

## **Effect of Bar Level and Queen Cells Position within Grafted Frame on the Quality of Produced *Apis mellifera carnica* Queen in Manzala Region**

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### **ABSTRACT**

The variations exist in acceptance percentage of grafted larvae, emergence percentage and weight of virgin queens according to different grafted bar level and queen cells position of local carniolan race (*Apis mellifera carnica*) in Manzala region were investigated. No significant differences were found in the acceptance percentage, emergence percentage and weight of virgin queen for the three bar levels. However, the acceptance percentage was positively affected by minimum temperature for the top bar. The weight of virgin queens was positively affected by the change in minimum temperature on all bar levels. On the other hand, the tested parameters were significantly increased on the middle position of bar than on the edge. In spite of the percentages of acceptance and queen emergence were positively affected by the ambient temperatures elements they were more pronounced factors on the weight of emerged queens on the edge position.

### **INTRODUCTION**

The quality of honeybee queen is a major current interest depends on her genotype and the environmental conditions of the grafted larvae and the dequeened rearing colonies. The suitable larvae for queen rearing is also affected the increase of acceptance honeybee queen cells (Sahinler and Kaftanoğlu, 2005 and Cengiz *et al.*, 2009).

The number of accepted larvae depends on quality, strength and developmental stage of the nurse colonies, age of the workers, age of the grafted larvae. Also, the queen presence or absence in the rearing colony and the period of the orphaning, open brood existence in the cell-starting colonies, number of grafted cells and methodology of rearing were considered. Moreover, weather conditions affect the process and good management can produce acceptance more than 80% of grafted larvae (Ruttner, 1983).

Weight at emergence and other characters were used to describe the queen reproductive potential (Woyke, 1971 and Tarpay *et al.*, 2000). However the environmental factors inside the colony such as the level and position at which the queen cells presented and the temperature around the queen cells are recently subjected a critical elements to produce a good queens (Woyke, 1971; Abd Al-Fatah, 1983; Degrandi-Hoffman *et al.*, 1993; Abd Al-Fatah *et al.*, 2003; Zeedan, 2002; Tarpay *et al.*, 2004; Abd Alhady, 2007; Abd Al-Fatah *et al.*, 2011; Sharaf El-Din, 2016). Therefore, the effect of bar level and queen cells position on the quality of produced queens was the aim of this research.

### **MATERIALS AND METHODS**

This study was carried out in a private apiary at Meet Salseel, Manzala region, Dakahlia governorate which is considered as isolated area for rearing Carniolan race (*Apis mellifera carnica*), during the period from February, 16 to May, 14, 2016, to Compare the effect of the different bar levels within grafted frame and queen cells position on honeybee queen rearing. The following procedure was carried out; six queenless colonies of the Carniolan honeybee race were used for this study. Each of these colonies consisting of two brood combs (sealed brood). All the combs were covered with bees (about 10 thousands bees). The rearing colonies were provided with sugar syrup (1:1) during the experimental period.

Young larvae less than 36 hours old (depending on the size) were collected from combs which were selected from the center of brood nest. Grafted larvae were transferred

from the edge area of the brood comb into wax queen cups containing a drop of a 50% aqueous solution of royal jelly (Laidlaw and Page, 1997). Each grafted frame was constructed of three removable bars and each bar carried 25 queen cups. The grafted operation was undertaken in a room maintained at 25°C and 60% RH.

Rearing colonies were supported with two combs of capped brood weekly and were fed continuously with sugar syrup to stimulate both the production of royal jelly and secretion of wax.

#### **Measurements**

The number of accepted queen cells were counted after 24 hours of inserted into queen rearing colonies and the acceptance percentages / bar / colony was calculated. The number of emerged virgin queens / bar/ colony was counted, individually weighed on balance 0.01 grams and the percentages of emergence was calculated.

#### **Statistical analysis**

Data were subjected to analysis of variance (ANOVA) using "SPSS" computer statistical program. The randomized complete Block Design was used and (T test) were used and the mean values were compared using Duncan's multiple range test (Duncan, 1955).

### **RESULTS AND DISCUSSION**

#### **1. Effect of different bar level within grafted frame on queen rearing.**

##### **1. The effect on the acceptance percentages cells / bar:**

The results showed no significant differences in the acceptance percentage of grafted larvae between the different bar level within grafted frame in the rearing colonies (Table 1 and Fig. 1). The mean acceptance percentage of the top bar was 89.2 % with a range from 74.0% to 100 %. The lowest mean was showed in February, 16, 2016, while the highest mean value was recorded in date May, 14, 2016. The mean acceptance percentage of the medium bar was 90.2% with a range from 74.4% to 100 %. The lowest mean was showed in February, 27, 2016, while the highest mean values were recorded in May, 14, 2016. The mean acceptance percentage of the bottom bar was 86.3% with a range from 53.0% to 100 %. The lowest mean was showed in February, 16, 2016, and the highest ones was recorded in May, 14, 2016.

The findings were not coincided with those obtained by Macicka (1985) who found that the percentage of accepted larvae varied between the top, middle and bottom bars.

Sarling (1992) in USA, mentioned that, a grafting frame with three cell bars, each bar with 15 to 18 cell cups, plastic cup is as effective as wax, although there are advantages and disadvantages to both.

Ali (1994) studied the type of cell bar and its level on the frame in the colony, the following four experiments were conducted; wide cell bar holding 45 queen cups and fixed on the upper position (A); wide cell bar holding 45 queen cups and fixed on the second position from the top (B), three normal cell bars each contained 15 queen cups were fixed on the three following position from the top (C) and three normal cell bars each contained 15 queen cups were fixed on the three following position from the bottom (D). The highest numbers of accepted queen cells were found in D, B and C

position, while the highest amount of royal jelly was obtained in C and D position.

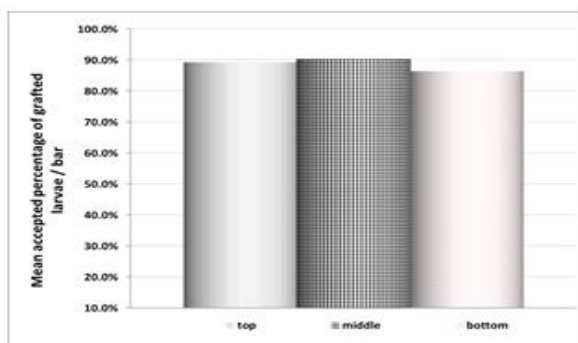
Ibrahim (1997) There was significant difference between the lower and upper positions of bars only, while no differences were found between each of (upper and middle) and (middle and lower position), regarding the acceptance percentages of queen cells. The lower position of bars gave the best result of acceptance (77.70%) followed by middle (64.44%) then upper bar which gave (51.10%).

Li (2000) used five wood bars on each royal jelly frame for a total of 125-170 queen cells, and 69-100 g of royal jelly can be collected from each frame within 2 hours cycle.

**Table 1. Effect of bar level within grafted frame on the acceptance of grafted larvae at various dates during the period from Feb., 16 to May,14/2016.**

Date	No. of grafted larvae/bar	Top bar		Medium bar		Bottom bar		Mean / date	
		Mean	%	Mean	%	Mean	%	Mean	%
16/02/2016	25	18.5	74.0	21.6	86.4	13.3	53.0	17.8	71.1
27/02/2016	25	20.5	82.0	18.6	74.4	17.1	68.2	18.7	74.9
09/03/2016	25	21.5	86.0	22.2	88.6	21.6	86.2	21.7	86.9
20/03/2016	25	21.5	86.0	22.2	88.8	23.2	92.6	22.3	89.1
11/04/2016	25	22.2	88.6	23.5	94.0	24.0	96.0	23.2	92.9
22/04/2016	25	24.2	96.6	23.2	92.6	24.0	96.0	23.8	95.1
03/05/2016	25	25.0	100.0	24.2	96.8	24.6	98.2	24.6	98.3
14/05/2016	25	25.0	100.0	25.0	100.0	25.0	100.0	25.0	100.0
Mean / treat.	25.0	22.3 ± 0.8127	89.2 A	22.6 ± 0.6915	90.2 A	21.6 ± 1.4917	86.3 A	22.1 ± 0.9339	88.5

Mean in each factor designated by the same letter are not significantly different at 5 % level using Duncan's Multiple Range Test.



**Fig. 1. mean accepted percentage of grafted larvae /bar in various grafted dates.**

**2. The effect on the emergence percentages of virgin queens / bar.**

No significant differences in the emergence percentage of virgin queens between the top, medium and bottom bar level of the tested colonies (Table 2 and Fig. 2) were observed. The mean of emergence percentage of the top bar was 93.1% with a range from 88.7% to 98.0%. The mean emergence percentage of the medium bar was 92.5% with a range from 84.2% to 100 %. The mean emergence

percentage of the bottom bar was 91.4% with a range from 81.9% to 98.0 %.

Sharaf El-Din *et al.* (2000) revealed that the production and quality of queens were affected by the rearing season and they recorded an increase in larval acceptance during the spring than summer season. Zeedan (2002) found that the highest level of building queen cups was during March (24.6 queen cups/colony), while the lowest level was during December (0.9 queen cups/colony). The building queen cups was higher during the spring followed by summer, winter and autumn seasons. There were significant differences in the mean of accepted larvae between both spring (84.2%) and summer (82.3%) from one side and both the autumn (73.4%) and winter (71.1%) from the other one.

Under environmental conditions of Giza region, Hammad (2007) recorded that production of queen cells started in the 25th of March 2006 and increased gradually till reached the maximum during the period from the 20th of May to the 17th of June, 2006 in the tested colonies, then the mean number gradually declined until the 9th of November, 2006 where no queen cells were present. The mean number of queen cells produced/colony during the spring season was higher than in the summer one.

**Table 2. Effect of bar level within grafted frame on virgin queen emergence at various dates during the period from Feb.,16 to May,14/2016.**

Date	Top bar		Medium bar		Bottom bar		Mean / date	
	Mean	%	Mean	%	Mean	%	Mean	%
16/02/2016	16.6	89.7	20.3	93.3	12.7	95.8	16.5	92.7
27/02/2016	19.6	95.4	17.7	95.2	15.1	88.6	17.5	93.2
09/03/2016	19.6	91.2	18.7	84.2	17.7	81.9	18.6	85.7
20/03/2016	20.1	93.3	20.1	90.5	20.2	87.0	20.1	90.2
11/04/2016	19.7	88.7	21.6	91.7	21.6	89.8	20.9	90.1
22/04/2016	22.0	91.1	20.6	89.0	22.5	93.8	21.7	91.3
03/05/2016	24.0	96.0	23.2	95.7	23.5	95.7	23.6	95.8
14/05/2016	24.5	98.0	25.0	100.0	24.5	98.0	24.7	98.7
Mean / treat.	20.7 ± 0.0116	93.1A	20.9 ± 0.8332	92.5 A	19.7 ± 1.4839	91.4 A	20.4 ± 1.0118	92.3

Mean in each factor designated by the same letter are not significantly different at 5 % level using Duncan's Multiple Range Test.

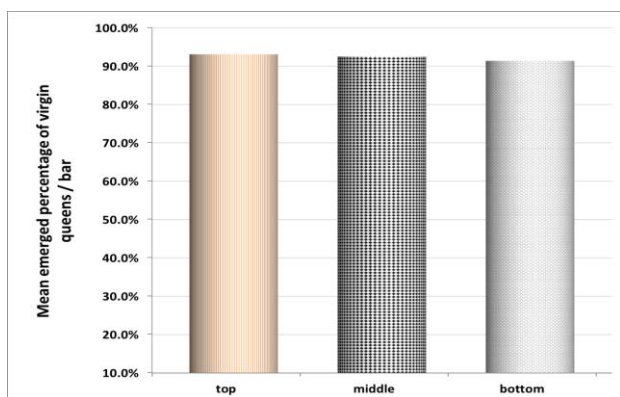


Fig. 2. mean emerged percentage of newly virgin queens /bar in various grafted dates.

### 3. The effect on the weight of emerged virgin queens.

The obtained results showed no significant difference in the weight of virgin queens (mg./queen) between the different bar level of the tested colonies (Table 3 and Fig. 3). The mean weight of virgin queens for the top bar was  $160.69 \pm 2.3827$  mg./queen with a range of 154.61 to 173.65 mg./queen. The mean weight of virgin queens for the medium bar was  $158.88 \pm 2.9025$  mg./queen with a range of 150.55 to 174.40 mg./queen. The mean weight of virgin queens for the bottom bar was  $160.75 \pm 2.9396$  mg./queen with a range of 154.56 to 175.71 mg./queen.

Abdellatif (1967) demonstrated that the average weights of the queen in Marsh, April and May were 153.8, 156.6, 153.5 mg, respectively, as there was a positive correlation between the queen weight and its cell size in March ( $r=0.7$ ), but no relationship existed in April and May ( $r = 0.4$  and  $0.3$ , respectively). Shaver *et al.* (1980) indicated that the body weight of virgin queens differed significantly according to the rearing season. The heaviest queens were produced in May and August. Queens reared in February, March and July had an intermediate weight, while those reared in April, June and October had the least body weight. Zeedan (2002) mentioned that under Giza environmental

conditions, the heaviest queens were produced during summer (178.7 mg. / queen) where this value was significantly higher than the other seasons which were 175.6, 171.8 and 168.2 mg./queen in spring, autumn and winter, respectively. Zohairy (2007) realized that the mean weight of emerged queens in a descending order were in May, June, September, August, April and March in 1998 and in May, June, September, April, August, July and Marsh in 1999. While Abdulaziz *et al.* (2013) shows that the general mean body weight of newly emerged virgin queens (less than 24 h) of *A. m. carnica* queens reared by the grafting method during spring season of 2010 and 2011 in Riyadh, were  $165.87 \pm 9.79$  ( $n=20$ ) and ranged between 153.0-195.0. Moustafa *et al.* (2014) studies the quality of virgin queens of honey bee that produced commercially from four large queen producers in Egypt, and results indicated that the mean body weight was  $127.6 \pm 0.017$  mg. in spring season and  $144.17 \pm 0.020$  mg. in summer season but for *Apis mellifera carnica*, the average weights of queens were  $208.40 \pm 15.31$ mg,  $209.49 \pm 9.82$ mg and  $201.83 \pm 15.85$  mg, during 2006, 2008 and 2010 respectively (Gregorc and Škerl 2015).

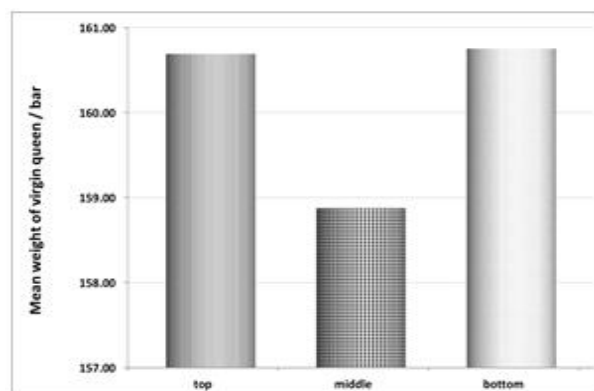


Fig. 3. mean weight of virgin queens (mg.) /bar in various grafted dates.

Table 3. Effect of bar level within grafted frame on weight of virgin queens (mg.) at various dates during the period from Feb., 16 to May,14/2016.

Date	Top bar		Medium bar		Bottom bar		Mean / date	
	no. of weighed	Mean	no. of weighed	Mean	no. of weighed	Mean	no. of weighed	Mean
16/02/2016	17	154.61	20	150.55	13	155.92	17	153.69
27/02/2016	20	157.00	18	158.01	15	159.41	18	158.14
09/03/2016	20	157.29	19	152.43	18	154.56	19	154.76
20/03/2016	20	155.83	20	155.98	20	155.41	20	155.74
11/04/2016	20	159.86	22	157.89	22	155.18	21	157.64
22/04/2016	22	158.42	21	153.43	23	157.91	22	156.58
03/05/2016	24	168.33	23	167.83	24	172.11	24	169.42
14/05/2016	25	173.65	25	174.40	25	175.71	25	174.59
Mean / treat.		160.69 A $\pm 2.3827$		158.88 A $\pm 2.9025$		160.75 A $\pm 2.9396$		160.10 $\pm 2.6978$

Mean in each factor designated by the same letter are not significantly different at 5% level using Duncan's Multiple Range Test.

### 2. Effect of interaction between bar level and ambient temperatures on acceptance, emergence and weight of virgin queens.

The obtained results showed significant influence in the acceptance percentage of grafted queen cups on the top bar level due to the minimum temperature (Table 4). No significant differences in the emergence percentage of virgin queens between the different bar levels and mean, minimum and maximum ambient temperature. In the mean weight of

virgin queens the results showed clear effect on the top bar level due to mean, minimum and maximum ambient temperature. The results showed clear effect on the medium and the bottom bar level due to minimum temperature.

### 3. Effect of different queen cups position within grafted frame on queen rearing.

#### 1. The effect on the acceptance percentages queen cups.

There were significant differences in the acceptance percentage of grafted larvae between the different queen cups

position within grafted frame of the rearing colonies were observed (Table 5 and Fig. 4). The mean acceptance percentage of the middle position was 97.0% with a range

from 88.5% to 100 %. The mean acceptance percentage of the edge position was 82.5% with a range from 51.4% to 100 %.

**Table 4. Simple correlation coefficients between ambient temperatures and acceptance of grafted larvae in honeybee queenless colonies with different populations during the period from Feb.,16 to May,14/2016.**

Temp. °c	acceptance			emergence			weight		
	Top	Medium	Bottom	Top	Medium	Bottom	Top	Medium	Bottom
Min. temp. Corr.(r)	0.746*	0.688	0.464	0.406	0.467	0.695	0.831*	0.711*	0.755*
Max. temp. Corr.(r)	0.455	0.541	0.221	0.254	0.398	0.626	0.754*	0.623	0.632
Mean temp. Corr.(r)	0.518	0.573	0.275	0.330	0.455	0.673	0.801*	0.679	0.696

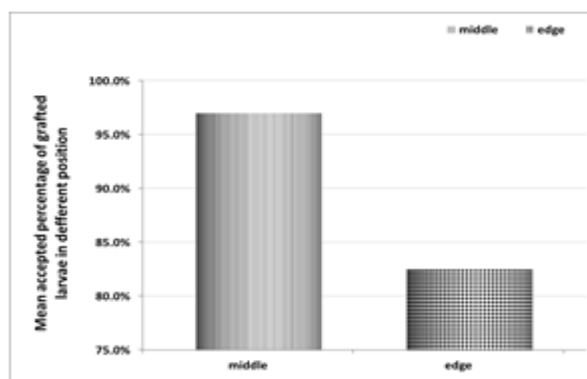
\* Correlation is significant at the 0.05 level  $r = .707$

**Table 5. Effect of queen cups position on bar strip on the acceptance of grafted larvae at various dates during the period from Feb., 16 to May, 14/2016.**

Date	Middle			Edge		
	No. of grafted larvae	Mean	%	No. of grafted larvae	Mean	%
16/02/2016	39	34.5	88.5	36	18.5	51.4
27/02/2016	39	35.5	91.0	36	20.5	56.9
09/03/2016	39	38.5	98.7	36	26.5	73.6
20/03/2016	39	38.5	98.7	36	28.0	77.8
11/04/2016	39	38.5	98.7	36	31.0	86.1
22/04/2016	39	39.0	100.0	36	32.0	88.9
03/05/2016	39	39.0	100.0	36	34.5	95.8
14/05/2016	39	39.0	100.0	36	36.0	100.0
Mean / treat.	39	37.8 ±0.626	97.0	36	29.7 ± 2.232	82.5

T value= 5.6325\*, df=7, P<0.05.

The finding were agreed with the results finding by Zeedan (2002) who reported that the grafted larvae in cups located in the middle of bar were significantly accepted (81.4%) more than those located on the bar edge. Also, Helaly (2018) found that the yield of royal jelly collected from grafted cups in the middle of bar was significantly increased than those collected from the edge queen cells.



**Fig. 4. Mean accepted percentage of grafted larvae at various dates with different position on bar strip.**

**2. The effect on the emergence percentages of virgin queens / bar.**

The results indicated high significant differences in the emergence percentage of virgin queens between middle and edge cups position of the tested colonies (Table 6 and Fig. 5). The mean of emergence percentage of middle position was 98.7% with a range from 95.7% to 100.0%. The mean emergence percentage of edge position was 82.9% with a range from 66.0% to 97.2 %. The results indicated high mean of emergence and the high minimum and maximum range recorded between the individual

experimental dates (Table 6) as well as in the variations between the tested colonies.

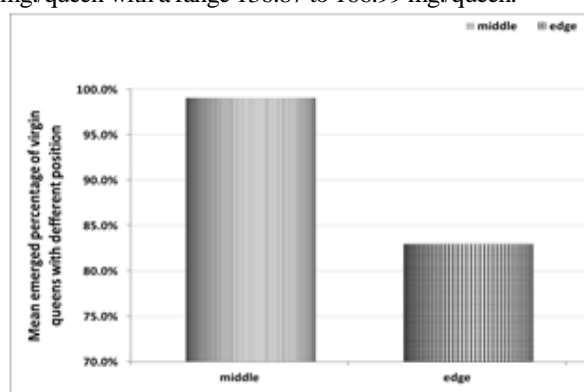
**Table 6. Effect of queen cups position on bar strip on the emergence of virgin queens at various dates during the period from Feb., 16 to May,14/2016.**

Date	Middle		Edge	
	Mean	%	Mean	%
16/02/2016	33.0	95.7	16.0	86.5
27/02/2016	34.5	97.2	17.5	85.4
09/03/2016	38.0	98.7	17.5	66.0
20/03/2016	38.5	100.0	21.5	76.8
11/04/2016	38.5	100.0	24.0	77.4
22/04/2016	39.0	100.0	26.0	81.3
03/05/2016	39.0	100.0	31.5	91.3
14/05/2016	39.0	100.0	35.0	97.2
Mean / treat.	37.4 ±0.826	98.7	24.6 ±2.445	82.9

T value= 5.6325\*, df=7, P<0.05.

**3. The effect of the weight of emerged virgin queens.**

The obtained results showed clear significant difference in the weight of virgin queens between the different queen cups position of the tested colonies (Table 7 and Fig. 6). The mean weight of virgin queens for the middle position was 166.90±2.8247mg./queen with a range 159.95 to 181.41 mg./queen. The mean weight of virgin queens (mg./queen) for the edge position was 148.30±3.6450 mg./queen with a range 136.87 to 166.99 mg./queen.



**Fig. 5. Mean emerged percentage of virgin queens at various dates with different position on bar strip.**

Zohairy (2007) realized that the mean weight of emerged queens in a descending order were in May, June, September, August, April and March in 1998 and in May, June, September, April, August, July and March in 1999. El-Barbary (2007) revealed that during summer the mean weight of queens emerged from cells located in the middle position of rearing frame was, (181.76 mg) significantly heavier than that produced on the edge position (162.54 mg). Similar results were obtained during spring where the means

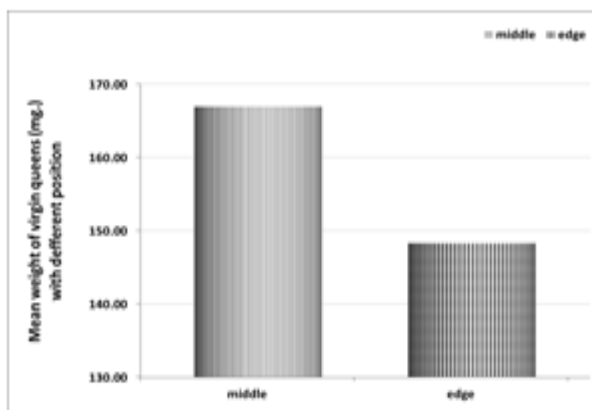
of queen weight were 176.62 mg and 166.05 mg for queen reared in the middle and the edge positions, respectively. Abd Al-Fatah *et al.* (2011) reported that the position at which the queen cell presented within building colony has a pronouncing effect on weight of the resulted queens throughout various seasons of the year. The queen cells that positioned in the middle areas of rearing frame gave a number of queens with heavy weight in relative frequencies significantly higher than those resulted from cells presented on the edge areas of rearing frame. The percentages of heavy queen weight were 69.7% & 44.8% and 25.8% & 31.5% for edge position and middle position during summer and spring seasons, respectively. The intermediate queen weight was significantly less during summer (27.2%) than those obtained

during spring (49.3%) for the middle position. For the edge position, little increase was recorded during summer, (45.2%), than spring (39.5%) for the relative queen weight distribution during summer and spring seasons, respectively. However, the lightest queens were frequented in significant rate in the peripheral areas (29.0 & 29.0%) than in the middle (3.1&5.9%) ones it is, also noticed from these results that the frequency percentages of heavy queens were significantly higher during summer season than those occurred during spring season. The extensive care and the consistency of brood nest temperature in the middle area than in the peripheral one may be the reason of harvesting high weights of honeybee queens.

**Table 7. Effect of queen cups position on bar strip on the weight of virgin queens (mg.) at various dates during the period from Feb., 16 to May,14/2016.**

Date	Middle			Edge		
	No. of weighed queen	Range	Mean	No. of weighed queen	Range	Mean
16/02/2016	33	140-190	159.95±13.13	16	110-150	136.87±10.88
27/02/2016	35	140-200	164.64±11.84	18	110-160	141.42 ±11.70
09/03/2016	38	140-180	160.19 ± 9.25	18	130-150	143.08 ±5.83
20/03/2016	39	150-180	161.00 ±8.04	22	140-160	146.16 ±5.59
11/04/2016	39	150-190	164.64±10.59	24	130-160	146.04 ±8.69
22/04/2016	39	140-190	166.54±12.88	26	110-170	141.28 ±15.70
03/05/2016	39	160-190	176.79±7.81	32	150-170	159.83 ±6.40
14/05/2016	39	170-190	181.41±5.97	35	160-180	166.99 ±5.21
Mean / treat.			166.90±2.825			148.30 ±3.645

T value= 13.1356\*\*, df=7, P<0.05.



**Fig. 6. Mean weight of virgin queens at various dates with different position on bar strip.**

**4. Effect of interaction between cell position and ambient temperature on acceptance, emergence and weight of virgin queens.**

The obtained results showed that no significant effect on the acceptance percentage of grafted larvae for the interaction between cell position and minimum, maximum and mean temperatures (Table 8). However, clear significant impact on the weight of virgin queens between the middle and edge positions and the tested temperatures.

The obtained results are agreement with those found by various authors who reported that the thermoregulation within honeybee colony is mainly depend on the population density of workers. They also, added that rearing brood need a constant nest temperature (Seeley and Heinrich, 1981; Eskov, 1990; Degrandi-Hoffman *et al.*, 1993; Chuda-Mickiewicz B., 1994; Jones *et al.*, 2004)

**Table 8. Simple correlation coefficients between ambient temperatures and acceptance of grafted larvae, emerged percentage and weight of virgin queens in honeybee queenless colonies with different populations during the period from Feb.,16 to May,14/2016.**

Temp. °c	acceptance		emergence		weight	
	Middle	Edge	Middle	Edge	Middle	Edge
Min. temp. Corr.(r)	0.463	0.670	0.623	0.598	0.731*	0.863*
Max. temp. Corr.(r)	0.304	0.397	0.603	0.504	0.625	0.778*
Mean temp. Corr.(r)	0.333	0.459	0.609	0.565	0.687	0.826*

\* Correlation is significant at the 0.05 level r = .707

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### تأثير مستوى سدابة التطعيم و موقع الكأس الملكي على جودة الملكات الناتجة في منطقة المنزلة

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تم اختبار النسبة المئوية لقبول اليرقات المطعومه , النسبة المئوية لفقس الملكات و كذلك وزن الملكات العذارى حديثة الفقس المرياة فى طوائف بنيمية وذلك على ثلاث مستويات مختلفة من سدابات التطعيم و موقعين مختلفين للكؤس المطعومة بمنطقة المنزلة المعزولة. كان هناك تأثير ايجابى لدرجات الحرارة الصغرى مستوى السدابة العلوى. كذلك كان هناك تأثير ايجابى لدرجات الحرارة الصغرى على اوزان الملكات الناتجة على كل مستويات السدابات. كذلك تأثرت جميع القياسات ايجابيا على موضع الحافة. وعلى الرغم من أن النسب المئوية لقبول وفقس الملكة تأثرت بشكل ايجابى بعناصر درجات الحرارة المحيطة ، فقد كانت التأثير أكثر وضوحاً في وزن الملكات الناتجة على موضع الحافة.