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Arab Univ.
J. Agric. Sci.,
Ain Shams Univ.,
Cairo, 26(1), 303-311, 2018

QUANTITATIVE POLLEN ANALYSIS OF BEE HONEY AT CERTAIN APIARIES IN QALYUBIA GOVERNORATE AND AVAILABLE HONEY IN LOCAL MARKET, EGYPT

[23]

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Keywords: Bee honey; Pollen analysis; Quantitative pollen analysis

ABSTRACT

A new proposal method for quantitative pollen analysis of pollen grains in each gram of bee honey was described, using a Neubauer improved chamber for leucocytes and erythrocytes counting. The study was conducted at the Bee Research Unit, Dept. of plant protection, Faculty of Agriculture, Ain Shams University, Shoubra El-kheimah, Al Qalyubia, Egypt **on** 36 samples of citrus and clover honeys. 18 samples were collected from 5 apiaries under study in Qalyubia Governorate; Kanater, Shalakan, Tookh, Khankah and Shoubra El kheimah and 18 samples were collected from local market in 5 Governorates in Egypt; Cairo, Giza, Qalyubia, Gharbia and Sharqia, during their seasons of production in 2013, 2014 and 2015. The results showed that 2.8 % of the samples were poor in their content of pollen grains (2000-10000 pollen grains /g honey), 77.8 % of the samples were rich in their content of pollen grains (10000-50000 pollen grains /g honey), 11.1% of the samples were more rich in their content of pollen grain (50000 -100000 pollen grains /g honey) and 8.3% of the samples were very rich in their content of pollen grain (>100000 pollen grains /g honey).

INTRODUCTION

Bee honey contains numerous pollen grains (mainly from plants bees feed on) of which provide exact information for the quality of honey. Quantitative pollen analysis is a branch of palynology (science of pollen and spores) and used in the microscopic investigation of bee honey. The microscopic analysis is a widely accepted assumption 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 **(Louveaux et al 1978).** The approved quantitative pollen analysis method was developed and proposed by the International Commission for Bee Botany in 1978 **(Louveaux et al 1978)**. The method was validated in 2004 **(von der Ohe et al 2004)**, and afterwards, widely used in European laboratories for bee honey analysis. It is among the methods used for description of European honey types in the beginning of the 21st century **(Persano Oddo and Piro, 2004)**.

Haemocytometer (Neubauer improved chamber) which used in routine laboratory practice for leucocytes and erythrocytes counting **(Heldrup et al 1992)**, also used for counting pollen grains from plants (Delaplane *et al.,* 2013), and to evaluate the number of pollen grains attached to bees **(Human et al 2013)**. Therefore, in the present work it was used as a new method for quantitative pollen analysis of pollen grains in each gram honey.

In Egypt, most of researches were conducted on chemical and physical properties **[El- Sherbiny et al (1980), Hassan (1985); Nour (1988); Gomaa (2004); Rateb (2005); Faraj (2007) and Ismail (2013)].** The accurate determination of bee honey type especially pollen analysis requires more detailed research. The proposed new method aims to determine the number of pollen grains in each gram of honey in all collected samples to evaluate their quality.

(Received 27 May, 2017)
(Revised 31 May, 2017)
(Accepted 4 June, 2017)

MATERIALS AND METHODS

36 samples of citrus and clover honeys were collected from the 5 apiaries under study in Qalyubia Governorate and local market from 5 Governorates of Egypt, during their seasons of production in 2013, 2014 and 2015 as follows:

1- 9 citrus honey samples from certain apiaries in Qalyubia Governorate situated in Kanater, Shalakan, Tookh, Khankah and Shoubra El kheimah included: H1, H2 and H3 in 2013, H13, H14 and H27 in 2014 and H20, H21 and H25 in 2015.

2- 9 clover honey samples from the same apiaries included: H7, H8 and H9 in 2013, H17, H29 and H32 in 2014 and H30, H31 and H33 in 2015.

3- 9 citrus honey samples from the local markets situated in Cairo, Giza, Qalyubia, Gharbia and Sharqia Governorates included: H4, H5 and H6 in 2013, H15, H18 and H22 in 2014 and H19, H24 and H26 in 2015.

4- 9 clover honey samples from the local markets situated in the same Governorates included: H10, H11 and H12 in 2013, H16, H36 and H37 in 2014 and H28, H34 and H35 in 2015.

For quantitative analysis of pollen grains in each gram of bee honey, 10 g of each honey sample were weighed in a graduated cylinder. Distilled water was added to a total volume of 20 cm^3.The obtained honey solution was divided into two aliquots of 10 cm^3 in graduated centrifuge tubes. The tubes were centrifuged at 2500×g for 10 min. With an automated pipette, 9 cm^3 of the supernatant were discarded from each tube. To the remaining amount of 1 cm^3 in each of the two tubes, 5 cm^3 distilled water were added to a total volume of 6 cm^3. The two tubes were centrifuged at 2500×g for 10 min. 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 (µl) samples were taken with automated pipettes from the bottom of each tube. The amount was chosen due to the experimentally established fact that a volume of .01 µl fills entirely, without leaking, the two semi-reflective segments of the Neubauer improved chamber after placing the cover glass.

Under direct light, the positions of the gridded areas were identified and the 0.1 µl samples were pipetted in the middle of grids, resulting in two drops on each grid. A thin coverslip (Cover glass, 24 x 32 mm, thickness 0.13-0.17 mm), was then carefully placed perpendicularly to the wide side of the chamber to cover entirely the gridded areas and to contact tightly the chamber edges **Fig. (1**).

Fig.1. Neubauer improved chamber

Using a light microscope, eyepiece lens **10x** and objective lens 10x/0.24, the two grids of the chamber were brought into focus and all pollen grains within them were counted using objective lens 40x/0.26, including those grains within each medium square and those that are over the top and right sides of the square (even when they are partially out) **Fig. (2).**

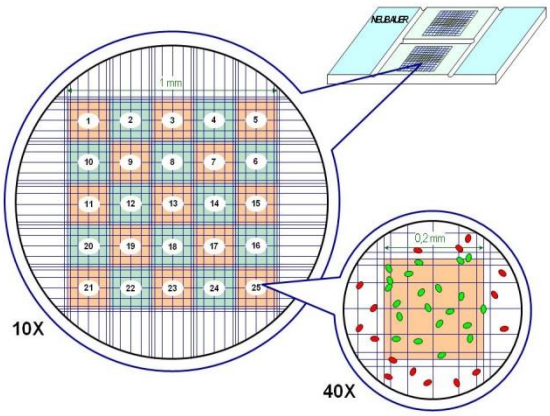

Fig. 2. Grid for pollen counting in Neubauer's improved cell counting chamber

Pollen grains in both segments of the chamber were counted and the arithmetic mean (A) was calculated. The number of pollen grains in **10 g honey** is calculated by the equation:

$$X = A \times 50000$$

where:

X – number of pollen grains in 10 g honey;

A – arithmetic mean of pollen grains counted in the two grids of the chamber (= (N1+ N2) /2).

50000– coefficient for calculation of sample volume

For obtaining the coefficient 50000

Neubauer 's improved chamber has 9 large squares each with a 1 mm^2 area, and each of them could hold 0.1 mm^3 of honey solution because when putting the sample under the coverslip, the cell suspension reaches a height of 0.1 mm (Neubauer's improved cell counting chamber, 2003). Taking these data into account, and considering one of the large squares, the volume will be:

$$1 \times 1 \times 0,1 = 0,1\ mm^3 = 10^{-4}\ ml$$

1. According to **Hornitzky, 2009** the counted N pollen grains in 5 of the medium squares (that is, in 25 medium squares **Fig. (3**), the concentration of our sample will be:

$$N \times 10^4 \text{ pollen grain/ml}$$

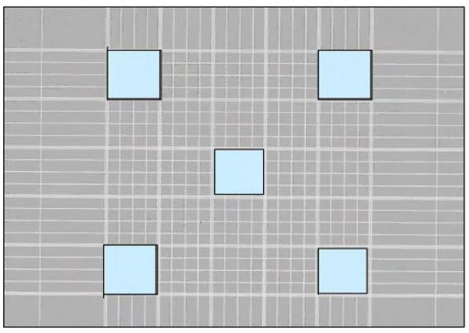

Fig. 3. Five medium squares **(Hornitzky, 2009)**

2. All the pollen grains within each medium square (total 25 square) and those that are over the top and right sides of the square (even when they are partially out) are counted so the final equation is: $N * 5 * 10^4 = N * 50000$

So, the number of pollen grains in **each gram honey** is calculated by the equation:

$$X = A \times 50000/10$$

where:

X – number of pollen grains in each gram honey

A – arithmetic mean of pollen grains counted in the two grids of the chamber (= (N1+ N2) /2).

5000– coefficient for calculation of sample volume

Example:

For obtaining the arithmetic mean ($\bar{x}$) of pollen grains counted in the two grids of the chamber for 10 tests ($A_{1\text{-}10}$) were as followed:

10 readings done in longer time intervals (1 month) were made from each honey sample, the results for pollen grains number in both chamber grids for the ten tests ($A_{1\text{-}10}$) were as followed:

$A_1 = 16 / 2$ (number of chamber grids) = 8

$A_2 = 15 / 2 = 7.5$

$A_3 = 15 / 2 = 7.5$

$A_4 = 16 / 2 = 8$

$A_5 = 15 / 2 = 7.5$

$A_6 = 15 / 2 = 7.5$

$A_7 = 16 / 2 = 8$

$A_8 = 15 / 2 = 7.5$

$A_9 = 16 / 2 = 8$

$A_{10} = 16 / 2 = 8$

$\bar{X} = 7.75$

s. e. (standard error) =0.08

The calculated pollen grain numbers in 1 g honey for each test run according to the formula ($X_{1\text{-}10}$) was as followed:

$X_1 = 8 \times 5000 = 4*10^4$ pollen grains/ 1 g honey

$X_2 = 7,5 \times 5000 = 37.5*10^3$ pollen grains / 1 g

$X_3 = 7,5 \times 5000 = 37.5*10^3$ pollen grains / 1 g

$X_4 = 8 \times 5000 = 4*10^4$ pollen grains / 1 g

$X_5 = 7,5 \times 5000 = 37.5*10^3$ pollen grains / 1 g

$X_6 = 7,5 \times 5000 = 37.5*10^3$ pollen grains / 1 g

$X_7 = 8 \times 5000 = 4*10^4$ pollen grains / 1g

$X_8 = 7,5 \times 5000 = 37.5*10^3$ pollen grains / 1 g

$X_9 = 8 \times 5000 = 4*10^4$ pollen grains / 1 g

$X_{10} = 8 \times 5000 = 4*10^4$ pollen grains / 1 g

$\bar{X} = 387500$

s. e. =4166.7

RESULTS AND DISCUSSION

The results from counting pollen grains in bee honey samples collected from experimental apiaries from **Qalyubia Governorate and local market in Egypt**, produced in 2013, 2014 and 2015 are presented in **Tables 1 to 6.**

The number of pollen grains of **citrus** honey samples collected during **2013** from different apiaries in Qalyubia Governorate and local market in Egypt were summarized in **Table (1**). The number of pollen grains in each gram of citrus honey collected from Kanater (H1) significantly came the first in order with an average of 365750 ± 16951.9 pollen grains / gram honey. Followed by the samples collected from Tookh (H3), Shalakan (H2), Cairo Univ. (H4), Ain Shams Univ. (H5) and Agri. Ministry

(H6) without any significant difference between them and their averages were 26750 ±2814.9, 26200 ± 2493.5, 25750 ±2445.2, 17000 ±1658.3 and 14750 ± 2187.4 pollen grains / g honey, respectively. Meanwhile, the number of pollen grains of **clover** honey samples collected during the same year **2013** from different apiaries in Qalyubia Governorate and local market in Egypt were summarized in **Table (2).** The number of pollen grains in each gram of clover honey collected from Shalakan (H8) significantly came the first in order and its average was 47750 ± 2056.5 pollen grains / g honey. Followed by the sample collected from Kanater (H7) which significantly came the 2nd in order and its average was 35750 ± 3051.6 pollen grains / g honey. The samples collected from Cairo Univ. (H11), Tookh (H9) and Agri. Ministry (H10) significantly came the 3rd in order, without any significant difference between them and their averages were 21000 ±2114.8, 15250 ±1416.7 and 14250 ±1750 pollen grains /g honey, respectively. While the sample collected from Ain Shams Univ. (H12) significantly came the last in order and its average was 11000 ±1715.9 pollen grains / g honey without any significant difference with the samples H9 and H10.

The number of pollen grains of **citrus** honey samples collected during **2014** from different apiaries in Qalyubia Governorate and local market in Egypt were summarized in **Table (3)**. The number of pollen grains in each gram of citrus honey collected from Kanater (H27) significantly came the first in order and its average was 88750 ± 2963.9 pollen grains / g honey. Followed by the sample collected from Agri. Ministry (H18) which significantly came the 2nd in order and its average was 54000 ± 1943.6 pollen grains / g honey. The samples collected from Ain Shams Univ. (H15) and Gharbia Governorate (H22) significantly came the 3rd in order and their averages were 33500 ± 1870.8 and 31000 ±1632.9 pollen grains / g honey, respectively. But the samples collected from Shalakan (H13) and Tookh (H14) were the last in order with averages of 18750 ± 1547.8 and 18750 ± 1070 pollen grains /g honey, respectively without any significant difference between them. Meanwhile, the number of pollen grains of **clover** honey samples collected during the same year **2014** from different apiaries in Qalyubia Governorate and local market in Egypt were summarized in **Table (4).** The number of pollen grains in each gram of clover honey collected from Shoubra El Khimah (H32) and Shalakan (H29) significantly came the first in order and their averages were 110250 ± 12469.1 and 109250 ± 5701.5 pollen grains / g honey, respectively. Followed by the sample collected from Agri. Ministry (H16) which significantly came the 2nd in order and its average was 70500 ± 4561.3 pollen grains / g honey. While the samples collected from Kanater (H17), Gharbia Governorate (H37) and Ain Shams Univ. (H36) significantly came the last in order and their averages were 23750 ± 1193.2, 17500 ± 2075 and 12750 ± 870 pollen grains /g honey, respectively.

The number of pollen grains of **citrus** honey samples collected during **2015** from different apiaries in Qalyubia Governorate and local market in Egypt were summarized in **Table (5)**. The number of pollen grains in each gram of citrus honey collected from Gharbia Governorate, Tanta (H24) significantly came the first in order and its average was 49500 ± 1658.3 pollen grains /g honey. Followed by the samples collected from Sharqia Governorate (H26) and Shalakan (H21) which significantly came the 2nd in order and their averages were 40500 ± 2438.1 and 37250 ± 1204.7 pollen grains /g honey, respectively. The samples collected from Kanater (H20) and Tookh (H25) significantly were the 3rd in order and their averages were 28750 ± 2334.8 and 22500 ± 1748 pollen grains /g honey, respectively. But the sample collected from Gharbia Governorate, Mahallah (H19) significantly came the last in order and its average was 20250 ± 946.5 pollen grains / g honey without any significant difference with the sample H25. Meanwhile, the number of pollen grains of **clover** honey samples collected during the same year **2015** from different apiaries in Qalyubia Governorate and local market in Egypt were summarized in **Table (6).** The number of pollen grains in each gram of clover honey collected from Gharbia Governorate, Mahallah (H28) significantly came the first in order and its average was 73000 ± 2549.5 pollen grains / g honey. Followed by the samples collected from Shalakan (H31) and Kanater (H33) which significantly came the 2nd in order and their averages were 43250 ± 2008.7 and 43000 ± 3452 pollen grains / g honey. While the samples collected from Sharqia Governorate (H35) and Tookh (H30) significantly came the 3rd in order and their averages were 32750 ± 3060.7 and 26250 ± 1356.6 pollen grains / g honey. But the sample collected from Gharbia Governorate, Tanta (H34) was the last in order and its average was 8750 ± 1455.3 pollen grains / g honey.

Table 1. Number of pollen grains / gram of **Citrus** honey samples collected from different locations at Qalyubia Governorate and local market in **2013**

Honey samp. / Rep.	Locations at Qalyubia			Local market in Cairo		
	H1 Kanater	H2 Shalakan	H3 Tookh	H4 Cairo Univ.	H5 Ain Shams Univ.	H6 Agri. Ministry
1	175004	25000	17500	30000	17500	5000
2	320000	15000	42500	15000	12500	15000
3	350000	32000	27500	20000	12500	7500
4	237500	20000	17500	15000	10000	10000
5	382500	20000	12500	22500	15000	7500
6	392500	40000	30000	30000	22500	17500
7	417500	35000	25000	32500	20000	22500
8	372500	20000	32500	22500	25000	20000
9	387500	30000	30000	35000	12500	17500
10	380000	25000	32500	35000	22500	25000
Mean($\bar{x}$) ± s. e.	**365750 [a] ± 16951.9**	**26200 [b] ± 2493.5**	**26750 [b] ± 2814.9**	**25750 [b] ± 2445.2**	**17000 [b] ± 1658.3**	**14750 [b] ± 2187.4**

L.S.D. 42617 pollen grains / g honey

Table 2. Number of pollen grains / gram of **Clover** honey samples collected from different locations at Qalyubia Governorate and local market in **2013**

Honey samp. / Rep.	Locations at Qalyubia			Local market in Cairo		
	H7 Kanater	H8 Shalakan	H9 Tookh	H11 Cairo Univ.	H12 Ain Shams Univ.	H10 Agri. Ministry
1	35000	52500	17500	20000	12500	15000
2	45000	55000	12500	20000	5000	12500
3	37500	50000	7500	20000	7500	10000
4	35000	57500	10000	30000	2500	10000
5	20000	45000	15000	15000	10000	12500
6	20000	42500	15000	25000	10000	10000
7	35000	50000	22500	17500	10000	10000
8	42500	37500	20000	10000	15000	27500
9	50000	40000	15000	20000	17500	17500
10	37500	47500	17500	32500	20000	17500
Mean($\bar{x}$) ± s. e.	**35750 [b] ± 3051.6**	**47750 [a] ± 2056.5**	**15250 [cd] ± 1416.7**	**21000 [c] ± 2114.8**	**11000 [d] ± 1715.9**	**14250 [cd] ± 1750**

L.S.D. 8702.4 pollen grains / g honey

Table 3. Number of pollen grains / gram of **Citrus** honey samples collected from different locations at Qalyubia Governorate and local market in 2014

Honey samp. / Rep.	Locations at Qalyubia			Local market in Gharbia and Cairo		
	H27 Kanater	H13 Shalakan	H14 Tookh	H22 Gharbia	H15 Ain Shams Univ.	H18 Agri. Ministry
1	75000	10000	15000	35000	45000	47500
2	77500	17500	15000	25000	35000	47500
3	87500	22500	17500	25000	37500	52500
4	85000	20000	17500	35000	27500	55000
5	100000	27500	17500	27500	32500	65000
6	95000	22500	22500	25000	27500	60000
7	92500	15000	17500	32500	27500	60000
8	102500	15000	17500	32500	40000	50000
9	92500	17500	22500	32500	32500	47500
10	80000	20000	25000	40000	30000	55000
Mean($\bar{x}$) ± s. e.	**88750 [a] ± 2963.9**	**18750 [d] ± 1547.8**	**18750 [d] ± 1070.4**	**31000 [c] ± 1632.9**	**[c]33500 ± 1870.8**	**54000 [b] ± 1943.6**

L.S.D. 7374 pollen grains / g honey

Table 4. Number of pollen grains / gram of **Clover** honey samples collected from different locations at Qalyubia Governorate and local market in **2014**

Honey samp. / Rep.	Locations at Qalyubia			Local market in Gharbia and Cairo		
	H17 Kanater	H29 Shalakan	H32 Shoubra El-khimah	H37 Gharbia	H36 Ain Shams Univ.	H16 Agri. Ministry
1	20000	90000	77500	22500	10000	50000
2	25000	102500	112500	5000	17500	65000
3	25000	120000	90000	20000	50001	85000
4	27500	85000	70000	15000	10000	57500
5	17500	97500	137500	17500	10000	62500
6	20000	127500	95000	10000	12500	55000
7	30000	140000	185000	15000	10000	82500
8	25000	112500	165000	20000	12500	72500
9	22500	95000	80000	22500	15000	90000
10	25000	122500	90000	27500	15000	85000
Mean($\bar{x}$) ± s. e.	**23750[c] ± 1193.2**	**109250[a] ± 5701.5**	**110250[a] ± 12469.1**	**17500[c] ± 2075**	**12750[c] ± 870**	**70500[b] ± 4561.3**

L.S.D. 25028 pollen grains / g honey

Table 5. Number of pollen grains / gram of **Citrus** honey samples collected from different locations at Qalyubia Governorate and local market in **2015**

Honey samp. / Rep.	Locations at Qalyubia			Local market Gharbia and Sharqeia		
	H20 Kanater	H21 Shalakan	H25 Tookh	H19 Gharbia (Mahallah)	H24 Gharbia (Tanta)	H26 Sharqeia
1	20000	32500	27500	20000	52500	27500
2	27500	35000	22500	20000	42500	37500
3	37500	32500	15000	17500	50000	42500
4	27500	35000	15000	15000	60000	30000
5	37500	45000	20000	22500	45000	47500
6	27500	37500	20000	17500	55000	35000
7	37500	40000	32500	22500	47500	42500
8	32500	40000	27500	20000	45000	45000
9	17500	37500	22500	25000	50000	50000
10	22500	37500	22500	22500	47500	47500
Mean(x^-) ± s. e.	**28750^c ± 2334.8**	**37250^b ± 1204.7**	**22500^{cd} ± 1748**	**20250^d ± 946.5**	**49500^a ± 1658.3**	**40500^b ± 2438.1**

L.S.D. 7541.9 pollen grains / g honey

Table 6. Number of pollen grains / gram of **Clover** honey samples collected from different locations at Qalyubia Governorate and local market in **2015**

Honey samp. / Rep.	Locations at Qalyubia			Local market in Gharbia and Sharqeia		
	H33 Kanater	H31 Shalakan	H30 Tookh	H28 Gharbia (Mahallah)	H34 Gharbia (Tanta)	H35 Sharqeia
1	35000	52500	22500	75000	20000	40000
2	35000	35000	27500	67500	7500	50003
3	37500	47500	17500	90000	5000	55000
4	42500	32500	25000	75000	10000	27500
5	67500	47500	30000	62500	7500	37500
6	35000	42500	25000	62500	12500	22500
7	42500	40000	25000	77500	7500	25000
8	37500	50000	27500	70000	5000	30000
9	57500	42500	30000	75000	7500	25000
10	40000	42500	32500	75000	5000	30000
Mean(x^-) ± s. e.	**43000^b ± 3452**	**43250^b ± 2008.7**	**26250^C ± 1356.6**	**73000^a ± 2549.5**	**8750^d ± 1455.3**	**32750^c ± 3060.7**

L.S.D. 10203 pollen grains / g honey

As shown in **Table (7)** the honey samples (collected during the 3 years 2103, 2014 and 2015) were classified according to the categories of **Leaveaux, et al 1978,** as follows:

- 2.8% of the samples were **poor** in their content of pollen grains (2000-10000 pollen grains/ g honey).
- 77.8% of the samples were **rich** in their content of pollen grains(10000-50000pollen grains/ g honey).
- 11.1% of the samples were **more rich** in their content of pollen grains (50000-100000 pollen grains/ g honey).

8.3% of the samples were **very rich** in their content of pollen grains (>100000 pollen grains/ g honey).

Table 7. Percentages of the 36 honey samples (collected during the 3 years 2013, 2014 and 2015) in each category of the **Leaveaux, et al 1978** categories, according to their content of pollen grains

Categories of Leaveaux, et al 1978 (pollen grains/ g honey)	No. of honey samples	Percentages of honey samples in each category
1. < 2000	0	0
2. 2000-10000	1	2.8
3. 10000-50000	28	77.8
4. 50000-100000	4	11.1
5. > 100000	3	8.3
Total	**36**	**100**

REFERENCES

Delaplane, K.S., Dag, A., Danka, R.G., Freitas, B.M., Garibaldi, L.A., Goodwin, R.M. and Hormaza, J.I. 2013. Standard methods for pollination research with *Apis mellifera*. **Cited from** Dikov, D. (2015). Quantitative melissopalynological analysis of bee honey using a Burker chamber. **International food research journal, 22 (4), 1538-1543.**

El-Sherbiny, G.A., Rizk, S.S., El-Ashwah, F.A. and Heikel, H.A. 1980. Chemical composition of citrus honey produced in **A.R.E. Agri. Res. Rev., 58, 289- 297.**

Faraj, R.M.A. 2007. Studies on Egyption honeys. M. Sc. Thesis, Plant Protection Dept., Fac. Agric., Cairo Univ., Egypt **183 p.**

Gomaa, W.M.K. 2002. Studies on honey quality with special reference to the Egyptian honey standard. M. Sc.Thesis, Plant Protection Dept., Fac. Agric., Alexandria Univ.,, Egypt **156 p**.

Hassan, Mona, I.M. 1985. Studies on food: Effect of storage on some physical and chemical characteristics of bee honey. M. Sc. Thesis, Plant Protection Dept., Fac. Agric., Alexandria Univ., Egypt **286 p.**

Heldrup, J., Kalm, O. and Prellner, K. 1992. Blood T and B lymphocyte subpopulations in healthy infants and children. **Acta Paediatrica; 81(2), 125–132.**

Hornitzy, M. 2009. Nosema diagnosis. Downloaded from http://www.dpi.nsw.gov.au/data/assets/pdf.

Human, H., Brodschneider, R., Dietemann, V., Dively, F., Ellis, G., Forsgren, J., Fries, E., Hatjina, F., Jaffè, R., Jensen, A.B., Köhler, A., Magyar, J., Özkýrým, C.W.W., Pirk, C.W.W., Rose, R., Strauss, U., Tanner, G., Tarpy, D. R., van der Steen, J.J.M., Vaudo, A., Vejsnaes, F., Wilde, J., Williams, G.R. and Zheng, H.Q. 2013. Miscellaneous standard methods for *Apis mellifera* research. **Cited from** Dikov, D. (2015). Quantitative melissopalynological analysis of bee honey using a Burker chamber. **International Food Research Journal, 22 (4), 1538-1543.**

Ismail, A.M., Owayss, A.A., Mohanny, K.M. and Salem, R.A. 2013. Evaluation of pollen collected by honey bee, *Apis mellifera* L. colonies at Fayoum Governorate, Egypt. Part : Botanical origin. **Journal of the Saudi Society of Agricultural Sciences, 12, 129–135.**

Louveaux, J., Maurizio, A. and Vorwohl, G. 1978. Methods of Melissopalynology. **Bee World; 59, 139–157.**

Neubauer's improved cell counting chamber 2003. Downloaded from http://insilico.ehu.es/counting_chamber/neubauer_improved.pdf.

Nour, M.E. 1988. Some factors affecting quality of Egyptian honey. Ph. D. Thesis, Plant Protection Dept., Fac. Agric., Cairo Univ., Egypt **252p.**

Oddo, L.P. and Piro, R. 2004. Main European unifloral honeys: descriptive sheets. **Apidologie; 35(1), S38–S81.**

Rateb, S.H. 2005. Studies on pollen spectrum, chemical and physical characters of honeys. Ph. D. Thesis, Plant Protection Dept., Fac. Agric., Assiut Univ., Egypt **332 p.**

von der Ohe, W., Oddo, L.P., Piana, M.L., Morlot, M. and Martin, P. 2004. Harmonized methods of melissopalynology. **Apidologie; 35, S18–S25.**