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## **EFFECT OF BROOD PHEROMONES ON SOME CHARACTERISTICS OF HONEY BEE QUEENS.**

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### **ABSTRACT**

The present study was carried out in private apiary in El-Kady village, Minia Governorate, from April to May in two seasons 2019 and 2020 to study the effect of brood pheromones on some characteristics of honey bee (*Apis mellifera* L.) queens. Six brood pheromones (Ethyl Palmitate, Methyl Linoleate, Methyl Linolenate, Methyl Oleate, Methyl Palmitate and Methyl Stearate) were added to melted wax in concentration of  $10^{-3}$  to make wax cups for queen rearing. The results indicated that Ethyl Palmitate, Methyl Linoleate, Methyl Linolenate, Methyl Palmitate and Methyl Stearate were effective in improving some characteristics of honey bee virgin queens such as body weight (mg), Length and width of head capsule (mm), number of ovarioles and spermatheca volume (SV) than the control. Methyl Linoleate, Methyl Palmitate and Methyl Stearate were more effective than the rest of the treatments in the two experimental seasons. Concerning the increasing percentages Ethyl Palmitate, Methyl Linoleate, Methyl Linolenate, Methyl Oleate, Methyl Palmitate and Methyl Stearate had increasing rate of 2.52, 4.41, 2.26, 0.55, 4.55 and 4.22 % for body weight, respectively but for length of head capsule (mm) were 2.59, 8.72, 2.86, 7.90, 7.63 and was decreasing for Methyl Oleate by -1.23 %. While the mean values percentages of head capsule width (mm) were 1.94, 4.85, 2.22, 4.44, 4.30 increasing and it was decreasing for Methyl Oleate -0.28 %. In regard to the number of ovarioles the mean values were 6.48, 16.93, 6.18, 13.46, 12.58 increasing and it was decreasing for Methyl Oleate -0.88 %; as for spermatheca volume ( $\text{mm}^3$ ) they were 5.91, 12.20, 5.12, 0.79, 11.42 and 11.02%, respectively compared with the control.

**Keywords:** Brood pheromones - queen rearing - fatty acid esters - queen cups.

## INTRODUCTION

Honey bees (*Apis mellifera* L.) play an important role in the world by pollinating both wild flowers and several crop plants because they feed mainly on nectar and pollen as well as manufacturing honey and secrete wax.

Queen is the most important member in bee colony. The colony efficiency can be recognized with the queen bee efficiency (Walsh and Rangel, 2016).

Honey bee larvae of queens are fed on the secretions formed by the nurse bees' hypopharyngeal and mandibular glands (Crailsheim *et al.*, 2013).

Getting quality queen bees may help to improve the efficiency of honey bee colonies. However, the quality of a queen differs depending on many physical properties, such as emergence weight, spermatheca diameter and sperm number stored in the spermatheca (Hatjina *et al.*, 2014). Physical characteristics influencing the efficiency of the queen were determined by different factors such as brood pheromones, feeding, age of transferred larvae, breeding season and genotype (Hu *et al.*, 2018; De Souza *et al.*, 2019; Njeru *et al.*, 2017; Uçak Koç and Karacaoğlu, 2011).

There is confirmation in the honey bee of a complex system of chemicals emitted by larvae to adapt the behaviour and physiology of workers to the requirements of the brood (Slessor *et al.*, 2005). A chemical signal on the surface of

larvae named brood pheromone (BP) is necessary for the communication between brood and worker bees. BP is a blend of ten fatty-acid esters (methyl palmitate, methyl oleate, methyl stearate, methyl linoleate, methyl linolenate, ethyl palmitate, ethyl oleate, ethyl stearate, ethyl linoleate and ethyl linolenate) (Le Conte *et al.*, 1990).

Modulation of the amounts and concentrations of these 10 esters, depending on the age of the larvae, is a chemical signature that allows adult workers to identify young or old larvae (Le Conte *et al.*, 1994).

The aim of the present work was to improve characteristics of honey bee reared queens in honey bee colonies in Minia government by using some brood pheromones.

## MATERIALS AND METHODS

This study was carried out in private apiary in El-Kady village, Minia Governorate, from April to May in two seasons (2019 and 2020).

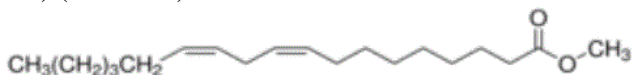
### 1. Experimental bees:

Twenty-one colonies of 1st Carniolan bees (*Apis mellifera* L.) were randomly chosen and equalized in strength (three brood combs and two honey and pollen combs all were covered with bees). The experimental colonies were divided to six treatments and control with three replicates each.

## 2. Treatments and its Preparation:

### 2.1. Brood pheromones:

- Methyl Linoleate (ML) (C<sub>19</sub>H<sub>34</sub>O<sub>2</sub>)



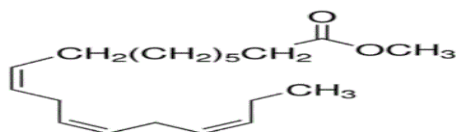
- Methyl Palmitate (MP) (C<sub>17</sub>H<sub>34</sub>O<sub>2</sub>)



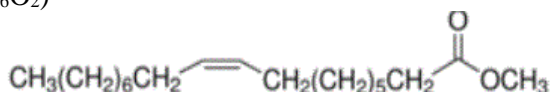
- Methyl Stearate (MS) (C<sub>19</sub>H<sub>38</sub>O<sub>2</sub>)



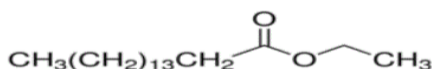
- Methyl Linolenate (MLN) (C<sub>19</sub>H<sub>32</sub>O<sub>2</sub>)



- Methyl Oleate (MO) (C<sub>19</sub>H<sub>36</sub>O<sub>2</sub>)



- Ethyl Palmitate (EP) (C<sub>18</sub>H<sub>36</sub>O<sub>2</sub>)



These pheromones were used in concentration of 10<sup>-3</sup> in bee wax where were obtained from Arabian Group for International Technology (Unit 10, 2<sup>nd</sup> Floor, Building S3, Downtown complex, 90<sup>th</sup> Street, Fifth Settlement, New Cairo-Egypt).

- Control: wax cups were used without brood pheromones.

### 3. Preparation of colonies for rearing virgin queens:

Queen rearing was carried out by using grafted method of **Laidlaw (1975)**

### 3.1. Queen cups:

Wax cups were prepared by melting bee wax in water bath at 60°C and the pheromones were added to melted wax at a concentration of 10-3 for each pheromone separately according to the method of Le Conte et al (1995). Queen cups forming stick was dunked in cold water then put it in melted wax. This step was repeated more than one time and dipped it in cold water again so that the internal diameter of these cups is 6.8 mm at base; 8.6 mm at the mouth and 8.8 mm depth (Abrol *et al.*, 2005). After the wax cups were formed, they were twisted and pulled out. Fifteen queen cups were fixed into grafting frame by slopping a thin layer to bee wax on the two wooden bars.

### 3.2. Grafting technique:

Langstroth frames with wax cups were placed in queenless colonies 24-hr. before grafting as nurse bees polish wax cups. In the next day, brood comb contained newly hatched larvae (24 hr.) was taken from breeding colony and translated to lab for grafting larvae in queen cups containing royal jelly. Frames containing larvae were placed in the middle of each queenless colony. The queen cups were sealed by bees after ten days from grafting and individually covered by half ball cages before virgin queen emergence to protect them from killing.

### 4. Measurements of reared queens:

- **Body weight of virgin queens (mg)** was weighted using sensitive electronic balance with 0.0001.
- **Length and width of head capsule (mm)** were measured by

stereoscopic microscope with micrometer eye piece (1/10 mm) (Atallah, *et al.*, 1987 and Eshbah, *et al.*, 1994)

- **Number of ovarioles** were counted in the right ovary of virgin queen according to the method of Hassona and Mourad (2016).
- **Spermatheca volume (SV)** was measured according to (Hatch *et al.*, 1999).  $SV = (4/3) (\pi) (r^3)$ . Where r = average radius of the r for the length and the r for the width of the spermatheca and  $\pi = 3.14$

### 5. Statistical analysis:

Data were statistically analyzed using Costat program software (Mead *et al.*, 1993). With ANOVA and the differences between means and their interactions were examined using Duncan's test. Variation (Increasing or decreasing) percentages than control was calculated by the following equation of Al-Daikh *et al.* (2017).

$$\text{Variation\%} = \frac{\text{Treatment-control}}{\text{control}} \times 100$$

### RESULTS AND DISCUSSION

The effect of brood pheromones on body weight of virgin queen, was presented in Table (1). Results indicated that usage of brood pheromones at a concentration of 10-3 per each pheromone lead to increased weight of virgin queens. The highest value (164.30 mg) of mean body weight of virgin queen was obtained when reared in wax cups mixed with Methyl Palmitate and Methyl Linoleate was followed by Methyl Stearate, Ethyl Palmitate, Methyl Linolenate and Methyl Oleate which gave 164.00, 161.07, 161.07 and

158.10 mg, respectively while the lowest one was 157.26 mg for the control during season 2019. On the other hand, the highest value was 164.60 mg for Methyl Palmitate followed by Methyl Linoleate (164.17), Methyl Stearate (163.88), Ethyl Palmitate (161.45), Methyl Linolenate (160.64) and Methyl Oleate (158.22) mg, while the lowest one was 157.34 mg for control during season 2020. Statistical analysis showed no significant difference among Methyl Linoleate, Methyl Palmitate and Methyl Stearate which gave the highest values also no significant differences between mean weights of virgin queens reared in wax cups mixed with Ethyl Palmitate and Methyl Linolenate. Also, no significant difference had been noticed between Methyl Oleate and Control. In fact, similar trend was noticed concerning the effect of the applied treatments on weigh of virgin queens in the two experimental seasons.

Results in Table (1) showed that increasing percentage in body weight of virgin queen than control ones in Methyl Palmitate, Methyl Linoleate, Methyl Stearate, Ethyl Palmitate, Methyl Linolenate and Methyl Oleate treatments were 4.55, 4.41, 4.22, 2.52, 2.26 and 0.55 %, respectively compared with control treatment.

Regarding the effect of brood pheromones on number of ovarioles (Table, 2), results showed that the highest number of ovarioles in the right ovary of virgin queens reared was 140.37 for Methyl Linoleate treatment followed by Methyl Palmitate (135.98), Methyl Stearate

(134.58), Ethyl Palmitate (127.50) and Methyl Linolenate (126.76) while the control and Methyl Oleate gave the lowest values (120.18 and 119.12, respectively) during season 2019. Similar trend was noticed in the second season (2020) where Methyl Linoleate scored the highest number (141.46) of ovarioles in the virgin queens reared followed by Methyl Palmitate, Methyl Stearate, Ethyl Palmitate and Methyl Linolenate which gave 137.47, 136.76, 129.15 and 129.15, respectively while the control and Methyl Oleate gave the lowest values which were 120.84 and 119.79, respectively. Statistical analysis in the two experimental seasons showed no significant difference among Methyl Linoleate, Methyl Palmitate and Methyl Stearate also no significant differences were found between Ethyl Palmitate and Methyl Linolenate. At the same tune, no significant difference had been noticed between Methyl Oleate and the Control.

Concerning the increasing percentage in number of ovarioles results in Table (2) showed that values of increasing percentage than control ones in Methyl Linoleate, Methyl Palmitate, Methyl Stearate, Ethyl Palmitate and Methyl Linolenate treatments were 16.93, 13.46, 12.58, 6.48 and 6.18 %, respectively while the treatment of Methyl Oleate showed a decreasing percentage of - 0.88 % compared with the control treatment.

Regarding the spermatheca volume shown in Table (3), Methyl Linoleate, Methyl Palmitate, Methyl Stearate, Ethyl Palmitate, Methyl

Linolenate, Methyl Oleate and control gave the descending values of 1.42, 1.42, 1.41, 1.34, 1.33, 1.28 and 1.27 mm<sup>3</sup>, respectively during season 2019 while they were 1.43, 1.41, 1.41, 1.35, 1.34, 1.28 and 1.27, respectively during season 2020. There were significant differences among all treatments as compared with control and Methyl Oleate.

Concerning the increasing percentages in spermatheca volume results in Table (3) showed that the values of increasing percentage than control ones for Methyl Linoleate, Methyl Palmitate, Methyl Stearate, Ethyl Palmitate, Methyl Linolenate

and Methyl Oleate treatments were 12.20, 11.42, 11.02, 5.91, 5.12 and 0.79 %, respectively.

The results concerning the number of ovarioles and spermatheca volume may be attributed to positive effect of the brood pheromones in increasing weight of virgin queen in the two seasons (Yun-Feng *et al.*, 2010).

The pervious increasing can agreement with Hu *et al.*, (2018) who stated that the number of ovarioles in one ovary was increased significantly when the queens were reared with the mixture of three brood pheromone esters.

**Table (1): Mean body weight (mg) of virgin queens reared in wax cups mixed with brood pheromones during seasons 2019 and 2020**

Pheromones	Mean weight (mg)			Variation ± %
	Season 2019	Season2020	Overall Mean	
Ethyl Palmitate	161.07	161.45	161.26 b	2.52
Methyl Linoleate	164.30	164.17	164.24 a	4.41
Methyl Linolenate	161.07	160.64	160.86 b	2.26
Methyl Oleate	158.10	158.22	158.16 c	0.55
Methyl Palmitate	164.30	164.60	164.45 a	4.55
Methyl Stearate	164.00	163.88	163.94 a	4.22
Control	157.26	157.34	157.30 c	

\* Means in the same column followed by the same letter are not significantly different (p>0.05).

**Table (2): Mean number of ovarioles of right ovary of virgin queens reared in wax cups with some pheromones during seasons 2019 and 2020**

Pheromones	Mean number			Variation ± %
	Season 2019	Season2020	Overall Mean	
Ethyl Palmitate	127.50	129.15	128.33 b	6.48
Methyl Linoleate	140.37	141.46	140.92 a	16.93
Methyl Linolenate	126.76	129.15	127.96 b	6.18
Methyl Oleate	119.12	119.79	119.46 c	- 0.88
Methyl Palmitate	135.98	137.47	136.73 a	13.46
Methyl Stearate	134.58	136.76	135.67 a	12.58
Control	120.18	120.84	120.51 c	

\* Means in the same column followed by the same letter are not significantly different (p>0.05).

**Table (3): Mean spermatheca volume (mm<sup>3</sup>) of virgin queens reared in wax cups with some pheromones during seasons 2019 and 2020**

Pheromones	Mean volume (mm <sup>3</sup> )			Variation ± %
	Season 2019	Season 2020	Overall Mean	
Ethyl Palmitate	1.34	1.35	1.35 b	5.91
Methyl Linoleate	1.42	1.43	1.43 a	12.20
Methyl Linolenate	1.33	1.34	1.34 b	5.12
Methyl Oleate	1.28	1.28	1.28 c	0.79
Methyl Palmitate	1.42	1.41	1.42 a	11.42
Methyl Stearate	1.41	1.41	1.41 a	11.02
Control	1.27	1.27	1.27 c	

\* Means in the same column followed by the same letter are not significantly different ( $p > 0.05$ ).

Regarding the length and width of head capsule, Results in Tables (4) and (5) indicated that significant positive effect for head capsule length was noticed between Methyl Linoleate, Methyl Palmitate and Methyl Stearate (3.99, 3.95 and 3.93 mm, respectively during season 2019 while in season 2020 the values were 3.99, 3.97 and 3.97 mm, respectively) and for head capsule width the values were (3.78, 3.76 and 3.75 mm, respectively during season 2019 while in season 2020 were 3.78, 3.77 and 3.77 mm, respectively) with the same treatments. Also there were significant differences between Ethyl Palmitate (3.77 mm and 3.76 mm for head capsule length and 3.68 mm and 3.67 mm for head capsule width during seasons 2019 and 2020, respectively) and Methyl Linolenate (3.76 mm and 3.79 mm for head capsule length and 3.67 mm and 3.70 mm for head capsule width during seasons 2019 and 2020, respectively) with control (3.66 mm and 3.68 mm for head capsule length and 3.60 mm and 3.61 mm for head capsule width during seasons 2019 and 2020, respectively) but was no significant

difference between Methyl Oleate (3.62 mm and 3.63 mm for head capsule length and 3.59 mm and 3.60 mm for head capsule width during seasons 2019 and 2020, respectively) with control.

Increasing or decreasing percentages of length and width of head capsule were clarified in Tables (4) and (5). Treatments of Methyl Linoleate, Methyl Palmitate, Methyl Stearate, Ethyl Palmitate and Methyl Linolenate showed increased percentage of length and width of head capsule where their increasing values were 8.72, 7.90, 7.63, 2.59 and 2.86 % for length of head capsule and 4.85, 4.44, 4.30, 1.94 and 2.22 % for width of head capsule, respectively while the treatment of Methyl Oleate showed a decreasing percentage in length and width of head capsule by -1.23 and -0.28%, respectively compared with the control treatment ones.

The Present results were in agreement with those reported by Hu *et al.*, (2018) who concluded that the body weight of the newly-emerged queens of *Apis cerana cerana* was significantly increased in treatments

with a mixture of three brood pheromone esters (3E, 1.0% methyl linoleate, 16.0% methyl linolenate and 35.0% methyl oleate). Increasing of length and width of head capsule may be attributed to the increase in body

weight of virgin queen by brood pheromones. Moreover, the brood pheromones lead to the increase in royal jelly quantity which are consumed by larvae (Le Conte *et al.*, 1995).

**Table (4): Mean length (mm) of head capsule of virgin queens reared in wax cups with brood pheromones during seasons 2019 and 2020**

Pheromones	Mean length (mm)			Variation $\pm$ %
	Season 2019	Season2020	Overall Mean	
Ethyl Palmitate	3.77	3.76	3.77 b	2.59
Methyl Linoleate	3.99	3.99	3.99 a	8.72
Methyl Linolenate	3.76	3.79	3.78 b	2.86
Methyl Oleate	3.62	3.63	3.63 c	-1.23
Methyl Palmitate	3.95	3.97	3.96 a	7.90
Methyl Stearate	3.93	3.97	3.95 a	7.63
Control	3.66	3.68	3.67 c	

\* Means in the same column followed by the same letter are not significantly different ( $p>0.05$ ).

**Table (5): Mean width (mm) of head capsule of virgin queens reared in wax cups with some pheromones during seasons 2019 and 2020**

Pheromones	Mean width (mm)			Variation $\pm$ %
	Season 2019	Season2020	Overall Mean	
Ethyl Palmitate	3.68	3.67	3.68 b	1.94
Methyl Linoleate	3.78	3.78	3.78 a	4.85
Methyl Linolenate	3.67	3.70	3.69 b	2.22
Methyl Oleate	3.59	3.60	3.60 c	-0.28
Methyl Palmitate	3.76	3.77	3.77 a	4.44
Methyl Stearate	3.75	3.77	3.76 a	4.30
Control	3.60	3.61	3.61 c	

\* Means in the same column followed by the same letter are not significantly different ( $p>0.05$ ).

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### تأثير فرمونات الحضنة على بعض صفات ملكات نحل العسل

حصافي محمد عشبّة، عبد السلام أنور محمد، حسني محمد شفيق عبد السلام،  
أفنان عباس توني سليمان

أجريت الدراسة في منحل خاص يقع في قرية القاضي- مركز المنيا. محافظة المنيا واستمرت من شهر أبريل حتى شهر مايو خلال موسمي الدراسة 2019م و2020م، استخدمت في هذه الدراسة 21 طائفة من نحل العسل الهجين أول كرينولى واختيرت هذه الطوائف عشوائيا من المنحل وكانت متساوية في القوة (3 أقراص حضنه واثنان عسل وحبوب لقاح وجميع هذه الأقراص مغطاة بالنحل). ووزعت طوائف التجربة الى ست معاملات بالإضافة الى معاملة الكنترول بحيث احتوت كل معاملة على ثلاث مكررات. وكانت الفرمونات المستخدمة في الدراسة هي:

الميثيل لينوليت، الميثيل بالميتيت، الميثيل استريت، الميثيل لينولينيت، الميثيل اوليت، الايثيل بالميتيت. واستخدمت هذه المواد الكيميائية بتركيز  $10^{-3}$  لشمع المستخدم للكؤوس الشمعية. بالإضافة لمعاملة الكنترول: شمع بدون إضافة أي فرمون من فرمونات الحضنة. تم تجهيز الكؤوس الشمعية وإجراء عملية التطعيم عن طريق نقل اليرقات عمر اقل من 24 ساعة الى الكؤوس الشمعية المحتوية على غذاء ملكي.

• تم تسجيل القياسات التالية للملكات العذارى:

• وزن الملكات العذارى (مجم): باستخدام ميزان إلكتروني حساس 0.0001

- طول وعرض الرأس (مم): تقاس بمجهر ذو عدسة ميكرو ميترية (10/1 مم)
- عدد فروع المبيض: يعد في المبيض الأيمن للملكات العذارى
- حجم القابلة المنوية (مم<sup>3</sup>): طبقا للمعادلة  $SV = (4/3) (\pi) (r^3)$
- وكانت اهم النتائج المتحصل عليها كالتالي:

#### أولا تأثير فرمونات الحضنة على وزن الملكات:

أدت خلط فرمونات الحضنة مع شمع الكؤوس الي زيادة في وزن الملكات مقارنة مع الكنترول وكانت اعلي المعاملات هي الميثيل بالميتيت والميثيل لينوليت والميثيل استريت بمتوسطات 164.24، 163.94، 160.86 مللجرام على التوالي يلي ذلك معاملات الايثيل بالميتيت والميثيل لينوليت بمتوسطات 161.26، 160.86 مللجرام على التوالي في حين كان الكنترول (157.30) مللجرام والميثيل اوليت (158.16) مللجرام هو اقل المعاملات تأثير على وزن الملكات.

#### ثانيا تأثير فرمونات الحضنة على طول وعرض رأس الملكات كمتوسط للموسمين:

أظهرت النتائج ان اعلى قيمة لطول وعرض رأس الملكات كانت في المعاملة بالميثيل لينوليت حيث أعطت 3.99 مم بالنسبة للطول و3.78 مم بالنسبة للعرض يليها المعاملة بالميثيل بالميتيت ثم الميثيل استريت ثم الميثيل لينوليت ثم الايثيل بالميتيت حيث كانت النتائج هي 3.96، 3.95، 3.78، 3.77 مم على التوالي بالنسبة للطول و 3.77، 3.76، 3.69، 3.68 مم بالنسبة للعرض على التوالي مقارنة بالكنترول، إما الميثيل أوليت فكانت اقل من الكنترول في الطول (3.63) مم والعرض (3.60) مم.

#### ثالثا تأثير فرمونات الحضنة على عدد فريعات المبيض الايمن في الملكات العذارى:

سجلت اعلى متوسطات لعدد فريعات المبيض في معاملات الميثيل لينوليت، الميثيل بالميتيت، الميثيل استريت حيث كانت النتائج هي 140.92، 136.73، 135.67 على التوالي، يتبعها معاملات الايثيل بالميتيت والميثيل لينوليت والتي أعطت 128.33، 127.96 على التوالي مقارنة بالكنترول.

#### رابعا تأثير فرمونات الحضنة على حجم القابلة المنوية للملكات العذارى:

أوضحت قياسات حجم القابلة المنوية أن معاملات الميثيل لينوليت ، الميثيل بالميتيت ، الميثيل استريت ادت الى زيادة معنوية في حجم القابلة المنوية حيث حققت 1.43، 1.42، 1.41 مم على التوالي ، يليها معاملات الايثيل بالميتيت ، الميثيل لينوليت، الميثيل أوليت حيث كانت النتائج كالتالي 1.35 ، 1.34، 1.28 مم على التوالي مقارنة بالكنترول.

أوضحت النتائج أن استخدام فرمونات الحضنة التالية: الميثيل لينوليت، الميثيل بالميتيت، الميثيل استريت أدت إلى زيادة وزن وعدد فريعات المبيض وحجم القابلة المنوية وطول وعرض رأس الملكات المرباة.