

## EGYPTIAN BEE PROPOLIS FOR CONTROLLING SOME HONEYBEE DISEASES IN HIVES

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**ABSTRACT:** *The current work was conducted in honeybee apiary located in Diarb Negm, Sharkia governorate during 2012-2013. Bee propolis (bee glue) was collected from honeybee colonies by using propolis traps. Ethanolic extract was used for this study to evaluate honeybee colony strength (brood area (cm<sup>2</sup>)), Varroa infestation level and chalkbrood infestation level. The obtained results summarized that Italian bees (Apis mellifera ligustica) significantly collected more propolis than Carniolan bees (Apis mellifera carnica). It also concluded that ethanolic propolis extract significantly decreased Varroa infestation level, meanwhile, it didn't effect neither brood area (cm<sup>2</sup>) nor chalkbrood infestation level. The data recommended that ethanolic propolis extract may be used for protecting honeybee colonies from Varroa infestation.*

**Key words:** *Apis mellifera , honeybee, bee propolis , bee glue ,ethanolic extract*

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### INTRODUCTION

The name "propolis" is the result of combining two terms, Latin and Greek: *pro* which means "in front of" or "before" and *polis* which means "fortress". Propolis was used especially in antiquity in Egypt and in old Greek. The famous Greek philosopher Aristotle mentioned that bees did not want to reveal their "secrets" and they covered the inside transparent wall with a dark substance probably propolis. Apimondia, 1978.

Propolis, or bee glue, is a brownish resinous material collected by worker bees from the leaf buds of numerous tree species. In order to manufacture propolis, bees may also use material actively secreted by plants, or exuded from wounds in plants (lipophylic material on leaves, mucilages, gums, resins, lattices, etc.)

Propolis results from the addition of the mandibular secretions of bees to resins collected by these insects from different parts of plants. It is a structurally complex resinous, gum-like balsamic substance (Ghisalberti, 1979; and Burdock, 1998).

The antibacterial and antifungal activities are the most popular and among the most extensively investigated biological actions of propolis (Marcucci, 1995). The biological activities of propolis include antibacterial

(Grange and Darvey, 1990; Kujumgiev, *et al.* 1993; Menezes, *et al.* 1997 and Christov, *et al.* 1999)

Propolis extraction methods may influence its activity, since different solvents solubilize and extract different compounds. The most common extracts used in biological assays are ethanol, in different concentrations, methanol and water (Cunha *et al.*, 2004). Its chemical composition is very complex: more than 300 components have already been identified, and its composition is dependent upon the source plant and local flora. Moreover, propolis composition is completely variable creating a problem for the medical use and standardization (Marcucci, 1995; DeCastro, 2001).

(Ghisalberti, 1979; and Burdock, 1998) found that bee propolis contains approximately 50–55% resins and balsams, 30% wax, 10% volatile oils, 5% pollen, and 5% other substances that vary according to the flora of the region and the bee species. Inside the hive, propolis is used by bees to line internal walls and seal possible openings to allow thermal control of the colony and prevent the entry of other insects. In addition, propolis is used to embalm dead insects and to prevent the proliferation of microorganisms in the colony.

It has been suggested that the therapeutic activities of propolis depend mainly on the presence of flavonoids (Havsteen, 1983). Flavonoids have also been reported to induce the immune system (Wleklik *et al.*, 1997; Orsolich and Basic, 2003),

Propolis usually contains different chemical compounds depending geographic regions such as phenolic acids and esters, flavonoids (flavones, flavanones, flavonols, dihydroflavonols, chalcones), terpenes, amino acids, caffeic acid phenyl esters, aromatic aldehydes and alcohols, fatty acids, stilbenes and steroids (Cirasino *et al.*, 1987; Monti *et al.*, 1983; Sorkun *et al.*, 2001). Flavonoids and various phenolic compounds are the most important pharmacologically active constituents in propolis and have been shown to be capable of scavenging free radicals, thereby protecting lipids and other compounds (such as vitamin C) from being oxidized or destroyed during oxidative damage (Hegazi and Abd El Hady, 2002).

Propolis cannot be used as raw material and it must be purified by extraction to remove the inert material and preserve the polyphenolic fraction. Indeed, this fraction is considered to contribute more to the therapeutic effects than the other components of propolis. Ethanolic propolis extracts were used as antioxidant capsules, free throat spray, and ingredient in cosmetics and toothpaste (Velikova *et al.*, 2001; Kosalec *et al.*, 2003; Marquele *et al.*, 2006).

Propolis has attracted researchers' interest in the last decades because of several biological and pharmacological properties, such as, antitumor, antimicrobial, antioxidant, among others (Banskova, *et al.* 2000).

Helmy, *et al.*, 2000 mentioned that there were relationship between varroa mite and chalkbrood fungus infestations in honeybees during variable ecological conditions and colony performance.

The ability to develop resistance to a wide range of pesticides is a widespread

phenomenon among the mites, so it was almost inevitable that Varroa would become resistant against the commonly used acaricides such as the pyrethroids, fluvalinate (Apistan) and flumethrin (Bayvarol), (Martin, 2004).

The current study aimed to evaluate Egyptian propolis as a protective material in hives to protect honeybee colonies from any honeybee diseases.

## **MATERIALS AND METHODS**

This current work was conducted in a private apiary located at Diarb Negm, Sharkia governorate during 2011-2013. Bee propolis was collected by using propolis traps to minimize their contamination.

### **1. Honey bee colonies:**

Eighteen honeybee colonies were prepared in this study. They were equal in strength. Each colony contained about eight combs (frames) (six brood combs, and two honey and pollen combs) and a side feeder. Nine of them were headed by Carniolan queens (*Apis mellifera carnica*) (group A) and the other nine were headed by Italian queens (*Apis mellifera ligustica*) (group B).

### **2. Propolis collection:**

Egyptian propolis was collected from experimental honeybee colonies by using propolis traps to minimize their contamination. Propolis samples were stored at 18°C for frozen in a deep freezer.

### **3. Preparation of ethanolic propolis extract:**

The propolis ethanol extract was prepared as described by (Alencar *et al.* 2007), with some modifications. Collected propolis was crushed in a blender and mixed with 100 ml. of ethanol 80g/100 ml. and then placed in water bath at 50°C under mechanical stirring for 30 min. Extracts were made by mixing 20 g crude propolis with 80g (96% ethanol) with intermittent shaking at room temperature in the dark for a week. Residues were kept in a closed color glass vial at 4°C until using. The filtrate was named ethanolic extract of propolis, about

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150 g sample of propolis were used in this study.

### **4. Propolis extract spraying:**

Ethanol propolis extract were used in this study. About 100 ml. of extract was used for each colony as spray, with 10 days intervals for 6 months to evaluate ethanol propolis extracts, the following measurements were examined.

- Brood rearing in cm<sup>2</sup>.
- Chalk brood infestation level.
- Varroa infestation level.

The obtained data were calculated and tabulated.

### **5. Brood area:**

Sealed worker brood area in cm<sup>2</sup> was measured in experimental honeybee colonies at 12-day intervals using a plastic sheet divided into square cm.

### **6. Varroa infestation level:**

About 5 x 5 cm of sealed worker brood area in the middle of brood comb in each experimental colony was used. The sealed brood cells were scratched and Varroa mites in each cell were counted and recorded. The infestation percentage (I.P.) was calculated (Ritter, 1981).

### **7. Chalkbrood infestation level:**

A piece of sealed worker brood area measured 5 x 5 cm in each experimental colony was used to determine chalk brood infestation level. The mummified larvae were recorded (Koenig *et al.* 1986). The percentage of infestation level on brood or number of infested cells/ cm<sup>2</sup>.

### **8. Experimental Design and analysis:**

The experimental design was a completely randomized design. Results were analyzed using SAS (SAS institute, 1988). The general liner modules procedures to test for differences (Alpha=0.05) and applied the least significant Differences (LSD) as a mean separation test.

## **RESULTS AND DISCUSSION**

### **1. Propolis collection:**

The results in Table (1) show the amounts of propolis collected by Italian and Carniolan bees during 2011 year. The data indicated that the total amount of propolis collected was , 144.30 g , and 113.78 g for the Italian and Carniolan colonies, respectively. The results summarized that Italian colonies collected the highest monthly amounts of propolis (17.05 g./colony) as compared with Carniolan bees (13.10 g / colony). There were significant differences between Italian and Carniolan bees in propolis collection. The monthly mean amounts of collected propolis per colony ranged between 4.10 g in January and 17.05g in September with a general mean of 12.025 g for Italian bees , meanwhile, the monthly mean amounts of collected propolis per colony ranged between 3.87g in January and 13.10 g in November with a general mean of 9.481 g for Carniolan bees. The high amounts of propolis collected in Italian bees was (17.05g / Colony) in September while, it was (13.10g/ colony) for Carniolan bees in November. The low amount of collected propolis was (3.90 g /colony) in December for Italian bees, and it was (2.55g / colony) in December for Carniolan bees.

### **2. Propolis extract spraying:**

The results in Table (2) show that the total amount of sealed brood areas were 20021 and 1101.7 (cm<sup>2</sup>) for Italian and Carniola bees during successive months of the 2012 year. The whole of sealed brood were 1945.5, 1840.5, 3262.8, 2461.3, 4358.8 and 6152 (cm<sup>2</sup>) at the period from March till August, respectively for the Italian colonies. With averages of 1251.3(cm<sup>2</sup>)/colony. While the sealed brood were 1911.3, 1652.3, 2922, 2103, 4146 and 4892.8 (cm<sup>2</sup>) at the period from March till August, respectively for the Carniolan colonies. With averages of 1101.7(cm<sup>2</sup>)/colony. The results indicated that Italian colonies produced the highest monthly amounts of sealed brood 1251.3 (cm<sup>2</sup>)/colony followed by Carniolan 1101.7 (cm<sup>2</sup>)/ colony then control 822.7 (cm<sup>2</sup>)/colony. There were significant differences between the Italian and between Carniolan and Control Colonies.

The results showed that there were increasing in the sealed brood areas during the treatment of propolis spraying till the end of the experiment compared with control colonies.

The results in Table (2) showed that in general for all the experimental colonies the monthly mean percentage of infecting with chalkbrood disease per colony ranged between 0.0%:1.9% during the experiment .Both Italian and Carniolan bees showed no infection percentage of chalkbrood during the treatment, while the control colonies showed appearance of chalkbrood disease during April, May and June with mean 2.9, 4.2 and 0.9, respectively. It may returns to the using of spray propolis in the colonies.

The results in Table (2) indicated that the mean percentage of Varroa infestation ranged between 3.7 and 27.9% during the

treatment, while it was 27.9, 12 , 7.9 , 3.7 , 7.7 and 8.2% at the period from March till August , respectively for the Italian colonies. With averages of 4.22%/colony. While the mean percentage of Varroa infestation were 24.3, 13.5, 11.5, 4.4, 5.11 and 6.8%/colony at the period from March till August, respectively for the Carniolan colonies. With averages of 4.12%/colony. The results indicated that Carniolan infected more than Italian with spraying propolis. While it was 10.6% for control colonies. There were significant differences between the Carniolan and between Italian and Control Colonies.

According to the above results may propolis spraying caused in decreasing in the Varroa infestation percentage, increased in the sealed brood areas production, and non infectious of chalkbrood .

**Table (1): Mean weights of collected propolis (g /colony) for Carniolan and Italian honeybees during 2011.**

Months	Italian bees	Carniolan bees	Mean
January	4.100bc	3.870c	3.985
February	6.900b	6.010b	6.455
March	14.75a	10.20a	12.48
April	8.100	8.700b	8.400
May	12.40ab	10.30a	11.35
June	16.40a	11.10a	13.75
July	14.10a	11.40a	12.75
August	15.30a	12.10a	13.70
September	17.05a	12.70a	14.88
October	15.40a	11.75a	13.58
November	15.90a	13.10a	14.50
December	3.900c	2.550c	3.225
Total	144.3	113.78	129.04
Mean ± SD	12.025 ± 4.91	9.48 ± 3.51	-

Means in each column followed by different letter(s) are significantly different at 5% level.

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**Table (2): Effect of spraying ethanolic extract propolis (whole hive) on sealed brood area (cm<sup>2</sup> / colony), chalk brood and Varroa infestation level.**

Strains	Italian bees			Carniolan bees			control		
	Sealed brood area (cm <sup>2</sup> )	Chalk brood %	V. mites/ 100 cell	Sealed brood area (cm <sup>2</sup> )	Chalk brood %	V. mites/ 100 cell	Sealed brood area (cm <sup>2</sup> )	Chalk brood %	V. Mites/ 100 cell
04/03/2012	471.5	0.0	9.2	513.3	0.0	8.9	446.5	0.0	14.2
15/03/2012	725.8	0.0	9.8	651.5	0.0	7.8	488.3	0.0	15.2
27/03/2012	748.3	0.0	8.9	746.5	0.0	7.6	539.0	0.0	15.7
March total	1945.5 e	0.0	27.9	1911.3e	0.0	24.3	1473.8de	0.0	45.1
08/04/2012	776.5	0.0	6.7	763.3	0.0	7.3	551.5	1.2	16.3
20/04/2012	1064.0	0.0	5.3	889.0	0.0	6.2	564.8	1.7	15.5
April mean	1840.5e	0.0	12	1652.3e	0.0	13.5	1116.3e	2.9	31.8
03/05/2012	1144.8	0.0	3.1	921.5	0.0	4.9	631.5	1.9	14.2
15/05/2012	1064.8	0.0	2.9	997.3	0.0	3.7	789.8	1.2	11.8
27/05/2012	1053.3	0.0	1.9	1003.3	0.0	2.9	814.0	1.1	12.7
May total	3262.8 c	0.0	7.9	2922.0c	0.0	11.5	2235.3c	4.2	38.7
08/06/2012	1204.0	0.0	1.8	1039.0	0.0	2.3	789.8	0.9	10.9
20/06/2012	1257.3	0.0	1.9	1064.0	0.0	2.1	900.3	0.0	7.8
June total	2461.3 d	0.0	3.7	2103.0d	0.0	4.4	1690.0d	0.9	18.7
02/07/2012	1279.0	0.0	2.1	1149.8	0.0	1.9	996.0	0.0	7.2
14/07/2012	1530.8	0.0	2.4	1489.8	0.0	1.7	1000.8	0.0	6.9
26/07/2012	1549.0	0.0	3.2	1506.5	0.0	1.8	1024.0	0.0	6.2
July total	4358.8 b	0.0	7.7	4146.0b	0.0	5.4	3020.8b	0.0	20.3
07/08/2012	1990.8	0.0	3.1	1539.8	0.0	1.9	1139.8	0.0	5.9
19/08/2012	1956.5	0.0	2.2	1613.3	0.0	2.1	1199.0	0.0	5.1
31/08/2012	2204.8	0.0	2.9	1739.8	0.0	2.8	1288.5	0.0	4.2
August total	6152.0a	0.0	8.2	4892.8a	0.0	6.8	3627.3a	0.0	15.2
Grand total	20021	0.0	67.4	17627	0.0	65.9	13163	8.0	169.8
Grand Mean	1251.3	0.0	4.22	1101.7	0.0	4.12	822.7	0.5	10.6
LSD for months	450.8	-	-	354.2	-	-	310.5	-	-

Means in each column followed by different letter(s) are significantly different at 5% level.

**3. Treated combs:**

The results in Table (3) show that the total amount of sealed brood areas were 747.6 and 662.9 (cm<sup>2</sup>) for Italian and Carniolan during successive months of the 2013 year. The whole of sealed brood were 200.6, 189.8, 336.5, 253.8, 414.5 and 474.3 (cm<sup>2</sup>) at the period from March till August,

respectively for the Italian colonies. With averages of 116.8(cm<sup>2</sup>/colony). While the sealed brood were 197,170.3, 301.3, 216.8, 362.3 and 409.5 (cm<sup>2</sup>) at the period from March till August, respectively for the Carniolan colonies with averages of 103.5 (cm<sup>2</sup>/colony).

**Table (3): Effect of spraying ethanolic extract propolis (one comb) on sealed brood area (cm<sup>2</sup>/ comb), chalk brood and Varroa infestation level.**

Strains	Italian bees			Carniolan bees			control		
	Sealed brood area (cm <sup>2</sup> )	Chalk brood %	V. mites/100 cell	Sealed brood area (cm <sup>2</sup> )	Chalk Brood %	V. mites/100 cell	Sealed brood area (cm <sup>2</sup> )	Chalk Brood %	V. mites/100 cell
04/03/2013	48.6	0.0	2.3	52.9	0.0	3.3	46.0	0.0	9.6
16/03/2013	74.8	0.0	2.6	67.2	0.0	3.6	50.3	0.0	8.9
28/03/2013	77.2	0.0	2.6	77.0	0.0	3.9	55.6	0.0	8.6
March total	200.6 cd	0.0	7.5	197.0 e	0.0	10.8	151.9 d	0.0	27.1
09/04/2013	80.1	0.0	1.9	78.7	0.0	2.9	56.9	0.0	8.3
20/04/2013	109.7	0.0	1.8	91.7	0.0	2.6	58.2	0.3	7.3
April mean	189.8 d	0.0	3.7	170.3 e	0.0	5.5	115.1 e	0.3	15.6
03/05/2013	118.0	0.0	1.3	95.0	0.0	2.6	65.1	0.7	7.6
15/05/2013	109.8	0.0	1.0	102.8	0.0	2.9	81.4	1.0	6.3
27/05/2013	108.6	0.0	1.3	103.4	0.0	2.9	83.9	0.3	5.9
May total	336.5 b	0.0	3.6	301.3 c	0.0	8.4	230.5 c	2.0	19.8
08/06/2013	124.1	0.0	1.3	107.1	0.0	2.6	81.4	0.0	5.6
20/06/2013	129.6	0.0	1.0	109.7	0.0	2.3	92.8	0.0	4.9
June total	253.8 c	0.0	2.3	216.8 d	0.0	4.9	174.2 d	0.0	10.5
02/07/2013	131.9	0.0	1.0	118.5	0.0	2.3	95.2	0.0	4.3
14/07/2013	137.8	0.0	1.3	118.6	0.0	2.6	98.2	0.0	3.9
26/07/2013	144.7	0.0	1.3	125.3	0.0	2.3	105.6	0.0	3.3
July total	414.5 a	0.0	3.6	362.3 b	0.0	7.2	299.0 b	0.0	11.5
07/08/2013	152.7	0.0	1.3	131.2	0.0	2.3	110.0	0.0	3.0
19/08/2013	159.2	0.0	1.3	136.3	0.0	3.3	118.6	0.0	6.3
31/08/2013	162.3	0.0	1.6	141.9	0.0	2.9	127.8	0.0	6.9
August total	474.3 a	0.0	4.2	409.5 a	0.0	8.5	356.5 a	0.0	16.2
Grand total	1869.0	0.0	24.9	1657.3	0.0	45.3	1327.0	2.3	100.7
Grand Mean	116.8	0.0	1.6	103.5	0.0	2.8	83.0	0.1	6.3
LSD5%	60.4	-	-	41.3	-	-	37.1	-	-

Means in each column followed by different letter(s) are significantly different at 5% level.

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The results indicated that Italian colonies produced the highest monthly amounts of sealed brood 116.8 (cm<sup>2</sup>/colony) followed by Carniolan 103.5 (cm<sup>2</sup>/colony) then Control 83.0 (cm<sup>2</sup>/colony). There were significant differences between the Italian and between Carniolan and Control Colonies.

The results showed that there were increasing in the sealed brood areas during the treatment of propolis spraying till the end of the experiment compared with control colonies.

The results in Table (3) showed that in general for all the experimental colonies the monthly mean percentage of infecting with chalkbrood disease per colony ranged between 0.0%:2.0% during the experiment. Both Italian and Carniolan bees showed no infection percentage of chalkbrood during the treatment, while the control colonies showed appearance of chalkbrood disease during April, and May with mean 0.3 and 2.0, respectively. It may returns to the using of spray propolis in the colonies.

The results in Table (3) indicated that the mean percentage of Varroa infestation ranged between 2.3 and 27.1% during the treatment, while it was 7.5 , 3.7 , 3.6 , 2.3 , 3.6 ,and 4.2 , % at the period from March till August , respectively for the Italian colonies. With averages of 1.6%/colony. While the mean percentage of Varroa infestation were 10.8 , 5.5 , 8.4 , 4.9 , 7.2 , and 8.5%/colony at the period from March till August, respectively for the Carniolan colonies. With averages of 2.8%/colony. The results indicated that Carniolan infected more than Italian with spraying propolis. While it was 6.3% for control colonies. There were significant differences between the Carniolan and between Italian and Control Colonies.

According to the above Data, the current research recommended to use propolis extracts as a protective material for honeybee colonies, not only as a material but as a spraying for all colonies, recommended researches to spot on the produced materials of colony in treatment.

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## استخدام صمغ النحل المصري للسيطرة على بعض الأمراض في خلايا نحل العسل

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### المُلخَص العربي

أجريت الدراسة الحالية في منحل خاص لنحل العسل بمركز ديرب نجم، بمحافظة الشرقية خلال فترة 2012-2013. البروبوليس (صمغ النحل) تم جمعه من طوائف نحل العسل باستخدام مصائد البروبوليس. تم استخدام المستخلص الكحولي للبروبوليس في هذه الدراسة لتقييم قوة طوائف نحل العسل (مساحة الحضنة/سم<sup>2</sup>) وكلا من مستوى الإصابة بطفيل الفاروا ومرض الحضنة الطباشيرى. وكان من أهم النتائج المتحصل عليها أن طوائف النحل الإيطالي جمعت كميات من البروبوليس (صمغ النحل) اكبر بفروق معنوية عن طوائف النحل الكرنيولى . كما خلصت النتائج إلى أن المستخلص الايثانولى للبروبوليس ادى لانخفاض مستوى الإصابة بالفاروا ، وفي الوقت نفسه، لم يؤثر على مساحة الحضنة المقفولة او مستوى الإصابة بمرض الحضنة الطباشيرى. وتوصى الدراسة باستخدام المستخلص الايثانولى للبروبوليس كمادة لحماية طوائف نحل العسل من الإصابة بالفاروا والامراض الاخرى.