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QUANTITATIVE POLLEN ANALYSIS OF NATURAL AND COMMERCIAL BRANDS OF HONEY

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ABSTRACT

Honey is a sweet, viscous liquid produced by honeybees. It is a very important food item referred by the physicians for the treatment of male infertility because honey contains fructose which is the ready source of food for spermatozoa. Pollen of the honey makes it incredibly healthy because pollen contains many vitamins, minerals and antioxidants which play an important role to decrease inflammation, as well as to improve immunity, menopausal symptoms and wound healing. The present study was conducted to analyze the commercial honey for pollen count. The honey samples of ten different brand were collected from the supper shops of Dhaka, Bangladesh and the natural honey samples from honeycomb were collected from different areas of Bangladesh. The widely accepted technique, microscopic analysis, was used to quantify the pollen of commercial honey and natural honey. In case of commercial honey, the results showed that 10% of the honey samples were very poor in pollen grains contents (< 2000 pollen grains / gm of honey), 30% of the samples were poor in their content of pollen grains (2000 - 10000 pollen grains /g honey), 20% of the samples were rich in their content of pollen grains (10,000 - 50,000 pollen grains /g honey), 30% of the samples were more rich in their content of pollen grains (50,000 - 100,000 pollen grains /g honey) and 10 % of the samples were very rich in their content of pollen grains (>100000 pollen grains /g honey). In case of natural honey, the results showed that 10% of the honey samples were more rich (50,000 - 100,000 pollen grains /g honey) and 90% were very rich in their content of pollen grains (>100000 pollen grains /g honey). Attention is required about pollen grains counts of commercial honey because without pollen grains, honey is nothing but a sugar syrup.

KEYWORD: Pollen, commercial honey, natural honey.

INTRODUCTION

Honey is a delicious food to the people of all ages. This sweet nectar is collected by honeybee (*Apis mellifera*) from flowers of plants to their comb, So numerous pollen grains are present in natural honey. Bee pollen has been a part of the human diet due to its high nutritional value and consists of approximately 40% carbohydrates, 35% proteins, 4 - 10% water, 5% lipids, and 5 - 15% other substances, such as amino acids, vitamins, minerals, antibiotics and antioxidant substances (Morgano et al., 2011). Bee pollen is loaded with a wide variety of antioxidants including flavonoids, carotenoids, quercetin, kaempferol and glutathione (Denisow & Denisow-Pietrzyk, 2016). Antioxidants in bee pollen may protect lipids from oxidizing so that it can prevent clumping of lipids together, smoothing blood vessels and lowering the risk of heart disease (Rzepecka-Stojko et al., 2018). Bee pollen may lower heart disease risk factors. A previous study showed that bee pollen extracts can lower blood cholesterol levels, especially "bad" LDL cholesterol in animal (Rzepecka-Stojko et al., 2017). Pollen of honey results in the reduction of the severity of allergies and enhancing immunity significantly by reducing activation of must cell (Ishikawa et al., 2018). The numerous benefits of honey pollen make honey worldwide favourite for maintaining good health. Many types of commercial honey are available in the market. The global honey market size was valued at USD 8.4 billion in 2018 and USD 9.79 billion in 2020 (Honey Market Size, Share, Trends: Global Industry Report,

2019-2025., 2019). In this study we performed quantitative pollen analysis by microscopic investigation to determine the number of pollen grains in each gram of honey samples to evaluate the quality of commercially available honey in Dhaka city and natural honey in different districts of Bangladesh. Honey pollen analysed by microscopic method is widely accepted by many scientists (Oddo and Piro, 2004). The method provides essential information about the hygienic aspects of bee honey production, its contamination with mineral dust, soot, and starch particles (Louveaux et al., 1978). The method described by Hassanien et al., (2018) was used to determine the number of pollen grains in each gram of honey in all collected samples to evaluate their quality.

Pollen Count Method

According to Hassanien et al. (2018) ten grams (10 g) of each honey sample was weighed in a graduated cylinder. Distilled water was added to a total volume of 20 cm^3.The collected honey solution was divided into two aliquots of 10 cm^3 in graduated centrifuge tubes. The tubes were centrifuged at 2500 rpm for 10 minutes. With an automated pipette, 9 cm^3 of the supernatant were discarded from each tube. The remaining amount of 1 cm^3 in each of the two tubes, 5 cm^3 distilled water was added to a total volume of 6 cm^3. The two tubes were centrifuged at 2500 rpm for 10 minutes. With an automated pipette, 5 cm^3 of the supernatant were discarded from each tube. From the remaining 1 cm^3 honey solution, after a thorough mixing with a glass rod, 0.1 mm^3 (0.1 µl) samples were taken with automated pipettes from the bottom of each tube and placed to the Improved Neubauer Counting Chamber.

Using 10x objective lens of a light microscope the two grids of the chamber were brought into focus and all pollen grains within them were counted using objective lens 40x including those grains within each medium square and those that were over the top and right sides of the square (even when they were partially out).

Calculation of Pollen Count: The number of pollen grains in 1 g honey is calculated by the equation:

$$X = A \times 5000$$

Where:

- X = Number of pollen grains in 1 g honey;
- A = Arithmetic mean of pollen grains counted in the two grids of the chamber (= (N1+ N2) /2).
- 5000 is the coefficient for calculation of sample volume.

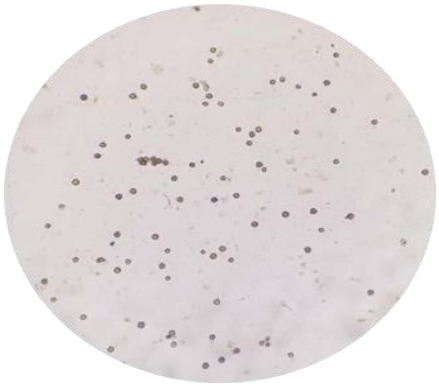

Fig. 1: Pollen under 10x of microscope

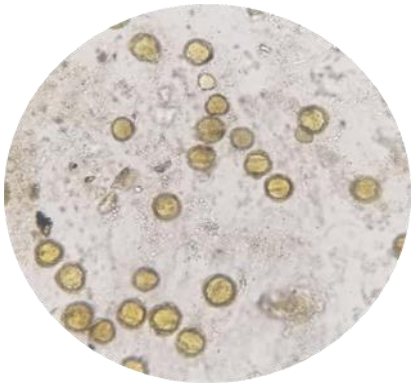

Fig. 2: Pollen at 40x magnification of microscope

RESULTS AND DISCUSSION

The results of counting pollen grains in commercial honey are presented in table 1. Data of table 1 contain pollen count of 10 samples at the beginning, 30 days, 60 days, 90 days and 120 days. During first analysis CHB_1 sample's pollen count was 15000 per gm of honey, while during 2nd, 3rd, 4th and 5th analysis CHB_1 showed 22500, 18500, 16000 and 17500 respectively. The average pollen count was 17900±2902.58 per gm of honey sample of CHB_1. During first analysis CHB_2 sample's pollen count was 60000 per gm of honey and during 2nd, 3rd, 4th and 5th analysis CHB_2 showed 45000, 55000, 50000 and 48000 respectively. The average pollen count was 51600±5941.38 per gm of honey sample of CHB_2. During first analysis CHB_3 sample's pollen count was 10000 per gm of honey whereas during 2nd, 3rd, 4th and 5th analysis CHB_3 showed 5000, 6000, 6500 and 4500 respectively. The average pollen count was 6400±2162.17 per gm of honey sample of CHB_3. During first analysis CHB_4 sample's pollen count was 56000 per gm of honey and during 2nd, 3rd, 4th and 5th analysis CHB_4 showed 58000, 49000, 48000 and 47000 respectively. The average pollen count was 51600±5029.91 per gm of honey sample of CHB_4. During first analysis CHB_5 sample's pollen count was 70000 per gm of honey and during 2^{nd}, 3^{rd}, 4^{th} and 5^{th} analysis CHB_5 showed 69000, 66000, 54000 and 47000 respectively. The average pollen count was 63400±7056.91 per gm of honey sample of CHB_5. During first analysis CHB_6 sample's pollen count was 7500 per gm of honey and during 2^{nd}, 3^{rd}, 4^{th} and 5^{th} analysis CHB_6 showed 10000, 8000, 7500 and 7000 respectively. The average pollen count was 8000±1172.60 per gm of honey sample of CHB_6. During first to 5^{th} analysis CHB_7 showed absence of pollen grains. During first analysis CHB_8 sample's pollen count was 25000 per gm of honey and during 2^{nd}, 3^{rd}, 4^{th} and 5^{th} analysis CHB_8 showed 17500, 19000, 18500 and 20000 respectively. The average pollen count was 20000±2936.83 per gm of honey sample of CHB_8.

During first analysis CHB_9 sample's pollen count was 138000 per gm of honey and during 2nd, 3rd, 4th and 5th analysis CHB_9 showed 145000, 134000, 147000 and 138000 respectively. The average pollen count was 140400±5412.94 per gm of honey sample of CHB_9.

During first analysis CHB_10 sample's pollen count was 5000 per gm of honey and during 2nd, 3rd, 4th and 5th analysis CHB_10 showed 10000, 6500, 8000 and 7000 respectively. The average pollen count was 7300±1857.41 per gm of honey sample of CHB_10.

Table 1: Number of pollen grains / gram of commercial honey samples collected from different super shops of Dhaka city, Bangladesh 2019.

Code of Brands	Pollen Count/gm of commercial honey					
	1st (At 0 Day)	2nd (After 30 Days)	3rd (After 60 Days)	4th (After 90 Days)	5th (After 120 Days)	Mean±SD
	01/11/2019	02/12/2019	3/01//2020	04/02/2020	05/03/2020	
CHB_1	15000	22500	18500	16000	17500	17900±2902
CHB_2	60000	45000	55000	50000	48000	51600±5941
CHB_3	10000	5000	6000	6500	4500	6400±2162
CHB_4	56000	58000	49000	48000	47000	51600±5029
CHB_5	70000	69000	66000	54000	58000	63400±7056
CHB_6	7500	10000	8000	7500	7000	8000±1172
CHB_7	0	0	0	0	0	0
CHB_8	25000	17500	19000	18500	20000	20000±2936
CHB_9	138000	145000	134000	147000	138000	140400±5412
CHB_10	5000	10000	6500	8000	7000	7300±1857

CHB = Commercial Honey of Bangladesh; SD= Standard deviation

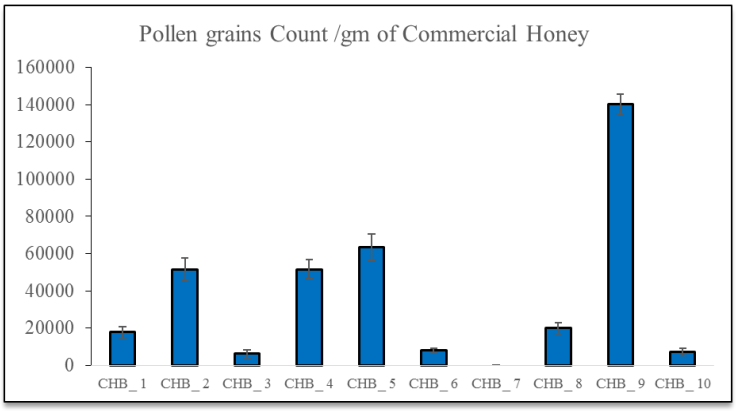

Fig. 1: Pollen grain counts of commercial honey of different brands of Bangladesh.

Comparing the pollen count with reference to pollen count by **Leaveaux, et al., (1978)** categories (Table - 3), sample CHB_7 was very poor in quality (< 2000 pollen grains / gm of honey); samples CHB_3, CHB_6 & CHB_10 were poor in quality (2000 - 10000 pollen grains /g honey); samples CHB_1, and CHB_8 were rich in their content of pollen grain (10,000 - 50,000 pollen grains /g honey); Samples CHB_2, CHB_4 and CHB_5 were more rich in their content of pollen grain (50,000 - 100,000 pollen grains /g honey) and sample CHB_9 was very rich in their content of pollen grain (>100000 pollen grains /g honey). Ten percent (10%) honey was very poor in quality (< 2000 pollen grains / gm of honey); 30% were poor in quality (2000 - 10000 pollen grains /g honey), 20% were rich in their content of pollen grain (10,000 - 50,000 pollen grains /g honey), 30% were more rich in their content of pollen grain (50,000 - 100,000 pollen grains /g honey) and 10% was very rich in their content of pollen grain (>100000 pollen grains /g honey) according to **Leaveaux, et al., (1978)** categories.

Table 2: Categorization of commercial honey according to Leaveaux, et al 1978, based on pollen grains counts.

Categorization of honey after Leaveaux, et al 1978. (Pollen grains/ g honey).		Frequency (n)	Percentages of honey samples in each category (%).
1. < 2000	Very Poor	1	10
2. 2000-10000	Poor	3	30
3. 10000-50000	Rich	2	20
4. 50000-100000	More rich	3	30
5. > 100000	Very rich	1	10
		N=10	**100%**

The results of pollen grains count in natural honey are presented in table 3. Data of table 3 contain pollen count of 10 samples at the beginning, 30 days, 60 days, 90 days and 120 days. During first analysis NHB_1 sample's pollen count was 142000 per gm of honey and during 2nd, 3rd, 4th and 5th analysis NHB_1 showed 145000, 138000, 152000 and 154000 respectively. The average pollen count was 146200±6723.09 per gm of honey sample of NHB_1. During first analysis NHB_2 sample's pollen count was 110000 per gm of honey while during 2nd, 3rd, 4th and 5th analysis NHB_2 showed 119000, 113000, 102000 and 98000 respectively. The average pollen count was 108400±8443.93 per gm of honey sample of NHB_2. During first analysis NHB_3 sample's pollen count was 105000 per gm of honey whereas during 2nd, 3rd, 4th and 5th analysis NHB_3 showed 108000, 100000, 97000 and 103000 respectively. The average pollen count was 102600±4277.84 per gm of honey sample of NHB_3. During first analysis NHB_4 sample's pollen count was 135000 per gm of honey and during 2nd, 3rd, 4th and 5th analysis NHB_4 showed 132000, 125000, 125000 and 132000 respectively. The average pollen count was 129800±4549.72 per gm of honey sample of NHB_4. During first analysis NHB_5 sample's pollen count was 98000 per gm of honey and during 2^{nd}, 3^{rd}, 4^{th} and 5^{th} analysis NHB_5 showed 102000, 110000, 106000 and 117000 respectively. The average pollen count was 106600±7334.84 per gm of honey sample of NHB_5. During first analysis NHB_6 sample's pollen count was 210000 per gm of honey and during 2^{nd}, 3^{rd}, 4^{th} and 5^{th} analysis NHB_6 showed 217000, 212000, 211000 and 196000 respectively. The average pollen count was 209200±7854.93 per gm of honey sample of NHB_6. During first analysis NHB_7 sample's pollen count was 189000 per gm of honey and during 2^{nd}, 3^{rd}, 4^{th} and 5^{th} analysis NHB_7 showed 170000, 187000, 173000 and 180000 respectively. The average pollen count was 179800±8348.85 per gm of honey sample of NHB_7. During first analysis NHB_8 sample's pollen count was 318000 per gm of honey, during 2^{nd}, 3^{rd}, 4^{th} and 5^{th} analysis NHB_8 showed 320000, 310000, 297000 and 320000 respectively. The average pollen count was 313000±9848.85 per gm of honey sample of NHB_8. During first analysis NHB_9 sample's pollen count was 80000 per gm of honey and during 2^{nd}, 3^{rd}, 4^{th} and 5^{th} analysis NHB_9 showed 74000, 77000, 84000 and 81000 respectively. The average pollen count was 79200±3834.05 per gm of honey sample of NHB_9. During first analysis NHB_10 sample's pollen count was 270000 per gm of honey and during 2^{nd}, 3^{rd}, 4^{th} and 5^{th} analysis NHB_10 showed 265000, 275000, 270000 and 285000 respectively. The average pollen count was 267600±6426.50 per gm of honey sample of NHB_10.

Table 3: Number of pollen grains / gram of natural honey samples collected from different honeycomb of different districts of Bangladesh.

Samples	Pollen Count/gm of natural honey					
	1st (At 0 Day)	2nd (After 30 Days)	3rd (After 60 Days)	4th (After 90 Days)	5th (After 120 Days)	Mean±SD
	07/6/2021	09/7/2021	13/08//2021	16/09/2021	18/010/2021	
NHB_1	142000	145000	138000	152000	154000	146200±6723
NHB_2	110000	119000	113000	102000	98000	108400±8443
NHB_3	105000	108000	100000	97000	103000	102600±4277
NHB_4	135000	132000	125000	125000	132000	129800±4549
NHB_5	98000	102000	110000	106000	117000	106600±7334
NHB_6	210000	217000	212000	211000	196000	209200±7854
NHB_7	189000	170000	187000	173000	180000	179800±8348
NHB_8	318000	320000	310000	297000	320000	313000±9848
NHB_9	80000	74000	77000	84000	81000	79200±3834
NHB_10	270000	265000	275000	270000	258000	267600±6426

NHB = Natural Honey of Bangladesh; SD= Standard deviation

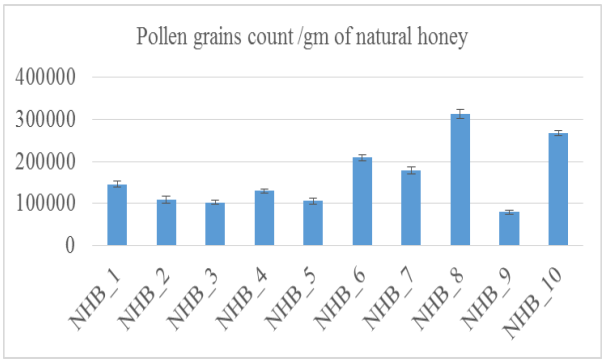

Fig. 2: Pollen grain counts of natural honey of different honeycombs.

Comparing the pollen count with reference to pollen count by Leaveaux, et al. (1978) categories (Table - 4), sample NHB_1 to NHB_8 and NHB_10 were very rich in pollen content (>100000 pollen grains /g honey). Only one sample NHB_9 was more rich in pollen grain (50,000 - 100,000 pollen grains /g honey), Ninety percent (90%) of natural honey of our experiments showed very rich (>100000 pollen grains /g honey) in pollen grain contents and 10% of natural honey was more rich (50,000 - 100,000 pollen grains /g honey) according to Leaveaux, et al., (1978) categories.

Table 4: Categorization of natural honey according to Leaveaux, et al 1978, based on pollen grains counts.

Categorization of Leaveaux, et al 1978 (pollen grains/ g honey)		Frequency (n)	Percentages of honey samples in each category (%).
1. < 2000	Very Poor	0	0
2. 2000-10000	Poor	0	0
3. 10000-50000	Rich	0	0
4. 50000-100000	More rich	1	10
5. > 100000	Very rich	9	90
		N=10	**100%**

CONCLUSION

Honey is a blessing product of nature. From ancient times honey is used in the treatment of different health ailments in traditional medicine. Honey contains a large amount of fructose which is a ready source of food for spermatozoa. It has not only nutritional value but also medicinal and industrial values. Pollen containing honey indicates its naturality. Natural honey is recommended rather than the commercial honey for maximizing benefits.

Ethical Statements

Ethical clearance was duly obtained from the Ethical Committee of Hamdard University Bangladesh.

Limitation

- Analysis of multiple samples of same brand.
- Large scale analysis might provide more accurate information about the pollen quantity of the commercial honey of Dhaka city.

Conflicts of interests

No conflicting interests are stated by the authors.

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