



**HARACTARISTICS OF PROPOLIS SAMPLES  
COLLECTED BY HONEYBEE COLONIES *APIS  
MELLIFERA L.* FROM LUXOR REGION, UPPER  
EGYPT**

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**ABSTRACT**

The present work was carried out in three private apiaries in Luxor governorate, Egypt. The conducted trials were extended over two successive years (July 2013-June 2015). Propolis samples were collected monthly and weighed. Collection process included scarping propolis by a sharp knife from the top of the frames. The obtained results showed that the highest Propolis amounts were collected in May, June and July, while the lowest one were collected in October, November and December, respectively. The location of the apiary was an effective factor on amount of harvest Propolis. Qurna district which the bees collected more propolis than Menshah and Bearaat districts, which the general means of districts were 2.85, 1.57 and 1.22 g/colony/year, respectively, in the first season 2013/2014, while in the second season 2014/2015 they were 3.57, 3.10 and 2.06 g/colony/year, for the Bearaat, Qurna and Menshah districts, respectively. Statistical analysis showed significant differences among all months in Bearaat district except November, they were significant differences among all months in Qurna district except August, March, May and June, also they were significant differences among all months in Menshah district except July, October and January.

Chemical composition determination indicated that the highest percentage of flavonoids was recorded in the samples collected from Bearaat district (10.12 %), while the lowest one was recorded in Qurna samples (2.01 %) in the first season. On the other hand the highest percentage of antioxidants was recorded in Qurna samples (91.56 %), while the lowest one was recorded in Bearaat samples (90.20 %). In the second season, the highest percentage of flavonoids (5.13 %) was recorded in Menshah samples, while the lowest one

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(1.13 %) was observed in Qurna samples . The highest percentage of antioxidants in Bearaat samples (93.50%) was recorded , while the lowest one in Qurna samples (86.27%).

**Key words:** honeybee, propolis.

## INTRODUCTION

The honey bee use propolis to cover cracks in their hive, to line interiors of a honey comb to ensure sterile environment for the queen to lay eggs, due to its antimicrobial properties. At the same time, the bees are lining propolis around entrance to the hive, so they can clean themselves before entering the hive. It is also used to mummify enemies killed in the hive, which are too big for the bees to throw out. (Ghisalberti, 1979)

Propolis (bee glue) is a complex mixture,formed from resinous and balmy material collected by bees from parts of plants and modified in the bee hive by addition of salivate secretions and wax(Pereira *et al.*, 2003).Before using it in the hive , honey bees take this sap combine it with nectar found in their own secretions and eventually end up with a mixture consisting of wax ,pollen and bee bread . For this reason , just chewing on awad of tree resin will not produce the same therapeutic results as propolis .Bees must transform the resin into propolis.

Chemical composition and pharmacological characteristics were studied by many authors (Ghisalberti, 1979, Omar, 1989, 1994). In Egypt ,Omar (1989) , studied composition of propolis samples collected from Upper Egypt at different times of the year .He noticed that autumn samples had the highest quality and quantity as

compared with other season .Also, results obtained by Omar(1994) ,indicated that propolis samples collected from different places of the hives significantly varied in its quantity and quality.

The aim of this study was to clarify the composition of propolis samples collected from Luxor region, Upper Egypt.

## MATERIALS AND METHODS

The present study was carried out in three private apiaries in Luxor governorate, Egypt. The trials of the study were conducted through two successive years from July 2013 to June 2015 as follows:

### 2.1. Propolis collection and sampling

For studying propolis gathering activity and chemical composition, five honeybees colonies for each apiary (*Apis mellifera* L.) were chosen . Each was headed by a mated queen of similar age .Propolis was gathered monthly and weighed during 2013/2014 and 2014/2015 seasons. Collection process included scarping propolis by a sharp knife from the end of the bars of the frames and the inner wall of the hives .The color of each propolis samples was recorded immediately after collection .All Propolis samples were kept in refrigerator until analyzed as mentioned by Muszynska *et al.*(1985).

## 2.2. Propolis Chemical composition:-

To determine chemical composition of propolis, six samples (each one weight about 5g) were taken. Three of them were collected in May of the first season 2013-2014 and the other three in September of the second season 2014-2015. These samples were sent to laboratory of Food Safety and Quality Control (FSQC), Faculty of Agriculture – Cairo University for chemical composition determination as follow:

### 2.2.1. Total Flavonoids determination:

Aluminum chloride colorimetric method was used to determine flavonoid content. 1 ml of sample extract (5g of propolis sample /100ml methanol 80%) was mixed with 3 ml of methanol, 0.2 ml of 10% aluminium chloride, 0.2 ml of 1M potassium acetate and 5.6 ml of distilled water and remained at room temperature for 30 min. The absorbance was measured at 420 nm. Rutin was used as standard (1mg/ml). Flavonoid content was calculated from the regression equation of the standard plot as follows: ( $y=3721x-17.065$ ,  $r^2=0.9899$ ) and were expressed as Rutin equivalent (g/100g of extracted compound). (Conti *et al*, 2015). where  $y$  is concentration of standard solution,  $r$  is the correlation coefficient and  $x$  is the absorbance concentration.

### 2.2.2. DPPH Radical-Scavenging activity:

DPPH is a common abbreviation for the organic chemical compound 2,2-diphenyl-1-picrylhydrazyl. It is a dark colored crystalline powder composed of stable free radical molecules. DPPH has two major applications, both in laboratory research, one is a monitor of chemical reactions involving radicals, most notably it's a common antioxidant assay and another is a standard of the position and intensity of electron paramagnetic resonance signals. Free radical scavenging activities of solutions of the sample extracts and synthetic antioxidant substances used in the study prepared in methanol at Concentration ranging from 5 to 200 ug/ml from each sample. (100 ul) extract and DPPH radical (100ul, 0.2 mM) dissolved in methanol. The mixture was stirred and left to stand for 15 min in dark. Then the absorbance was measured at 517 nm comparing with the blank. (Da Silva *et al*, 2013).

Percentage scavenging effect was calculated as:

$$[(A_0-A_1)/A_0] \times 100$$

Where  $A_0$  is the absorbance of the control (without sample) and  $A_1$  is the absorbance in the presence of the sample (Laura Stan *et al.*, 2011).

### 2.2.3. Wax content determination:

Samples were prepared by mixing propolis with methanol (1:1, w/v) and were left over-night in agitation. After this step, the obtained solution was filtered (Whatman N 4 filter paper). Two other methanolic extractions were performed using the

same procedures. The combined methanolic extracts were placed at low temperatures and, after 12h, filtered to remove wax. The methanol was evaporated with a rotary evaporator. The extracts were evaporated by reduced pressure (Rotavapor Buchi RE 111 with a Buchi 461 water-bath, 2002), re-dissolved in the corresponding solvent at a concentration of 50 mg/mL and analyzed for their content in total phenols. (Barth *et al.*, 1999).

#### 2.2.4. Determination of ash content :( AC)

Two gm of propolis sample was weighted to the nearest 0.001g (m0) and put it into an ash dish and reweighted (m1). place the dish in the preheated furnace and heat for at least 2 hour. Cool the ash dish in the desiccators and reweighted. Continue the ashing procedure until constant weight is reached (m2).it is calculated as follows:

$$AC = (m1 - m2) / m0 \times 100$$

Where:

m0 = weight of propolis sample.

m1 = weight of dish + propolis.

m2 = weight of dish+ ash.

#### 3. Statistical analysis:

The obtained data was statistically analyzed through application of one-way analysis of variance. Differences among treatment means were compared through using the least significant difference (LSD) test according methods of Mead *et al.*, (1993).

## 4. RESULTS AND DISCUSSION

### 2.1. Activity of Propolis collection

The obtained results were presented in Table (1) and illustrated in Fig. (1). Data in the first season showed that the highest amount collected was in June, 2014, while lowest one was recorded in October, 2013 in Qurna district. The honey bee colonies in Qurna collected more propolis than those of Menshah district followed by Bearaat district ,which showed the general means 2.85 , 1.57 and 1.22 g/colony/year, respectively,. In the second season 2014/2015 the general means of total propolis quantities were 3.57 , 3.10 and 2.06 g/colony/year , for Bearaat , Qurna and Menshah districts, respectively, with significant differences among all months in Bearaat district except November , while the differences were significant among all months in Qurna district except August, March ,May and June . Also they were significant differences among all the amounts of propolis collected in months of Menshah district except July, October and January. The highest monthly mean quantities of propolis were collected in June ,July and August which showed 3.37, 3.55 and 3.48 g/colony/month ,when the max temperature were 40.6, 39.5 and 40.2, respectively.

The results of propolis amounts collected by honeybee colonies were came in agreement with Ashour ,(1989) who cleared that the amount of propolis collected by honeybee colonies increase during warm and hot seasons (1.4-3.9g/hive/month) which

Table (1): Quantity of propolis collected (g/colony/month) by honey bee colonies from different districts in 2013 / 2014 season in Luxor Governorate .

Locations	2013						2014						General mean
	July	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	
Bearaat	1.99b	1.04b	0.63b	0.17b	0.31b	0.29b	0.38b	0.46b	0.46b	0.36b	1.07b	7.44a	1.22
Qurna	7.08a	6.50a	4.45a	3.85a	2.45a	0.52b	1.43a	1.28b	1.62a	0.57b	3.21b	1.27b	2.85
Menshah	1.57b	2.91b	1.25b	0.61b	0.44b	1.63a	0.30b	1.70a	1.32b	1.54a	4.17a	1.39b	1.57
Monthly mean quantit:	3.55	3.48	2.11	1.54	1.07	0.81	0.70	1.15	1.13	0.82	2.81	3.37	
<i>L.S.D at 5%</i>	1.25	2.34	1.44	0.53	0.43	0.57	1.00	0.83	0.64	0.58	1.67	1.32	

L.S.D. (Least significant difference at5%)

Table (2): Quantity of propolis collected (g/colony/month) by honey bee colonies from different districts in 2014 / 2015 season in Luxor Governorate .

Propolis quantity	2014						2015						General mean
	July	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	
Bearaat	13.44a	4.05b	2.26b	1.53b	0.52b	2.37a	2.63a	3.25a	2.43b	1.88a	3.25b	5.17a	3.57
Qurna	3.50b	5.17a	3.26a	0.90b	0.40b	2.32b	5.17a	9.03a	1.86b	1.23a	2.26b	2.08b	3.10
Menshah	1.69b	0.89b	2.46b	1.07b	0.97a	0.31b	0.59a	0.60a	2.75a	3.15a	5.37a	4.86b	2.06
Monthly mean quantity	6.21	3.37	2.66	1.16	0.63	1.66	2.80	4.29	2.35	2.09	3.63	4.04	
<i>L.S.D at 5%</i>	2.37	1.53	1.41	0.64	0.19	0.77	1.08	1.24	0.84	0.54	1.38	1.49	

L.S.D.( Least significant difference at5%)

could be obtained during the months from May to September, while lower amounts (0.185-0.582 g/hive/month) were obtained in cold weather through December to January. However, observation of Omar (1989) showed that honeybees start propolis collection early in February - March and become more active in October-November in Assiut and Sohage governorates. A

notable decrease in the activity was noticed during the main honey flows. Krupicka (1978) reported that bees propolize mostly in August-October when pollen is not abundant. Also, results obtained by Omar (1994) indicated that propolis collected from different places of the hives significantly varied with its quantity and quality.

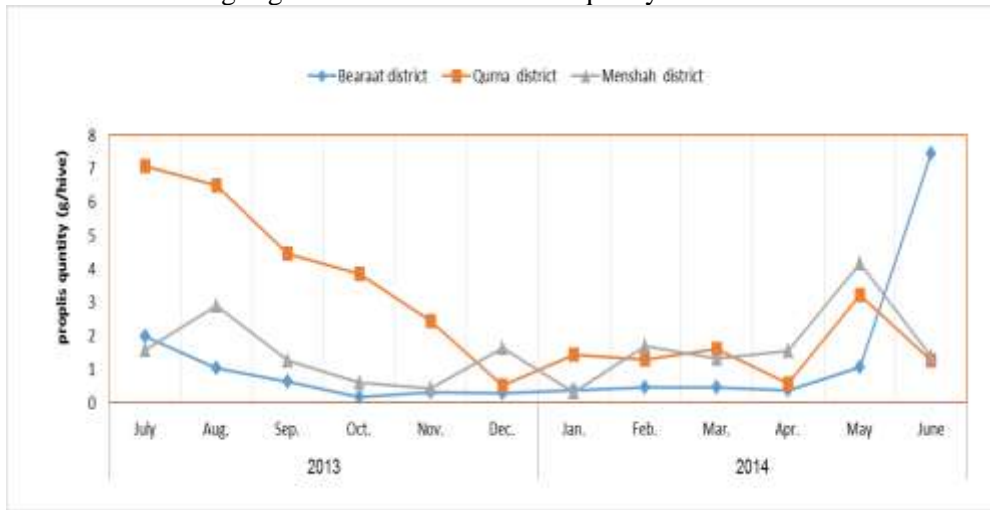


Fig. (1):Quantity of propolis collected (g/colony/month) by honey bee colonies from different districts of 2013 / 2014 season in Luxor governorate .

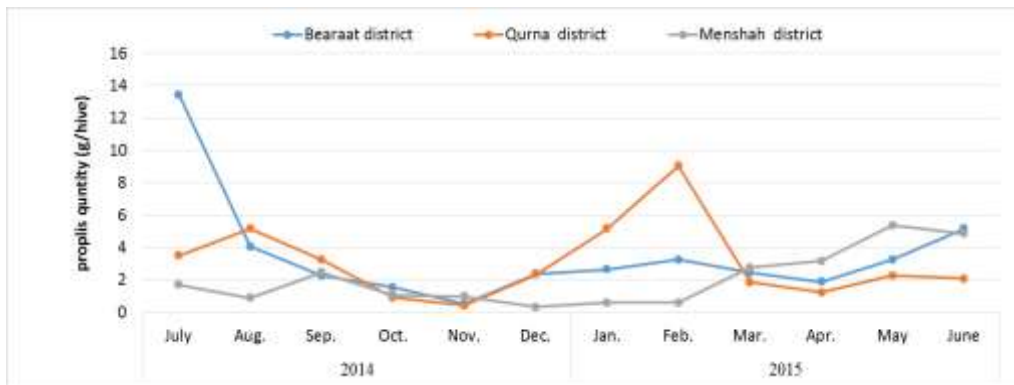


Fig. (2):Quantity of propolis collected (g/colony/month) by honey bee colonies from different districts of 2014 / 2015season in Luxor governorate

## 2.2. Propolis Chemical composition

Data presented in Table (3) and illustrated in Fig. (3) indicated that the results of chemical composition of propolis which showed that the highest percentage of flavonoids of (10.12 %) in samples of Bearaat district ,while the lowest percentage observed in samples of Qurna district ( 2.01 )% in the first season 2013/2014 . The results of antioxidants recorded the highest activity of propolis (91.56 %) in samples collected from Qurna district, with brown color, while the lowest recorded in Bearaat district (90.20 %). In the second season 2014/2015, the highest percentage of flavonoids was 5.13 % observed in Menshah district, while the lowest percentage observed also in Qurna district (1.13 %). About the results of antioxidants which recorded highest percentage in Bearaat district (93.50%) , with brown color , while the lowest in Qurna district(86.27%) . About the results of waxes which recorded

highest percentage in Bearaat district 25% and the highest percentage of ash observed in Menshah district , while in second season 2014/2015 the highest percentage of waxes recorded in Menshah district 20 % and also the highest percentage of ash is 2% . Omar, 1994 reported that both honeybee wax and impurities in propolis determine its quality.

These results were came in agreement with Almeida-Aguiar *etal.*( 2015) who said brown propolis found in New Zealand has higher antioxidant or polyphenol than propolis from other regions. New Zealand propolis averaged 237mg per gram of Polyphenol and 152mg per grams of flavonoids. Flavanoids are well known for their antioxidant capabilities but the polyphenols in New Zealand .So stay tuned and check out some New Zealand propolis for a natural way to help with inflammation, digestive disorders, candida and more.

Table (3): The percentages of chemical composition of propolis samples collected during the first season 2013/2014 in Luxor Governorate .

Location	Propolis color	Flavonoids %	Waxes %	Ash %
Bearaat district	light brown	10.12	25.00	1.96
Qurna district	Brown	2.01	17.80	1.00
<i>Menshah district</i>	Dark brown	3.20	17.00	2.00

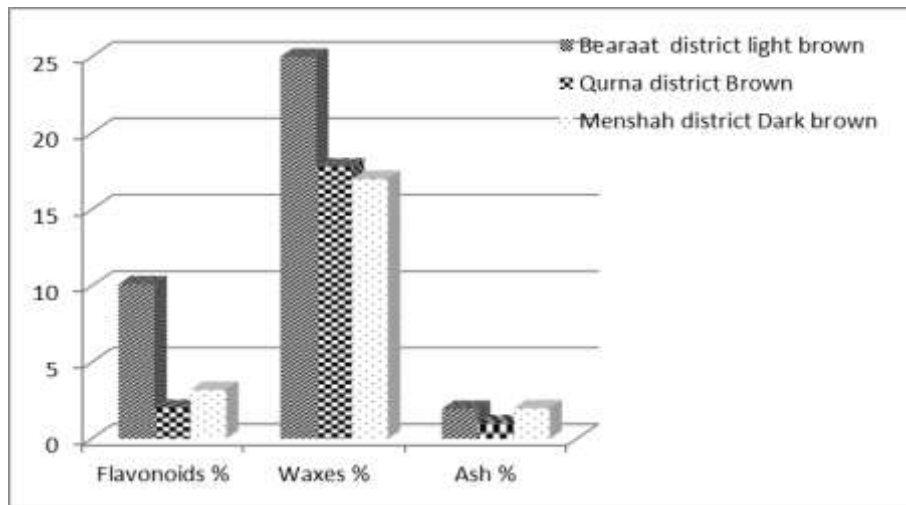


Fig (3): The percentages of chemical composition of propolis samples collected during the first season 2013/2014 in Luxor governorate .

Table (4): The percentages of chemical composition of propolis samples collected during the first season 2014/2015 in Luxor Governorate .

Location	Propolis color	Flavonoids %	Waxes %	Ash %
Bearaat district	Brown	3.88	11.10	1.96
Qurna district	Dark brown	1.13	17.80	0.99
<i>Menshah district</i>	White brown	5.13	20.00	2.00

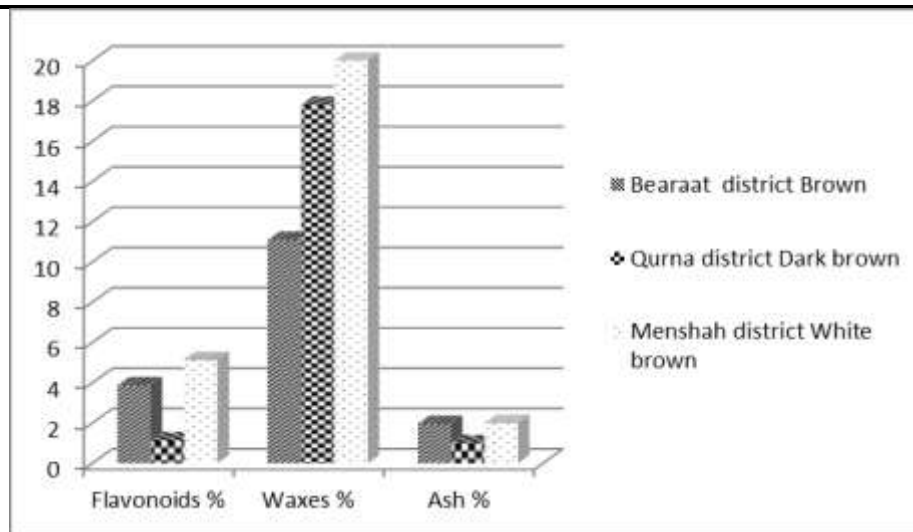


Fig (4): The percentages of chemical composition of propolis samples collected during second season 2014/2015 in Luxor governorate



Table (5): The percentages of chemical composition of Antioxidants in propolis samples collected during the first and the second season 2013/2014, 2014/2015 in Luxor Governorate .

Location	Propolis color	Antioxidants	
		first season 2013/2014	second season 2014/2015
Bearaat district	light brown	90.20	93.50
Qurna district	Brown	91.56	86.27
Menshah district	Dark brown	90.88	92.13

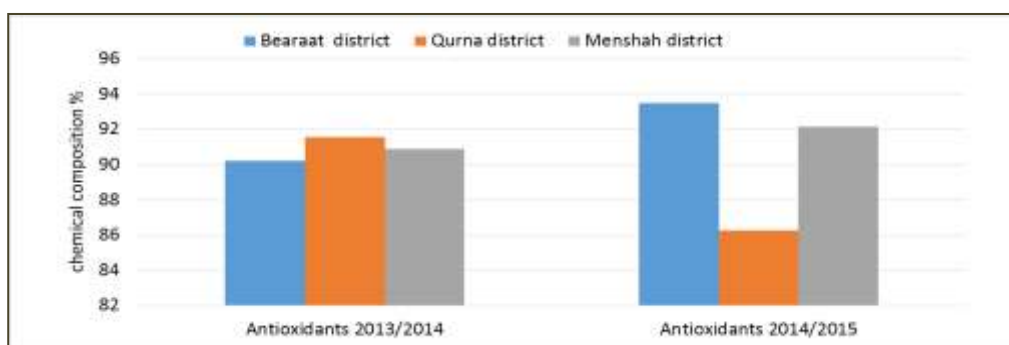


Fig (5): The percentages of chemical composition of Antioxidants in propolis samples collected during the first and the second season 2013/2014, 2014/2015 in Luxor Governorate .

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## الملخص العربي

خصائص البروبوليس الذي تجمعه طوائف نحل العسل *Apis mellifera L* في منطقة الأقصر – مصر العليا

تناولت الدراسة العديد من العوامل التي تؤثر على إنتاجية البروبوليس ، وقد استغرقت الدراسة موسمين (2013/2014 و 2014/2015) حيث اجريت الدراسة على ثلاث مناحل خاصة في مناطق (البعيرات ،القرنة والمنشأة) بمحافظة الأقصر .

وقد استهدفت الدراسة المحاور التالية:

- 1-مقارنة كميات البروبوليس التي جمعتها طوائف نحل العسل على مدار العام في مناطق الدراسة.
- 2-دراسة التحليل الكيماوي للبروبوليس المنتج من الثلاث مناطق وتقدير نسبة الفلافونيدات ومضادات الاكسدة والشمع والرماد .

وقد أوضحت النتائج ما يلي:

بالنسبة للكميات المجمعة من البروبوليس في الموسم الأول 2013/2014 فقد أظهرت النتائج أن أكبر متوسط لكمية البروبوليس جمعت في شهر يونيو حيث كانت 7,44 جم / طائفة في منطقة البعيرات .اما الموسم الثاني 2014/2015 ان أكبر متوسط لكمية البروبوليس هو ما تم جمعه خلال شهر يوليو (13,78 جم / طائفة) في منطقة البعيرات ايضا .

اما بالنسبة للتحليل الكيماوي لكميات البروبوليس المتحصل عليها على مدار السنة فقد تم إجراء بعض التحليلات الكيماوية للبروبوليس في معامل جامعة القاهرة- كلية الزراعة- معمل جودة وسلامة الغذاء لتقدير النسب المئوية لكل من الفلافونيدات- مضادات الاكسدة - الشمع - الرماد .

أن أعلى نسبة مئوية للفلافونيدات سجلت في عينات البروبوليس في منطقة البعيرات حيث كانت 10,12%. بينما كانت أقل نسبه (2,01%) في منطقة القرنة. أما فيما يخص النسبة المئوية لمضادات الاكسدة فقد أظهرت النتائج أن أعلى نسبة وجدت في منطقة القرنة (91,56%)، وأقل نسبة (90.22%) وجدت في منطقة البعيرات. كما أظهرت النتائج خلال الموسم الثاني 2014/2015 ان أعلى نسبة مئوية للفلافونيدات سجلت في عينات البروبوليس في منطقة المنشأة حيث كانت 5,13%. بينما كانت أقل نسبه (1,13%) في منطقة القرنة ايضا .

أما بخصوص النسبة المئوية لمضادات الاكسدة فقد أظهرت النتائج أن أعلى نسبة قدرت في منطقة البعيرات (93,50%) ، وأقل نسبة وجدت في عينات منطقة القرنة بنسبة 86,27% .

الكلمات الدالة: جمع البروبوليس ، التحليل الكيماوي لشمع النحل ،