antibiotics and other chemicals[23-24] and such honey is rarely contains pollen grains. In this regard, only few published reports are available on palyonological study i.e., pollen analysis in honey.[15] has conducted the pollen analysis in New Zealand honey.[39-45] have made investigations on the palynological observations in the honey samples collected from different parts in India and other parts of the world.

All these investigations revealed the importance of pollen analysis to have honey with natural ingredients, collected from different plant sources amidst diversified ecosystems.[19-20] Few published reports are available from the honey samples collected from the commercial market centres at different ecosystems of Karnataka. In most of the cases, honey is contaminated or adulterated with many additives[23-24] and affects the consumers. Hence, literature on pollen analysis is quite diffuse. Published reports on how pollen would discriminate the contaminated or uncontaminated honey due to the presence or absence of foreign matter or on the basis of different types of pollen analysis is not clear. Moreover, reports on pollen analysis in honey samples of Uttar Kannada and Shivamogga Districts in Karnataka are fragmentary. Hence, the present investigation was necessitated.

MATERIALS AND METHODS

Study Area: The Uttar Kannada District ($13^{0}55^{1}$ 02^{11} to 15^{0} 31^{1} 01^{11} N latitude and $74^{0}0^{1}35^{11}$ to $75^{0}10^{1}$ 23^{11}E longitude) has tropical, evergreen and semi evergreen forests with coastal area. The District is known for its rich floral diversity that helped many people to conduct beekeeping activity using *A. cerana* and *A. mellifera* species throughout the year. While, Shivamogga District (13^{0} 27^{1} to 14^{0} 39^{1} N latitude to 74^{0} 38^{1} to 76^{0} 04^{1} E longitude) is the gate way to 'Malnad' has tropical forests, pretty hilly areas with good floral source. Many people are practising beekeeping using *Apis cerna* and *A. mellifera* species during different seasons.[46]

Methodology: Altogether 12 honey samples were collected randomly from different places in Uttar Kannada and Shivamogga Districts of Karnataka by following standard methods. The collected honey samples were brought to the Laboratory and stored in air tight vials as per.[47] Before, pollen analysis, the physical properties such as colour and electrical conductivity was conducted as per.[18] The honey colour was determined by simple method using standard colour strip. Then, the colour of honey was confirmed by using spectrophotometer at 560 nm by taking glycerine as a blank. The value was recorded and colour was determined by comparing with Pfund scale as per.[48] The electrical conductivity was measured by using conductivity meter. Ten gram of honey was weighed using standard Dhona analytical balance and transferred into the 50 ml volumetric flask and made up to the mark using distilled water. The conductivity meter was calibrated using 0.01N KCl solution, then the readings were taken in milli Siemens as soon as the conductivity stabilised as per.[18]

Pollen Analysis: Pollen was analysed in different honey samples by using acetolysis method as per[49-50] and pollen grains were counted using haemocytometer. Ten gram honey was weighed and dissolved in 10 ml distilled water and centrifuged at 3000 rpm for 10 minutes and the supernatant was discarded. Then one gram sediment was mixed with 5ml glacial acetic acid and allowed for two minutes. From this, one gram (8 drops) sample was taken and mixed with four ml (100 drops) double distilled water and stored at 1% Safranine stain mixed with glycerine jelly and it is used as standard for pollen count. A drop of standard sample was taken using pasture pipette and transferred on to the sterilized haemocytometer and observed under microscope. The pollen grains were recorded from all the nine chambers as per,[45] and sum of all the pollen grains were multiplied by 100, where 100 is the dilution factor for one gram honey sediment after slight modification.[15] The pollen shape, pollen size and pollen aperture was recorded as per[49] and the aberrant pollen grains were ignored from the count, as they were in small percentage. Observed pollen grains data was multiplied by 100 to reveal the analytical standards as per[15] after slight modification.

Statistical Analysis: The collected data was complied by following standard methods using arithmetic mean and per cent calculation. Analysis of variance (ANOVA) was used as per.[50] The pollen plants diversity based on the pollen grains found in the honey samples was calculated by using PAST version 2.10. The £ diversity of pollen plants was calculated by using Shannon Diversity Index

(H^1) that combines the number of pollen plant species within Uttar Kannada and Shivamogga Districts with the relative abundance of each pollen plant species as per.[51] The Shannon Diversity Index (H^1): $H^1 = -\Sigma(pi\ In\ pi)$, where, pi is the proportion of the i^{th} species in the total sample and in pi is the natural log of pi. Moreover, the pollen plant species richness in different Districts and their evenness in abundance are the two parameters that define 'H'. The evenness of plant species within two Districts was calculated by using Pielov's Evenness Index (J^1) to identify the variation within the Districts among the species. Pielovu's Evenness Index: $J^1 = H^1/In\ S$, where, S is the number of pollen plant species present in the study site and H^1 is the diversity index.

RESULTS

Colour of honey: The honey samples from Uttar Kannada and Shivamogga Districts showed extra light amber (35-50 mm) to light amber (51-80 mm) colour. Moreover, few honey samples showed light colour (18-34mm) and it was compared with the honey colour chart as per Carolinahoneybees.com (Table 1).

Electrical Conductivity: Table 2 show the electrical conductivity of different honey samples collected from Uttar Kannada and Shivamogga Districts. The honey samples had the electric conductivity ranged between 0.092 and 0.198 which lie within the Codex Alimentations Standards.

Pollen grains: On average 32,033 pollen grains with a range between 26,900 and 39,500 obtained in 10 g honeys collected from Uttar Kannada District. However, in the honey samples collected from Shivamogga District had an average 47,666 with a range between 41,100 and 52,100 pollen grains (Table 3). Table 4 show the analysis of variance of pollen grains in honey samples collected from different places in Uttar Kannada District. For every 1 g of honey, 395 pollen grains were observed in sample 1 and it was followed by sample 3 (297) and sample 2 (269). However, the analysis of variance between pollen grains in different honey samples didn't indicate significant variation ($F=1.223$; $P<0.05$) (Table 4). Similarly, the analysis of variance of pollen grains in different honey samples collected from different places in Shivamogga District is depicted in Table 4. For every 1 g of honey, 521 pollen grains were observed in sample 2 and it was followed by sample 3 (498) and sample 1 (411). However, the analysis of variance between the pollen grains in different honey samples didn't indicate significant variation ($F=0.421$; $P<0.05$) (Table 4). Further, collected data of pollen grains in all the honey samples were subjected to analysis of variance to find out if any difference existed between and within the pollen grains density in different honey samples of Uttar Kannada and Shivamogga Districts and the results indicated no significant difference ($F=0.617$; $P<0.05$) between the Districts (Table 4).

Diversity indices of pollen grains in honey samples: Table 5 shows the pollen grains diversity index in different honey samples collected from Uttar Kannada District. The pollen grains dominance index ('D') was high (0.254) in sample 1 and it was followed by sample 2 (0.244). However, sample 3 indicated lowest dominance index (0.181) compared to samples 1 and 2 (Table 5). Further, the Shannon Index ('H') and Sorenson's Index were calculated as diversity indices, which incorporated the different pollen grains richness and abundance into a single value. The Shannon index 'H' value ranged between 2.208 and 1.753 and Fisher alpha value ranged between 4.839 and 2.831 and suggested a variation existed between the different pollen grains diversity indices. Moreover, Simpson and Shannon 'J' (Equitability) indices revealed that distribution of majority of pollen grains within the honey samples collected from different places in Uttar Kannada District was 0.818 to 0.745 and 0.764 to 0.635 and suggested unevenness between these honey samples (Table 5). Further, the Sorenson's (ß diversity) index values indicated considerable variation existed between the honey samples and the values ranged between 0.8235 and 0.9473 and confirmed the considerable difference existed among the pollen grains in different honey samples collected from various places in Uttar Kannada District (Table 6). Further, Table 5 show the pollen grains diversity index in different honey samples collected from various places in Shivamogga District. The pollen grains dominance index ('D') was high (0.852) in sample 3 and it was followed by sample 2 (0.346). However, sample 1 has indicated lowest dominance index (0.339) compared to samples 2 and 3 (Table 5). Further, the Shannon Index ('H') and Sorenson's Index were calculated as diversity indices, which incorporated the different pollen grains richness and abundance into a single value. The Shannon index 'H' value ranged between 0.367 and 1.623 and Fisher alpha value ranged between 4.127 and 1.560 and suggested a variation existed between the different pollen grains diversity indices. Moreover, Simpson and Shannon 'J' (Equitability) indices revealed that the distribution of majority of pollen grains species within the honey samples was 0.660 to 0.147 and 0.541 to 0.167 suggested unevenness existed in the pollen grains between the honey samples (Table 5). Further, the Sorenson's (β diversity) index values indicated considerable variation existed between the three honey samples with the values ranging between 0.888 and 0.620 and confirmed the considerable difference between the honeys samples collected from different places in Shivamogga District (Table 6).

Pollen plants: Altogether, 33 plant species which belong to 23 families were recorded based on the pollen grains identified in honey samples of Uttar Kannada and Shivamogga Districts (Table 7). Only, three plant species namely *Arabidopsis* sp. (Family: Brassicaceae), *Psidium guajava* and *Eucalyptus* sp. (Family: Myrtaceae) were common in the honey samples of both Uttar Kannada

and Shivamogga Districts. Interestingly, honey from Uttar Kannada District had 22 plant species pollen grains and in Shivamoga District honey, only 14 plant species pollen grains were recorded. Further, the common name, scientific name and plant family are depicted in Table 7.

Table 1: Colour of honey samples.*

Honey sample	Uttar Kannada District (in mm)	Shivamogga District (in mm)
1.	18-34	18-34
2.	35—50	35—50
3.	51-80	51-80

*Values are as per honey chart of Californiahoney.com

Table 2: Electrical conductivity of honey samples.*

Sl. No.	Honey samples					
	Uttar Kannada			Shivamogga		
	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3
1.	0.096	0.112	0.119	0.115	0.205	0.194
2.	0.095	0.106	0.117	0.113	0.200	0.195
3.	0.093	0.101	0.116	0.111	0.197	0.189
4.	0.091	0.100	0.116	0.111	0.196	0.190
5.	0.089	0.100	0.115	0.110	0.196	0.189
Mean	0.092	0.103	0.116	0.112	0.198	0.191

*Values are in Millie Siemens Units. Each value is a mean of two observations.

Table 3: Pollen grains obtained in honey samples.*

Sample	Uttar Kannada	Shivamogga
1.	39.500	41,100
2.	26,900	52,100
3.	29.700	49,800
Mean	32,033	47,666

*Values are as per analytical standards of Moar (1985). Each value is a mean of two observations.

Table 4: Analysis of variance of honey samples.

Sl. No.	Uttar Kannada			Shivamogga		
	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3
1.	65	92	91	158	40	3
2.	13	59	111	171	59	27
3.	7	15	7	55	293	459
4.	35	6	4	8	38	1
5.	171	9	5	1	5	2
6.	68	5	21	1	36	1
7.	3	15	2	1	2	2
8.	12	9	15	1	1	1
9.	3	15	9	2	8	2
10.	2	8	4	2	2	-
11.	4	8	2	5	8	-
12.	5	6	2	1	3	-
13.	4	4	2	1	15	-
14.	3	4	5	2	2	-
15.	-	2	1	1	1	-
16.	-	2	6	1	1	-
17.	-	6	3	-	3	-
18.	-	4	5	-	1	-
19.	-	-	1	-	2	-
20.	-	-	1	-	1	-
Total	395	269	297	411	521	498
'F' value	1.2235*			0.421*		
	0.6172*					

Note: *Values are not significant.

Table 5: Pollen grains diversity index in different honey samples.

Sl. No.	Diversity Indices	Uttar Kannada			Shivamogga		
		Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3
1.	Dominance 'D'	0.254	0.181	0.244	0.339	0.346	0.852
2.	Shannon 'H'	1.753	2.208	1.902	1.327	1.623	0.367
3.	Simpson 1_D	0.745	0.819	0.755	0.660	0.653	0.147
4.	Equitability _J	0.664	0.764	0.635	0.478	0.541	0.167
5.	Fisher_ alpha	2.831	4.346	4.839	3.314	4.127	1.560

Note: Data is based on Table 4.

Table 6: Beta diversity indices of pollen grains in different sampling areas.

Uttar Kannada				Shivamogga			
Sample	**1**	**2**	**3**	**Sample**	**1**	**2**	**3**
1	-	-	-	1	-	-	-
2	0.875	-	-	2	0.888	-	-
3	0.823	0.947	-	3	0.720	0620	-

Note: Data is based on Tables 3 & 4.

Table 7: Pollen plants identified based on pollen grains in honey samples

Sl. No.	Family	Sl. No.	Common Name	Scientific Name	Uttar Kannada	Shivamogga
1.	Acantheceae	1.	Black-eyed Susan vine	*Thunbergia alata*	-	+
2.	Amaranthaceae	2.	Careless weed or Palmer amaranth	*Palmer amaranthus*	+	-
3.	Asteraceae	3.	Beauty head	*Baltimora* sp.	+	-
4.	Apocynaceae	4.	Blackboard tree or Devil's tree	*Alstonia scholaris*	+	-
5.	Balsaminaceae	5.	Garden balsam	*Impatiens balsamina*	-	+
6.	Brassicaceae	6.	Mouse-ear cress	*Arabidopsis* sp.	+	+
7.	Bromiliaceae	7.	Vase plant	*Aechmea* sp.	+	-
8.	Combrtaceae	8.	Kwandari	*Terminalia macroptra*	+	-
9.	Caprifoliaceae	9.	Adusoge	*Adatoda zeylanica*	-	+
10.	Fabaceae	10.	Cassia	*Senna alata*	-	+
		11.	Golden shower tree	*Cassia fistula*	-	+
		12.	-	*Polyphylla* sp.	+	-
		13.	Heartwood plant	*Lonchocarpus* sp.	-	+
		14.	Japanese pagoda tree	*Sophora japonica*	+	-
		15.	Narra plant	*Pterocorpus* sp.	-	+
		16.	Tamarind Tree	*Tamarindus indica*	-	+
11.	Lamiaceae	17.	Spur-flower plant	*Plectranthus* sp.	+	-
12.	Myrtaceae	18.	Bayberry plant	*Myrica* sp.	+	-
		19.	Common Guava	*Psidium guajava*	+	+
		20.	Niligiri tree	*Eucalyptus* sp.	+	+
13.	Marsiliaceae	21.	Water clover or Four-leaf clover	*Marselia aegyptica*	+	-
14.	Malaceae	22.	Cheese weed	*Malva parviflora*	+	-
		23.	Sleepy morning	*Waltheria indica*	+	-
15.	Malpighiaceae	24.	Peanut butter fruit	*Bunchosia cordifolia*	+	-
16.	Phyllanthaceae	25.	-	*Phyllanthus* sp.	-	+
17.	Poaceae	26.	Wild grass	*Streptochaeta spicata*	+	-
		27.	Maize plant	*Zea mays*	+	-
18.	Polygonaceae	28.	Black wheat	*Fagopyrum* sp.	.-	+
19.	Rosaceae	29.	Almond plant	*Prunus* sp.	+	-
20.	Scropulariaceae	30.	Mullein	*Verbascum* sp.	+	-
21.	Solanaceae	31.	Pepper plant	*Capcicum parvifolium*	+	-
22.	Verbenaceae	32.	Lantana	*Lantana camara*	-	+
23.	Vitaceae	33.	Grape vine	*Vitis* sp.	+	-

Note: + : Present; - : Absent.

DISCUSSION

Honey is a sweet substances produced by honeybees, considered as one of the international commodities and used as food by man around the world. Regular analysis of honey, which is available at different geographical regions, is necessary to assure the quality and meet the consumers demand around the world.[1] Therefore, various physico-chemical and biological methods are essential to assess regularly the quality and contamination in honey.[39-45, 47, 53-61] During the present investigation, physical properties such as colour and electrical conductivity and biological method such as pollen analysis revealed the natural state of honey which was devoid of any foreign matter. The extra light amber (35-50 mm) to light amber (51-80 mm) colour was recorded in honey samples collected from Uttar Kannada and Shivamogga Districts. Moreover, few honey samples had light colour (18-34mm) and it was compared with the honey colour chart as per.[48] The honey can range anywhere from nearly colourless to dark brown and show different readings in millimeters which is in correspondence with the different colours as per Pfund Scale. In honey literature, distinction has been made between natural honey and synthetic or adulterated honey. Introspectively, pollen content of honey is translated into mathematical terms, using colour constants in terms of approximate wave lengths assigned to practically all hues. Using a standard colour chart, anther and corbicular pollen type was identified by colour-matching in honey.[48] Thus, different colour in honey was due to anther and corbicular pollen[62] and hence recording the colour of honey is one of the important factors revealing the different types of anther and corbicular pollen[48] in honey. Further, the honey samples of Uttar Kannada and Shivamogga Districts had the electric conductivity ranged between 0.092 and 0.198 which was within <0.8ms/cm as per Codex Standards.[1] Since, electrical conductivity is one of the most important factors, determine the physical characteristics of the honey, which was closely related to the concentration of mineral salts, organic acids, proteins/amino acids[2] and it is used as a good criterion for determining the botanical origin of honey as well.[13-14,63] Thus, during the present investigation, electrical conductivity was considered to show indirectly the source of proteins/amino acids which could get into honey via pollen in the honey and it was almost nearer to the Codex Standards.[1]

The pollen grains in different honey samples collected from both Uttar Kannada and Shivamogga Districts indicated not much difference. This shows the good content of pollen grains which were ranged in between 395 and 269 per every one gram honey in Uttar Kannada District. In Shivamogga District, the pollen grains were ranged between 521 and 411 per every one gram honey. Hence, the honey samples collected from Uttar Kannada and Shivamogga Districts indicated good source of pollen grains and didn't indicate significant differences. Thus, in both Districts, the collected honey samples had rich source of carbicular pollen and didn't contain any foreign matter. However, statistical analysis of the pollen grains dominance index ('D') was not even within and between the honey samples. It was high (0.254) in sample 1, little less in sample 2 (0.244) and very less in sample 3 (0.181) of honey collected from Uttar Kannada District. But, in Shivamogga District the pollen grains dominance index ('D') was high (0.852) in sample 3, less (0.339) in sample 1 and very less in sample 2 (0.346). Further, the Shannon diversity index (H) was <1 and indicated the more unevenness of pollen grains among the honey samples in both Uttar Kannada and Shivamogga Districts. Moreover, the Sorenson's similarity coefficient values in honey samples collected from Uttar Kannada District was ranged between 0.8235 and 0.947, and in the honey samples of Shivamogga District, it was ranged between 0.888 and 0.6220 and indicated dissimilarity of specific plant species pollen present in the honey samples. Thus, the different carbicular pollen grains abundance was uneven among different honey samples as elucidated by the Shannon and Sorenson's Index and incorporated the different pollen grains abundance into a similar status. Further, the Fisher alpha value ranged between 4.839 and 2.831 in Uttar Kannada District, and 4.127 and 1.560 in Shivamogga District that has suggested a considerable variation existed between the different pollen grains diversity indices. Furthermore, Simpson and Shannon 'J' (Equitability) indices was <1 and confirmed the considerable unevenness of pollen grains within honey samples collected from different places in Uttar Kannada and Shivamogga Districts. However, in synthetic or adulterated honey the pollen content would be less and accordingly the honey differs in colour from its foreign matter.[62] Because honeybees collect pollen as a protein source to raise their brood and to provide protein/amino acids source to queen from their forage from a range of different plant species.[64] Naturally, little quantity of corbicular pollen get into honey and that becomes a good predictor for a plant by which honeybees collected pollen and it could help calculate the pollen producing plants availability during different days in a year.[13-14, 65] Hence, pollen analysis in a honey samples authenticate the natural or synthetic or adulterated honey. Unfortunately, pollen literature ignores to a great extent the important factor of colour in honey.[62] All these observations clearly demonstrate the diversity of pollen grains in the natural honey. Thus, the pollen analysis in honey could help reveal the origin of honey in terms of locality, floral source and this information could be used to develop analytical standards for pollen to authenticate the quality of honey for export or indigenous use.[15] If honey to be graded within the normal category, it should have 20,000 to 1,00,000 pollen grains in 10 g honey sample (e.g. Lycopodium honey, Clover honey). Similarly, if pollen grains exceeds > 1,00,000, it should be graded as above normal category (e.g. Manuka honey) and the pollen grains count is <20,000, it is graded as below normal category (e.g. Thyme honey).[15] In the

present study, pollen grains count was in the range between 32,033 and 47, 666 per 10 g honey which was within the normal category. Thus, pollen composition assures quality and authenticate that the honey is natural. Thus, based on pollen grains present in honey samples, it is possible to identify the pollen plants also. It was evidenced by observing total 32 different types of pollen grains, which belong to 22 plant species and 18 families in Uttar Kannada District. Similarly, in Shivamogga District, the honey samples revealed 14 different types of pollen grains which belong to 14 plant species and nine families. Obviously, the present study facilitates us to understand the pollen grains which belong to specific plant species and becomes a part of honey. While collecting pollen, honeybees visit different plant species and based on the diversity of pollens it enables to assess the potentiality of natural honey production. Since, honeybees are primary pollinators of the floral world, play a crucial role both for wild and cultivated plant species, especially in the tropics where insect pollination is vital.[58] People living at various ecosystems are fully or partial depended on the flora to produce honey by using honeybees in the wild as well as under domesticated conditions. Because, honeybees collect nectar and pollen from different plant species as their main source of diet, while doing so they transform and produce honey. Pollen is a protein/or amino acid source for honeybees present abundantly in natural honey. So, pollen grains present in the honey and their analysis help understand the foraging source and enable to distinguish natural or synthetic or adulterated honey.[15] Therefore, by conducting pollen analysis in the honey samples collected from commercial centres, it is possible to reveal or verify honey authenticity.[45] Thus, pollen analysis is vital to maintain the natural quality in the honey[8,15, 40-41, 45, 52-61, 66-68] All these investigations revealed the importance of pollen analysis to have honey with natural ingredients, collected from different plant sources amidst diversified ecosystems. The present findings could provide preliminary evidences to suggest that there is considerable differences exist between natural honey and the synthetic or adulterated honey due to presence or absence of different types pollen composition. Our observations are corroborating the observations of.[13-15, 19-22, 61, 62-63, 65, 69-70] However, we afraid to reveal the details of different types of pollen grains morphology in the present paper, which is not in the purview of the present study and such details will be published elsewhere.

CONCLUSION

More the different types of pollen grains, honey would be multifloral and rich with its constituents. The abundance of different pollen grains in the honey samples would help authenticate the natural honey. To confirm the assured quality, adulteration tests are necessitated. However, our aim is to show the naturally occurring honey, which is rich with different types of anther and corbicualr pollen grains used as source of proteins/amino acids to adult honeybees and developing brood as well. However, in synthetic or adulterated honey, anther and corbicular pollen grains would be sparse and mayn't be with different types of corbicular pollen grains. Thus, pollen grains analysis is not only reveal the floral source, but it also suggest the status of natural honey.

ACKNOWLEDGEMENT

Authors are thankful to the beekeepers of Uttar Kannada and Shivamogga Districts, who have provided the honey samples to conduct the present study. Thanks are also due to the Chairperson, DOS in Zoology for the facilities. Some part of the work is benefited from the Laboraory/PSFS grants sanctioned to DOS in Zoology, University of Mysore, Mysore.

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