

# Pollen Source's Effects on Honeybee Queen Rearing

Hala A.S. Abdel-Sattar<sup>1</sup>, Ibrahim I.H. Mesbah<sup>2</sup>, Khaled M.A. Abdel-Hameed<sup>1</sup>, Haitham M. Ramadan<sup>2\*</sup>

## ABSTRACT

Protein-rich diets are known to promote mandibular glands development in nurse workers of the honeybee, hybrid of *Apis mellifera carnica*. Since the main source of protein for honeybees is pollen, its quality, and digestibility might be important dietary factors determining glands secretions. Since the nurse workers feed the queens, the queen ovaries are affected by the nurse worker's nutrition. Also, the hemolymph is essential for immunological protection, honeybee chemical transport, and physiological state monitoring. The present work has compared the effect of four types of pollen (clover, Eucalyptus, Corn plant, and Brazilian pepper tree) upon morphological and physiological characteristics of honeybee queens reared at the previous pollen sources. In general, the highest mean of acceptance and mating rate of reared queens were recorded in the Eucalyptus pollen, and the highest mean of queens and virgin weight were observed in the Brazilian pepper pollen. Furthermore, there was a significant difference among means of head width, thorax, and ovarioles length which have a highest mean in the clover pollen, but a number of ovarioles and spermatheca size have the highest mean in the Brazilian pepper pollen, while the highest mean of abdominal length recorded in the *Zea mays*. In addition, the greatest means of haemocytes was in larvae fed on the *Zea mays* pollen and then the clover pollen. The predominant types of haemocytes observed were the plasmatocyte cells followed by granulocytes, then the prohaemocytes, coagulocytes, and oenocytes. This study provides a guide for Egyptian beekeepers to know the best seasons of plants for rearing and obtaining high-quality queens.

**Keywords:** Queen rearing, acceptance rate, mating rate, morphometric measurements, Hemolymph.

## INTRODUCTION

Honeybee colonies obtain proteins, lipids, minerals, and vitamins required for brood rearing, maturation, and development from pollen (Omar *et al.*, 2017). It is the main diet to nurse bees to develop hypopharyngeal glands (HPGs) and produce royal jelly to feed larvae (Renzi *et al.*, 2016). Furthermore, pollen quality and digestibility might be important dietary factors determining reproductive capacity (Human *et al.*, 2007). On the other side, the amount and quality of pollen consumed by nurse bees affect the development of hypopharyngeal glands that produce royal jelly which

are used to feed the queen larvae (Chuda-Mickiewicz and Samborski, 2019). Without this initial pollen which was consumed during 1<sup>st</sup> 10 days after the emergence of new adults, the brood rearing ability of these bees is drastically decreased, since the glands responsible for producing the food fed by nurse bees to the larvae remain underdeveloped and nonfunctional (Omar, 2006).

The success and quality of queen production depend on strong well-fed healthy nurse colonies (Büchler *et al.*, 2013). However, the colony's condition has a significant impact on the acceptance rate of queens or queen cells (Smilga-Spalvina *et al.*, 2024). Thus, the standards related to the queen quality are the hints such as weight at emergence, ovarian weight, number of ovarioles, and the diameter and volume of spermatheca (Gamal Eldin *et al.*, 2018). In addition, the reported range for ovariole number per ovary in honeybee queens is wide, from 100 to 180 ovarioles per ovary (Snodgrass, 1956).

Antimicrobial factors, mostly produced by the fat body and, to a lesser extent, haemocytes, enable it to play a defensive function by preventing the growth of germs through an encapsulation pathway (Feng *et al.*, 2014). Therefore, the availability of circulatory immune cells, plasmatocytes and granulocytes, was the physiological mechanism responsible for the effects of phagocytosis, encapsulation, and other related defense processes (Sanjayan *et al.*, 1996). On the other hand, the different blood cells, which constitute the foundation of an insect's immune system, are the most significant parts of blood (Fatehe *et al.*, 2021). Thus, changes in both the number of circulating haemocytes and in the relative proportions of different haemocyte types in the hemolymph have been accompanied by cellular immune reactions (Hink, 1970). This study tested the effect of varying pollen sources that the bees were fed (Clover (*Trifolium alexandrinum*), Eucalyptus (*Eucalyptus sp.*), Corn plant (*Zea mays*), and Brazilian pepper (*Schinus terebinthifolius*) tree) on the blood cell numbers of honeybee workers' larvae, and studied the numbers of different haemocytes at each season of these different pollen sources. Also, the present work was carried out to study the effect of the same different pollen sources

DOI: 10.21608/asejaiqsae.2025.417847

<sup>1</sup>Apiculture Department, Plant Protection, Institute Agriculture Research Center, Egypt

<sup>2</sup>Plant Protection Department, Faculty of Agriculture, Tanta University, Egypt.

\*Corresponding author: Dr. Haitham Mahmoud Ramadan

E-mail: hmamramadan@agr.tanta.edu.eg

Received February 05, 2025, Accepted March 16, 2025..

on the morphological and physiological characteristics of honeybee queens.

## MATERIAL AND METHODS

### 1. Apiary

This study was established at the experimental apiary of the El-Sabaheia research station, Alexandria (27.2831°N and 30.7632°E). The apiary was surrounded with Eucalyptus, Casuarina, and the Brazilian pepper trees for protection from winds during winter and early spring. The flora found in the apiary area comprised Eucalyptus sp., Schinus terebinthifolius, Zea mays, and clover. The tested honeybee was the hybrid of *Apis mellifera carnica*. The hives were one chambered typical Langstroth type. The study was conducted during season 2021 on the pollen flow season of the clover (*Trifolium alexandrinum*), that its blooming period is from after mid-May to mid-June, Eucalyptus (*Eucalyptus* sp.) from late June to mid-July, the Corn plant (*Zea mays*), which blooms from late July to early September and the Brazilian pepper (*Schinus terebinthifolius*) tree, that's blooms from late September to the end of October.

### 2. Queen rearing

In each pollen flow season of the four previous plants, the Doolittle method was used for queen rearing. The plastic cups were attached to the wooden bars of the standard grafting frame by means of molten beeswax. Each grafting frame had 3 bars with 15 cups each with a total of 45 cups. The wooden bars frame was placed in three queen rearing hives. On the second day, the number of queen cell accepted out of 45 cups was recorded and expressed either as a number of cups per 45 cups or as a percentage acceptance.

On the ninth day, all sealed queen cups were removed from their grafting frames and reintroduced into their experimental nucleus colony by direct insertion into the honeycomb. Individually, each queen cell was caged by a plastic half ball cage until queen emergence. Then, each newly emerged queen was individually caged in a wooden Benton cage with some attendants and a small piece of candy and quickly transferred to the laboratory to be weighed. Then, the weighted newly emerged queens returned to the nucleus colony (24 nuclei) and released them out of cages to allow this queen to mate. On the fifteenth or seventeenth day after release, the number of mated queens was counted either as a number of mated queens per 24 virgin queen or as a mating percentage.

### 3. Morphometric measurements of queen

Newly mated queens were obtained for morphological studies. The measured morphological parameters were the queen's weight as well as her abdominal length, head capsule width, thorax length, antennal length, front and hind wings lengths, and hind

legs lengths. Each queen was carefully dissected in a saline solution (NaCl, 0.09%). The right and left ovaries were removed and carefully isolated. In formalin solution (formalin 40%, ethyl alcohol 40%, and glacial acetic acid 100%) where ovaries were placed for two hours to separate the ovarioles, which were carefully washed with water to remove any residues. The loosened ovarioles were counted by a binocular microscope per ovary. The spermathecae were removed, freshly mounted, and examined under a binocular microscope and the diameters were measured by a standardized ocular micrometer.

### 4. Hemolymph

The hemolymph samples were collected from the worker's larvae on the 3rd or 4th larval stage. For hemolymph collection from the larvae aged days 4–5, a disposable glass micro-capillary pipette (5  $\mu$ L) was inserted to one side (two-thirds down from the head in the body) of the larva avoiding deep cuts and drawing hemolymph liquid by capillary action. We Used a clean pipette tip to transfer 10  $\mu$ l of hemolymph sample mixed with the incubation solution onto a hemocytometer, and used a compound microscope, to count, and record the number of haemocytes in at least 5 of the large squares of the central grid of the hemocytometer. The types of the haemocytes (prohaemocyte, plasmatocytes, granulocytes, coagulocyte, and oenocytoid) were differentiated based on the classification of Cho and Cho (2019). We calculated the mean number of haemocytes per ml of hemolymph volume for each pollen source reared larvae as follows:

The total volume of each square of the hemocytometer grid: (0.04 mm<sup>2</sup> area)  $\times$  (0.1 mm depth) = 0.004 mm<sup>3</sup>  $\times$  5 squares = 0.02 mm<sup>3</sup> = the total volume of all 5 counted squares.

(Sum of the total number of haemocytes in the 5 squares counted / the total volume of all 5 counted squares)  $\times$  2 dilution factor = the number of haemocytes/mm<sup>3</sup>  $\times$  1,000 = the number of haemocytes/ml of hemolymph.

### 5. Statistical analysis

The data were analyzed by one-way (ANOVA) for Completely Randomized Design (CRD) to determine the significance of differences among the mean values, least significant difference (L.S.D.) values were done at ( $P < 0.05$ ) level of significance (Steel and Torrie, 1981).

## RESULTS AND DISCUSSION

### 1. The acceptance rate of queen cups and mating success rate of queens:

Queen rearing activity was examined in four different seasons as shown in Table (1) the highest level of queen cups acceptance was during the Eucalyptus

season followed by the clover season then the Brazilian pepper and the less acceptance rate was in the *Zea mays* season within the total accepted queen cups 78, 71, 48 and 24, respectively.

On the same pattern, (Table 1) Eucalyptus pollen source recorded the highest level of the percentage of queen cells acceptance from the 45 queen cells presented to the queen less colony was 57.78%, followed by the clover pollen source, then the Brazilian pepper pollen source and the lowest level of percentage of queen cups acceptance was in the *Zea mays* pollen source with 52.29%, 35.56%, and 17.78%, respectively.

In the Table (1) the most effective plant source on the acceptance rate was the Eucalyptus season with mean of 26, followed by the clover season with a mean of 23.67 then, the Brazilian pepper season with mean of 16, and the lowest mean was *Zea mays* season of the 8, with significant differences between the plant sources.

Also, the mating rate of the queen was recorded. The greatest mating rate was at the Eucalyptus pollen source with a total of 18 cells, with the percentage of 75.00% followed by the clover pollen source with a total of 17 cells with the percentage of 70.83%, then the Brazilian pepper pollen source with a total of 16 with the percentage of 66.67%, and the lowest mating rate was at

the *Zea mays* pollen source with a total of 12 cells, with percentage of 50.00%. 24 colonies that were tested. There was a very high significance correlation between acceptance and mating rate ( $r = 0.965$ ,  $P = 0.035$ ) according to Cohen and Holliday (1982).

## 2. Evaluation of the honeybee queen and virgin queen' weight within different pollen sources.

The weights of honeybee queen and virgin queen which were reared at different pollen sources explained in Table (2) the highest rate of virgin queens' weight was in the Brazilian pepper followed by the clover, then Eucalyptus and the lowest rate was in the *Zea mays*, with a mean weight of 0.176 g, 0.147 g, 0.144 g and 0.130 g, respectively.

However, the highest rate of queen' weight was recorded in the Brazilian pepper with a mean weight of 0.240 g, followed by the clover with mean weight of 0.231 g, then the *Zea mays* with a mean weight of 0.211 g, and the lowest rate of queen' weight was in the Eucalyptus with a mean weight of 0.181 g. With significant differences among the means of the queen' weights and the virgin queen' weights, as shown in Table (2).

**Table 1. The acceptance rate of queen cups and mating success rate of queens**

Pollen sources	Acceptance rate		Mating rate	
	Total	Percentage	Total	Percentage
Clover	71a	52.59%	17	70.83%
Eucalyptus	78a	57.78%	18	75.00%
<i>Zea mays</i>	24c	17.78%	12	50.00%
Brazilian pepper	48b	35.56%	16	66.67%

For acceptance rate

L.S.D at 5% between plant source = 3.56

Pollen sources	Clover (23.67)	<i>Zea mays</i> (8)	Brazilian pepper (16)
Eucalyptus (26)	2.33	18*	10*
<i>Zea mays</i> (8)	15.67*		
Brazilian pepper (16)	7.67*	8*	

**Table 2. Effect of different pollen sources at the mean of queen and virgin queen' weight**

Pollen sources	Virgin queen		Queen	
	Mean weight (g)	SD	Mean weight (g)	SD
Clover	0.147 b	0.010	0.231 ab	0.024
Eucalyptus	0.144 b	0.015	0.181 c	0.018
<i>Zea mays</i>	0.130 b	0.009	0.211 bc	0.033
Brazilian pepper	0.176 a	0.017	0.240 a	0.014

### 3. The morphometric measurements of queens reared in different pollen sources

The included results in Table (3) explain that the greatest calculated means of ovarioles number was 188.67 branches counted at the Brazilian pepper pollen source, followed by 176.83 branches counted at the clover pollen source, then 145.83 branches counted at the *Zea mays* pollen sources, and the lowest mean was 112.83 branches recorded at Eucalyptus pollen source, with significant difference among means. There was a very high significant correlation between a number of ovarioles and queen weight ( $r= 0.997$ ,  $P= 0.003$ ) according to Cohen and Holliday (1982).

The highest mean of head capsule width was 3.65 ml, 3.53 ml, and 3.42 ml, and the lowest mean was 3.37 ml were recorded at the clover, *Zea mays*, Brazilian pepper, and Eucalyptus pollen sources, in respect Table (3), with significant differences among the means.

Also, the results in Table (3) revealed that the highest mean of thorax length was recorded at the clover pollen source at 4.70 ml, and the lowest mean was 4.20 ml at the Brazilian pepper pollen source, there was a significant difference among means.

The highest mean of abdomen length was registered at the *Zea mays* pollen source 11.28 ml, followed by the Brazilian pepper pollen source at 10.88 ml, then the clover pollen source at 10.62 ml, and the lowest mean was 9.13 ml at the Eucalyptus pollen source (Table 3), with a significant difference.

However, the highest mean of antennal length was observed at the clover pollen source 4.03 ml, and the lowest mean was recorded at the Eucalyptus pollen

source 3.57 ml (Table 3), with no significant differences among antennal length means.

The exhibited data in Table (3) illustrated that the highest mean of front wing length was observed at 9.91 ml, followed by 9.68 ml, 9.58 ml, and 9.53 ml recorded in the clover, *Zea mays*, Brazilian pepper, and Eucalyptus pollen sources, respectively. There were no significant differences in the front wings' mean length.

According to our results, the highest mean of hind wings was 6.95 ml observed at the clover, 6.87 ml at the *Zea mays*, 6.78 ml at the Brazilian pepper and the lowest mean was 6.66 at the Eucalyptus pollen sources (Table 3).

The results show that the highest mean of hind legs length was registered at the clover pollen source 12.52 ml, followed by the *Zea mays* pollen source 12.18 ml, then the Eucalyptus pollen source 11.73 ml, and the lowest mean was 11.57 ml recorded at the Brazilian pepper pollen source (Table 3), with no significant differences among the means of all measures mentioned.

Also, the results in Table (3) revealed that the highest mean of ovarioles length was 18.72 ml, followed by 17.50 ml, then 15.64 ml, and the lowest mean was 13.39 ml, at the clover, *Zea mays*, Eucalyptus, and Brazilian pepper pollen sources, respectively. With significant difference among means.

Moreover, the obtained results in Table (3) revealed that the highest mean of spermatheca size was 0.89 ml<sup>3</sup> recorded at the Brazilian pepper pollen source, followed by the *Zea mays* 0.82 ml<sup>3</sup>, then 0.65 ml<sup>3</sup>, and the lowest mean was 0.41 ml<sup>3</sup>, observed at the clover and Eucalyptus pollen source, respectively, with significant differences among means.

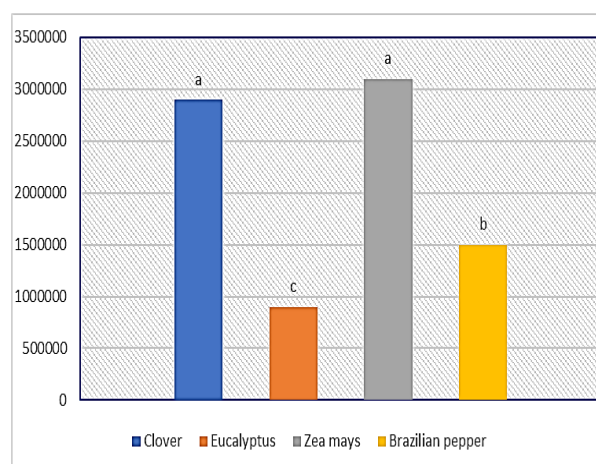
**Table 3: Effect of pollen sources on certain morphological characteristics of queens**

Plant source	Clover	Eucalyptus	<i>Zea mays</i>	Brazilian pepper	L.S.D at 5%
Morphometric measurements	Mean ± (S.D)	Mean ± (S.D)	Mean ± (S.D)	Mean ± (S.D)	
No. of ovarioles	176.83±40.05 ab	112.83±18.11 c	145.83±14.24 cb	188.67±36.16a	38.70
head capsule (ml)	3.65±0.08 a	3.37±0.05 cd	3.53±0.07 ab	3.42±0.21 cb	0.16
Thorax (ml)	4.70±0.34 a	4.47±0.25 ab	4.51±0.16 ab	4.20±0.38 b	0.39
Abdomen (ml)	10.62±0.71 a	9.13±0.27 b	11.28±0.48 a	10.88±0.85 a	0.82
Antenna (ml)	4.03±0.65	3.57±0.59	3.85±0.28	3.85±0.27	N.S
Front wings (ml)	9.91±0.52	9.53±0.45	9.68±0.62	9.58±0.24	N.S
Hind wings (ml)	6.95±0.29	6.66±0.20	6.87±0.17	6.78±0.31	N.S
Hind legs (ml)	12.52±0.68	11.73±0.62	12.18±0.69	11.57±1.02	N.S
Ovarioles length (ml)	18.72±3.14 a	15.64±2.17 ab	17.50±4.29 a	13.39±1.26 b	3.88
Spermathecal size (ml <sup>3</sup> )	0.65±0.08 b	0.41±0.06 a	0.82±0.12 a	0.89±0.16 a	0.15

#### 4. Haemocytes' immune impact in Honeybee larvae fed on different pollen sources

##### 4.1. Number of haemocytes in hemolymph

The included results in Fig. (1), showed that the greatest mean number of haemocytes were 3100000 cell/ml at the *Zea mays* pollen source, followed by the mean of the clover pollen source which was 2900000 cell/ml, then the Brazilian pepper pollen source mean it was 1500000 cell/ml, and the lowest mean number of haemocytes were 900000 cell/ml at Eucalyptus pollen source. There were significant differences among the means.



**Fig. 1. Number of haemocytes in hemolymph of larvae fed on different pollen sources**

##### 4.2. Number of different types of haemocytes in each plant source

As shown in Table (5) the highest mean of prohaemocytes was at the *Zea mays* pollen source 568850 cell/ml, followed by the clover pollen source, then the Brazilian pepper pollen source, and the lowest mean was at Eucalyptus pollen source, 348000 cell/ml, 199950 cell/ml, 90990 cell/ml, respectively, with a significant difference among means.

However, the highest mean of coagulocytes was at the *Zea mays* pollen source, then the mean at the clover pollen source, and the lowest means were at the Brazilian pepper and the Eucalyptus pollen source, there was 300080 cell/ml, 258100 cell/ml, 200100 cell/ml, and 99000 cell/ml, consecutively, with a significant difference.

Also, the highest mean of plasmatocytes was at clover pollen source, 1300070 cell/ml, and 1269140 cell/ml at the *Zea mays* pollen source, the lowest mean was at the Brazilian pepper, and the Eucalyptus pollen source, 499950 cell/ml, and 491040 cell/ml, respectively, with a significant difference among means.

While the highest mean of granulocytes was 941920 cell/ml recorded at the clover pollen source, and 699980 cell/ml, 499950 cell/ml, 199980 cell/ml recorded at the *Zea mays*, Brazilian pepper, and Eucalyptus pollen source, in respect, with a significant difference.

Oenocytes were observed with a highest mean number at the *Zea mays* pollen source, 261950 cell/ml, followed by 100050 cell/ml, and 51910 cell/ml recorded at the Brazilian pepper and clover pollen source, and the lowest mean was recorded at the Eucalyptus pollen source. It was 18990 cell/ml, with a significant difference.

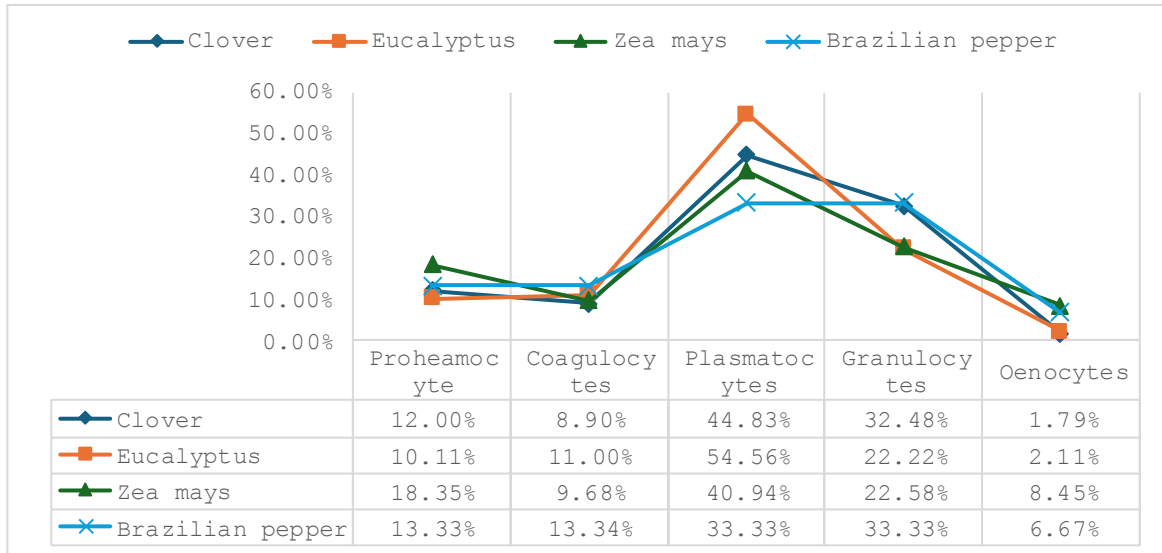
##### 4.3. Percentage of different types of haemocytes in each plant sources

According to the results presented in Fig. (2), the highest level of haemocytes percentage of larvae hemolymph fed on different plant sources was plasmatocytes in all the plant sources it was 54.56%, 44.83%, 40.94%, and 33.33% in the Eucalyptus, clover, *Zea mays*, and Brazilian pepper in respect. Plasmatocyte percentage is equal to Granulocyte percentages in the Brazilian pepper source only.

Plasmatocytes followed by Granulocytes percentage in all plant sources was 33.33%, 32.48%, 22.58%, and 22.22% in the Brazilian pepper, clover, *Zea mays*, and Eucalyptus, respectively.

**Table 4. Number of different types of haemocytes in each plant sources**

Plant sources	Clover	Eucalyptus	<i>Zea mays</i>	Brazilian pepper	L.S.D at 5%
Type of Hemocytes	Mean (Cell/ml) $\pm$ SD	Mean (Cell/ml) $\pm$ SD	Mean (Cell/ml) $\pm$ SD	Mean (Cell/ml) $\pm$ SD	
Prohaemocytes	348000 $\pm$ 29393.88b	90990 $\pm$ 16509.56d	568850 $\pm$ 44948.14a	199950 $\pm$ 43535.60c	81954.43
Coagulocytes	258100 $\pm$ 21800.46ab	99000 $\pm$ 17962.93c	300080 $\pm$ 23711.06a	200100 $\pm$ 43568.26b	65815.46
Plasmatocytes	1300070 $\pm$ 109810.63 a	491040 $\pm$ 89096.11b	1269140 $\pm$ 100282.1a	499950 $\pm$ 108855.32b	236020.6
Granulocytes	941920 $\pm$ 79559.43a	199980 $\pm$ 36285.11d	699980 $\pm$ 55309.48b	499950 $\pm$ 108855.32c	173161.3
Oenocytes	51910 $\pm$ 4384.58664c	18990 $\pm$ 3445.62c	261950 $\pm$ 20698.19a	100050 $\pm$ 21784.13b	35238.51



**Fig. 2. Percentage of different types of haemocytes in each plant sources**

Then proheamocytes only in the *Zea mays* was 18.35% and clover it was 12.00% but in the Brazilian pepper and Eucalyptus proheamocytes followed coagulocytes it was 13.33%, 13.34% in the Brazilian pepper, and it was 10.11% and 11.00% in the Eucalyptus pollen source, in respect.

And the lowest level of percentage was oenocytes in all pollen sources it was 8.45%, 6.67%, 2.11% and 1.79% in the *Zea mays*, Brazilian pepper, Eucalyptus and clover, respectively.

### 1. The acceptance rate of queen cups and mating success rate of queens

Our results showed that the greatest acceptance percentage was at the Eucalyptus pollen source (from late June to mid-July), followed by the clover pollen source (from after mid-May to mid-June) then the Brazilian pepper pollen source, and the lowest acceptance percentage was the *Zea mays* pollen source, with significant differences among acceptance percentages. These results were synchronized with those of Eissa *et al.* (2012) who found that there were significant differences in acceptance percentage among spring, summer, and late summer. And to some extent, it is consistent with the results of Ramadan (2023) that the highest rate of queen cells acceptance was during May and July followed by April and August then March and June while the less acceptance rate was in January followed by February and September in all the examined races and genotypes. The early summer season was the best period for acceptance of queen cells followed by spring then, the late summer period. There is a great significant acceptance rate between different

sources we reared the queen at them, and this result was close to Al-ghzawi and Zaitoun (2008) showed that the acceptance rate varied significantly by season but their result that the most successful mating took place in May, and the least successful between July and August is not close to our result that the greatest mating success rate was achieved at Eucalyptus pollen source and the lowest mating rate was at the *Zea mays* pollen source.

There was a high significance correlation between acceptance rate and mating rate, this result was close to that of Koç and Karacaoglu (2004) they noticed that when there is the high acceptance rate of grafted larvae, high mating success of queens occurred.

### 2. Evaluation of the honeybee queen and virgin queen' weight

The present study demonstrated that the mean weight of queens was significantly affected by rearing them in different seasons. This result was agreed with those of Gamal Eldin *et al.* (2018) who found that there was a significant difference in queen weight fed at different pollen diets. The highest mean weight of queen was recorded when queens were reared during the Brazilian pepper pollen source season. Mustafa *et al.* (2002) also mentioned that the mean weight of queens was significantly affected by rearing them in different seasons. He recorded the maximum weight in late summer (August and September). Also, the findings agreed with those of Elaidy *et al.* (2010) in which there was a significant difference among means of queen weight fed the different diets.

While statistical analysis indicated that the virgin weight was significantly affected by rearing them in different seasons. That was close to the results of Mustafa et al. (2002) who found that the weight of virgin queens was significantly affected by rearing them during different seasons.

### 3. The morphometric measurements of queens reared in different pollen sources

Generally, most of the morphometric characteristics of queens were significantly affected by rearing them at different pollen sources (clover, Eucalyptus, *Zea mays* and Brazilian pepper) except, antennal length, front wings, hind wings and hind legs have no significant difference. Also, Mustafa et al. (2002) showed that certain characteristics of the queens were significantly affected by rearing them in different seasons.

The mean length of the queen's abdomen was significantly affected by different pollen sources. Our results closed with those of Elaidy *et al.* (2010) who recorded a significant difference in abdominal length of queens reared in Winter fed a different diet. In Autumn, there was no significant difference among means.

However, there were significant differences among the means of spermatheca size recorded in this study, that not coincide with Chuda-Mickiewicz and Samborski (2019) they not find significant differences in the volume of spermatheca. Also, Koç and Karacaoglu (2004) recorded that the volume of spermatheca was not affected by season.

It could be noticed that there was a significant difference in the number and length of queen ovarioles. The obtained results may agree with those reported by Elaidy *et al.* (2010) who found that there was a significant difference in ovary length means in Autumn and Winter at queens fed on different diets, while there was a significant difference in Winter at means of ovarioles number but in Autumn there were no significant differences. Generally, there was a significant correlation between the number of ovaries and queen weight. It was close to the result of Mustafa et al. (2002) who recorded a relation between queen weight and a number of ovarioles. Versus, Hatjina *et al.* (2014) they noticed the absence of significant correlations between queen weight and ovarioles numbers.

### 4. Hemolymph

Changes in both the number of circulating haemocytes and in the relative proportions of different haemocyte types in the hemolymph have been accompanied by the cellular immune reactions (Hink, 1970). From the previous results, it was noticed that the predominant type of haemocytes of bees fed with the four tested pollen source types was the plasmatocytes

followed by granulocytes, then prohemocytes, coagulocytes, and oenocytes. These results agree with those of previous studies which mentioned that plasmatocytes were the most numerous cells in the hemolymph of young honeybee (Szymas and Jędruszek, 2003). Through the phagocytic process, bee hemolymph can directly destroy bacteria, fungus spores, and other tiny foreign molecules (Götz, 1986). Our results recorded the greatest means of hemolymph at the *Zea mays* and the clover pollen source.

### CONCLUSION

In conclusion, for beekeepers who are interested with queen rearing, Eucalyptus pollen source according to our study was the best in queen cells acceptance and mating activity among the other examined sources followed by the clover, Brazilian pepper, and *Zea mays* source. Furthermore, the best time for obtaining high quality queens during the Brazilian pepper source, clover, *Zea mays* and Eucalyptus source for all examined sources according to the data of the highest queen weight during the study. In addition, the Brazilian pepper recorded the highest rate of virgin queens' weight followed by the clover, Eucalyptus, and *Zea mays* source.

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## الملخص العربي

### تأثير مصادر حبوب اللقاح على تربية ملكات نحل العسل

هالة أبو المجد سليمان عبد الستار<sup>1</sup>، إبراهيم إبراهيم مصباح<sup>2</sup>، خالد محمد أحمد عبد الحميد<sup>3</sup>، هيثم محمود رمضان<sup>4</sup>

البرازيلي. علاوة على ذلك، كان هناك فرق كبير بين متوسطات عرض الرأس، وطول الصدر، وطول المبايض التي سجلت أعلى متوسط في مصدر حبوب لقاح البرسيم، في حين أن عدد المبايض وحجم القابلة المنوية كان لهما أعلى متوسط في مصدر حبوب لقاح الفلفل البرازيلي، بينما أعلى متوسط لطول البطن سجل في حبوب لقاح الذرة. بالإضافة إلى ذلك، كان أكبر متوسط لعدد خلايا الهيموليمف في البرقات التي تم إطعامها بحبوب لقاح الذرة، ثم حبوب لقاح للبرسيم. كانت الأنواع السائدة من خلايا الهيموليمف هي الخلايا البلازمية، تليها الخلايا المحببة، ثم الخلايا الأولية، والبلعمة، ثم خلايا الايونساييت. توفر هذه الدراسة دليلاً للمربين المصريين على أفضل مواسم النباتات لتربية الملكات والحصول على ملكات عالية الجودة.

الكلمات المفتاحية: تربية الملكات، معدل القبول، معدل التلقيح، قياسات مورفومترية، الهيموليمف.

تعرف التغذية الغنية بالبروتين انها تعزز تطور الغدد الفكية في الشغالات الحاضنة في نحل العسل، وبما أن المصدر الرئيسي للبروتين لنحل العسل هو حبوب اللقاح، فقد تكون جودتها وقابليتها للهضم عوامل مهمة تحدد إفرازات الغدد. وبما أن الشغالات الحاضنة هي من تقوم بتغذية الملكات، فإن مبايض الملكة تتأثر بتغذية الشغالات الحاضنة. أيضا يُعد الهيموليمف أساسياً للحماية المناعية، ونقل المواد الكيميائية في نحل العسل، ومراقبة الحالة الفسيولوجية. وقد قمنا بمقارنة تأثير أربعة أنواع من حبوب اللقاح وهي حبوب لقاح البرسيم، والكافور، ونبات الذرة، الفلفل البرازيلي على الخصائص المورفولوجية والفسيولوجية لملكات نحل العسل التي تم تربيتها على هذه المصادر المختلفة من حبوب اللقاح. بشكل عام، تم تسجيل أعلى متوسط لمعدل القبول ومعدل التلقيح للملكات التي تم تربيتها على حبوب لقاح الكافور، في حين تم ملاحظة أعلى متوسط لوزن الملكات والملكات العذراء في مصدر حبوب لقاح الفلفل