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## Quantity and Quality of Commercially Reared Queens as Affected by Number and Distribution of Artificially Grafted Queen Cells Within Queen-Less Honey Bee Colonies in the Middle Delta Region, Egypt

Abd Al-Fattah, M. A.<sup>1</sup>; A. A. Ghanim<sup>2</sup>; A. A. Eissa<sup>3</sup> and M. A. Ali<sup>3\*</sup>



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<sup>1</sup>Dept. of Economic Entomology & Pesticides, Agriculture Faculty, Cairo University, Giza, Egypt.

<sup>2</sup>Department of Economic Entomology, Faculty of Agriculture, Mansoura University.

<sup>3</sup>Department of Apiculture, Plant Protection Research Institute, Agriculture Research Center, Dokki, Giza, Egypt

### ABSTRACT

This work was conducted throughout the queen rearing process using starter-finisher queen-less honey bee colonies to evaluate the impact of different numbers of grafted larval queen cups (24 and 48 grafted cups/colony), the level of bar they hung on it (top, middle, and bottom), and their positions they located on the bar (peripheral and middle) on the percentages of accepted queen cells and queen emergence, as well as the weight of emerged queens during three successive years (2021 to 2023). The number of grafted queen cups significantly influenced the studied traits in all years. The rates of acceptance and emergence were generally influenced by both bar level and cell position. The obtained values were significantly higher for the top (94.3 % and 97.7%) and middle (94.0 % and 92.0%) levels than in the bottom (89.4% and 87.0%) ones for the two characters, respectively. The middle position (93.6%) was positively higher than peripheral (90.8%) ones in case of accepted percent but not for rate of emergence (90.8%) and 88.9%, respectively. The weight of emerged queens was pronouncedly decreased in the bottom than both the top and middle levels and in the peripheral than middle position during study years. The general mean weights were 176.9, 172.8, and 162.8 mg/queen in the middle, top, and bottom levels of the bar and were 174.6 and 167.0 mg/queen for the middle and peripheral positions, respectively.

**Keywords:** queen rearing, number of grafted cups, bar level and position, quantity and quality of queen



### INTRODUCTION

It is known that the key to survive the honey bee colonies and success the beekeepers is the reproductive potentiality of their owned bee queens. The reproductive ability of queen is mainly depends on two basics. The first is relating to the genetic origin (Hoopingamer & Farrar, 1959; Morse, 1979; Breed, *et al.*, 1985; Al Ghzawi & Zaitoun, 2008; El-Kady, *et al.*, 2021; Facchini, *et al.*, 2021; Frost, *et al.*, 2021). The second is environmental influences which may divide into two groups: a, the external surrounding circumstances such as weather factors, botanical sources, geographic location and elevation above sea level...etc., (Mohanna, 1969; Taber, 1979; El-Sarrag and Nagi, 1985; Spivak, *et al.*, 1992; Zeedan, 2002; Taha, 2005; Amiri, *et al.*, 2017; Hamada, 2019; Arslan & Cengiz, 2020; Arslan, *et al.*, 2021; Abd Al-Fattah, *et al.*, 2021; Canverdi, *et al.*, 2023; Guda, *et al.*, 2023), and b, which included all internal factors related to rearing colonies as the number, level and position of introduced queen cells, enough food, age of nursery workers, colony health, optimum nest temperature, ...etc., (Johansson & Johansson, 1973; Nasr, 1976; Weiss, 1983; Visscher, 1986; Spivak, *et al.*, 1992; DeGrandi-Hoffman, *et al.*, 1993; Abd Al-Fattah and El-Shemy, 1996; Tarpy, *et al.*, 2000; Abd Al-Fattah, *et al.*, 2003; Hassan & Mazed, 2003; Fathy, *et al.*, 2019; Yi, *et al.*, 2020; Abd Al-Fattah, *et al.*, 2021).

Many investigators agreed that, in the artificial queen rearing through grafting process, the commercial mass production of honey bee queens is mostly correlate with the

accepted, sealed and emerged numbers of the total queen cells received by the rearing colony, (Zhu, 1981; Rawash, *et al.*, 1983; Macicka, 1985; Abd Al-Fattah, *et al.*, 2007; Hamada, 2019; Abd Al-Fattah, *et al.*, 2021). However, the qualitative features of the future queen are mainly correlate with its weight at emergence, thus the weight of newly emerged queen was considered as reliable criterion for the evaluation of queen quality as reported by Woyke, (1971); Szabo, (1973 & 1977); Szabo, *et al.*, (1987); Chuda-Mickiewicz & Samborski, (2015); Cengiz, *et al.*, (2019); Yi, *et al.*, (2020) and Guda, *et al.*, (2023).

The objective of this research is to evaluate the quantity and quality of newly emerged queens that reared within starter - finisher queen-less colonies which received different numbers of grafted queen cups distributed at various places in the frame of grafted cells.

### MATERIALS AND METHODS

This experiment was carried out in a private apiary located in Tanta district, Gharbia Governorate, during summer months, (June to September) of three successive years, (2021, 2022 and 2023).

Six Carniolan hybrid colonies similar in their strength were selected to serve as starter and finisher colonies simultaneously. Each colony contained seven to eight combs covered with bees, four sealed and unsealed brood combs, one comb filled with bee bread and two combs of sealed and unsealed honey. These colonies were provided with sugar syrup (1:1 w/v) for two weeks before

\* Corresponding author.

E-mail address: [ma922169@gmail.com](mailto:ma922169@gmail.com)

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and during the queen rearing process. The six colonies were divided into two groups of three colonies each. Queens of those colonies removed 24 hours before inserting the frames of grafted larvae; so, the queen rearing operation had completely occurred in queen-less colonies.

**A - Studying factors**

The quantity and quality of the produced queens were evaluated in relation to the following factors:-

**1 - Number of grafted cups introduced into queen-less rearing colonies**

Two sets of grafted queen cells, (24 and 48 cells / colony) were examined in queen-less rearing colonies with nearly identical strength. The inserted larval queen cups from each set were equally distributed on three bars hanging within a rearing frame. Each set was repeated in three colonies as replicates.

**2- Level of bar containing larval queen cells within rearing frame**

Each of the two previous sets of grafted queen cells were equally distributed on wooden bars at three levels within each rearing frame. So each bar carried 8 or 16 grafted queen cells when the treatment of 24 or 48 inserted cups was applied. The top bar was hung about 5 cm from the head of the rearing frame. The middle and lower bars were hung below the top bar, with the same distance between them. Each of the three bars had an equal number of inserted queen cells from each queen cell category as described by Abd Al-Fattah, et al., (2011).

**3- The position of queen cell on the bar within rearing frame**

Each horizontal bar was vertically divided into three parts. The two lateral parts of the frame were considered as a peripheral position, (included 4 or 8 queen cells / bar in treatment of 24 or 48 grafted cups, respectively). The medium part was regarded as a middle position which

included the same previous numbers of queen cells according to the evaluated treatment. Therefore, the distance between each two adjacent queen cells on the bar varies depending on the treatment to which they belong, (Abd Al-Fattah, et al., 2021).

**B - Parameters of evaluation**

To assess the impact of the preceding factors on the quantity and quality of the resulted queens, the following parameters were selected:

a. The acceptance percentage of grafted larval queen cups, which calculated by the following formula:

$$\text{Accepted queen cells \%} = \frac{\text{No. accepted queen cells}}{\text{No. grafted queen cups}} \times 100.$$

b. The emergence percentage of queens from sealed queen cells, which calculated by the following formula:

Percentage of emerged virgin queens:

$$\text{Queen emergence \%} = \frac{\text{No. emerged queens}}{\text{No. grafted queen cells}} \times 100.$$

c - Weight of newly emerged virgin queens:

This parameter was recorded using an electric balance to the nearest 0.01 mg.

**Statistical analysis:**

Data were subjected to analysis of variance (ANOVA) using “Co-stat” computer statistical program, (Version 6.303.). The randomized complete Block Design was used and the mean values were compared using Duncan’s multiple range test (Duncan, 1955).

**RESULTS AND DISCUSSION**

Data in Tables (1 and 2) showed that the effect of numbers of grafted larvae and their distribution within grafting frame, (as position and level) on certain quantitative, (acceptance and emergence percentages) and qualitative, (weight of virgin queens at emergence) traits of produced virgin queen during three successive years, (2021, 2022 and 2023).

**Table 1. Effect of number of grafted larval cups and their position within grafting frame on the acceptance and emergence percentages and weight of newly emerged queens in starter-finisher queen-less rearing colonies during 2021, 2022 and 2023 years**

Year	No. of grafted cups	Acceptance %			Emergence %			Weight(mg)		
		Peripheral	Middle	Mean	Peripheral	Middle	Mean	Peripheral	Middle	Mean
2021	24	97.9	95.8	96.9 A	95.8	93.8	94.8 A	179.8	185.3	182.5 A
	48	91.7	89.6	90.7 B	91.7	85.4	88.6 B	147.5	155.5	151.5 B
	Mean	94.8 a	92.7 a	93.8	93.8 a	89.6 a	91.7	163.7 b	170.4 a	167
2022	24	93.8	100	96.9 A	91.7	95.8	93.8 A	173.8	181.8	177.8 A
	48	87.5	91.7	89.5 B	85.4	89.6	87.5 B	144.8	153.3	149.0 B
	Mean	90.7 b	95.9 a	93.2	88.6 a	92.7 a	90.7	159.3 b	167.6 a	163.4
2023	24	89.6	95.8	92.7 A	87.5	93.8	90.6 A	189.0	196.5	192.8 A
	48	84.4	88.5	86.5 B	81.3	86.5	83.9 B	167.3	175.3	171.3 B
	Mean	87.0 b	92.2 a	89.6	84.4 b	90.2 a	87.3	178.2 b	185.9 a	182.1
General mean		90.8 B	93.6 A	92.2	88.9 A	90.8 A	89.9	167.0 B	174.6 A	170.8

Means in the same column followed by the same capital letter are not significant at 0.05 level according to Duncan’s Multiple Range test. Means in the same row followed by the same small letter are not significant at 0.05 level according to Duncan’s Multiple Range test.

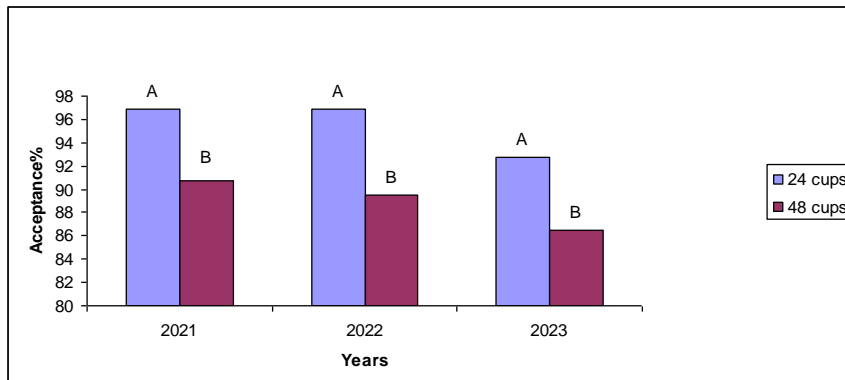
**Effect of the number of grafted larvae on:**

**1 - Acceptance and emergence percentages**

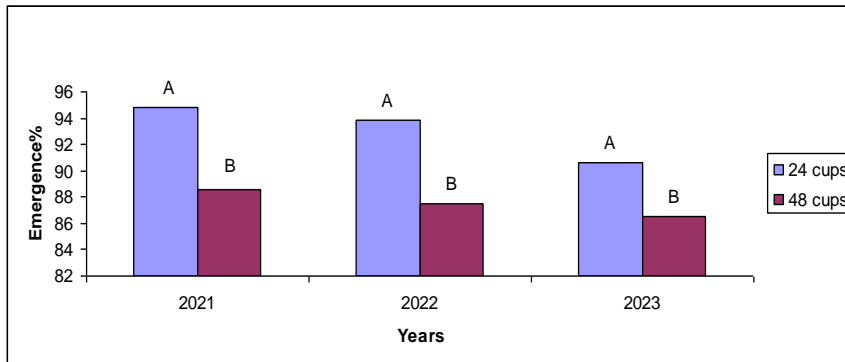
The percentages of accepted and emerged queen cells are the most important quantitative traits in limited and commercial production during artificial queen rearing. The present results in Tables (1 and 2) revealed that there was significant difference between the two groups of introduced grafted queen cups (24 and 48 grafted cups/colony) for larvae acceptance during the three studied years. They recorded acceptance rates by 96.9± 2.1 and 90.7± 2.7 %; 96.9± 3.98

and 89.5± 6.1 % ; 92.7±7.12and 86.5 ± 6.49 % for the two inserted numbers of grafted larvae throughout the three successive years, respectively., as shown in Fig., (1).

Likewise, the percentage of emerged queens was significantly affected by the numbers of grafted larvae where it was 94.8± 2.05%, 93.8 ± 5.37% and 90.6± 9.22% for 24 graft cup/ colony against 88.6± 1.21%, 87.5± 5.37% and 83.9 ± 6.92% in case of 48 graft cup/ colony throughout the three successive years, respectively,(Fig. 2).



**Fig. 1. Effect of the number of grafted larvae on acceptance percentage**



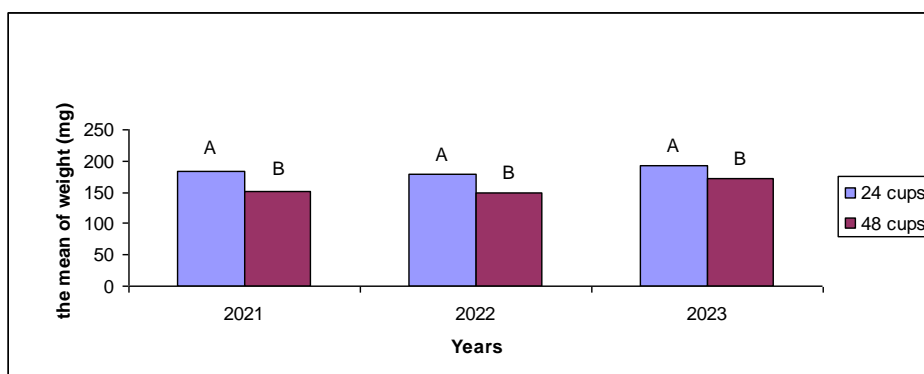
**Fig. 2. Effect of the number of grafted larvae on emergence percentage**

These results are consistent with those of many researchers in different countries. Lawrence and Cobey (1991) in USA, reported that the number of queen cells given to a cell builder colony is important and graft 30 cups was preferable than 80 cups per cell builder. El-Barbary, (2007) at Giza governorate, registered pronouncing percentages of sealed, (94.5 and 88.1%) and emerged, (93.3 and 89.3 %) queen cells when 24, than 42, graft cups were introduced into starter queen-less colony, respectively. In the same respect, Abd Al-Fattah, *et al.*, (2021) under North Egypt conditions, found that there was significant increase in the accepted and emerged percentages of queen cells

when introducing 32, (73.5% and 98.1%) than 48, (65.8% and 96.0%) larval queen cups into finisher queen rearing colony, respectively.

**2 – Fresh weight of virgin queens at emergence**

The findings in Table (1) indicated that the weight of newly emerged virgin queens was significantly higher, ( $182.5 \pm 4.79$ ,  $177.8 \pm 8.02$  and  $192.8 \pm 6.1$  mg.) with introducing a number of 24 graft cups/ colony than those attained, ( $151.5 \pm 3.87$ ,  $149.0 \pm 4.76$  and  $171.3 \pm 15.13$  mg.) with 48 graft cups/colony throughout the three years, respectively, ( Fig. 3)



**Fig. 3. Effect of the number of grafted larvae on fresh weight of virgin queen at emergence**

These results appeared that the fresh weight of newly emerged virgin queens was significantly affected by the number of grafted cups on the queen-less builder colony received. This conclusion is in accordance with Lawrence and Cobey (1991) in USA, where they stated that cell builder colonies given more than 45 graft cells may produce inferior queens. These raised weak queens, in case of introduced high numbers of graft cups, May attributed to

inadequate provided food by nursery workers where the mean weight of royal jelly / cell decreased with increasing number of graft cups as reported by Chen and Chang (1993). Besides, the small numbers of larval queen cells will have the optimal temperature required to complete all physiological processes with high efficiency, which increase the reproductive efficiency of the resulting queens, (DeGrandi-Hoffman, *et al.*, 1993; Mohammad, *et al.*, 2002;

Abd Al-Fattah et al., 2011; Sagili, et al., 2018; Dhaliwal, et al., 2019; Sakla and El-shafeiy, 2022; Abd Al-Fattah et al.,2021; Guda, et al., 2023).

**B – Effect of queen cell position within grafting frame on: 1 - Acceptance and emergence percentages**

Results presented in Table (1) revealed that there was no significant difference in the mean acceptance rate between the middle, (92.7%) and peripheral, (94.8 %) queen cup positions on bars within grafting frame during queen rearing process in the year of 2021. These rates were significantly different and recorded 95.9% & 90.7% and 92.2 % and 87.0 % for middle and peripheral positions, during the years of 2022 and 2023, respectively, (Fig. 4).

The same output was recorded in the case of emerged queen percentage by values of (89.6 % and 93.8 %) and (92.7% & 88.6 %) for the middle and peripheral positions throughout the years of 2021 and 2022, respectively. However, this character was significantly increased in the middle, (90.2%) against peripheral, (84.4%) positions in the year of 2023, (Table, 1).

In general, the rate of emerged queens in middle position was higher than those occurred in peripheral ones. These results are in agreement with what has been

mentioned by many researchers. They stated that the middle position within grafting frame offer more favorable conditions that enhance and increase the acceptance rate of grafted larvae and queen emergence during queen rearing program, (Visscher, 1986; Zeedan, 2002; Abd Al-Fattah, et al., 2007; Abd Al-Fattah et al.,2011 ; Helaly, 2018; Hamada, 2019; El-Kady, et al., 2021).

**2 –Fresh weight of virgin queens at emergence**

Data recorded in Table (1) obvious that queens reared in the middle area of rearing frame attained higher quality, depending on their weight at emergence, than those resulted in the peripheral ones. The mean weights at emergence in middle position were 170.4, 167.6 and 185.9 mg compared with 163.7, 159.3 and 178.2 mg. in the peripheral ones during the three experimental years, respectively, (Fig. 5).

These findings are similar with those demonstrated by several investigators that numerous heavy queens could be obtained when the grafted larvae were positioned in the middle region of the rearing frame where intensive care was provided by the nursery workers, (Gracy, 1985; Spivak, et al., 1992; Fathy, et al., 2019; El-Kady, et al., 2021).

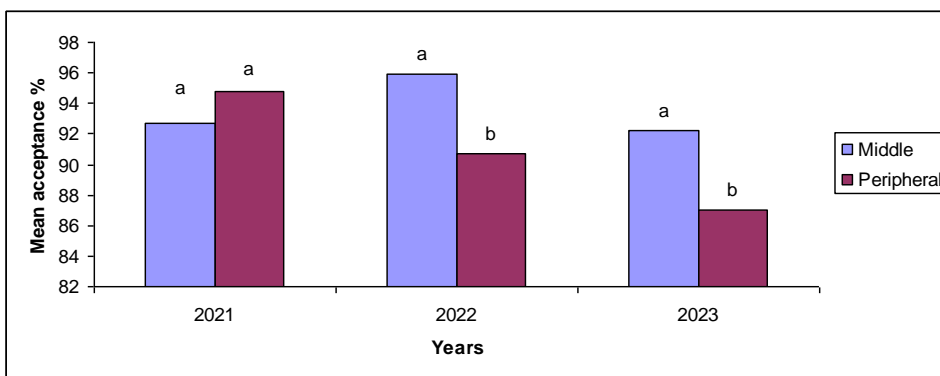


Fig. 4. Effect of queen cell position within grafting frame on acceptance percentages

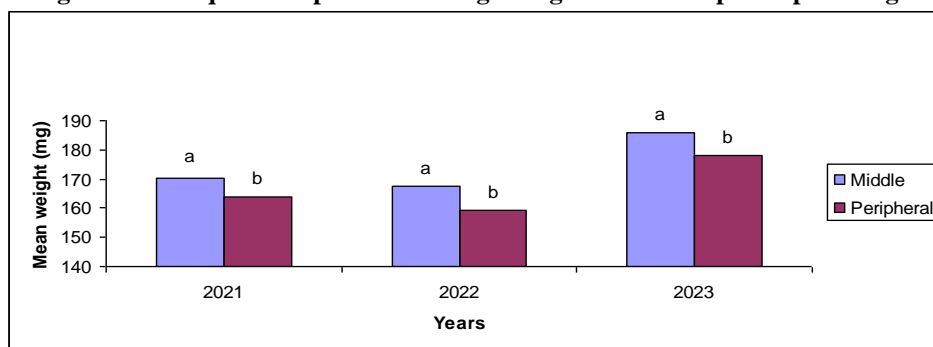


Fig. 5. Effect of queen cell position within grafting frame on fresh weight of virgin queen at emergence

**C – Effect of queen cell level within grafting frame on: 1 - Acceptance and emergence percentages**

Results presented in Table (2) showed significant increase in the percent of acceptance of grafted larvae located on the top, (96.9 and 91.5%) and middle, (93.0 and 91.5%) bars from one side and those on the bottom,(91.5 and 86.0%) bar from the another ones during years of 2021 and 2023, respectively. But this increase was not valuable through 2022 year where the acceptance values were 94.6, 94.6 and 90.7% for the top, middle and bottom bars, respectively. However, the effect of bar level over the three studied years appeared a significant superiority of the

acceptance rate for the top, (94.3 %) and middle, (93.0 %) bars over the lower, (89.4 %), ones, (Fig. 6).

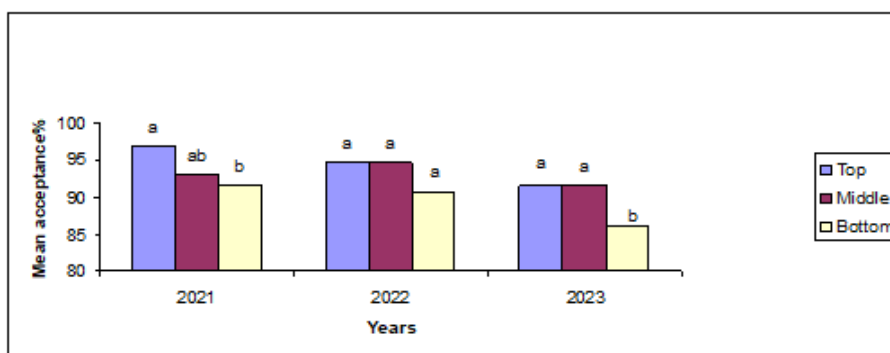
In the same context, the percentages of newly emerged virgin queens do not significantly differed between the bar levels except in year of 2023 where the emergence rate on bottom bar registered a lower value, (80.5 %) than those on the top, (89.9 %) and middle, (91.5 %) bars as shown in Table, ( 2 ) and Fig. ( 7 ).

The overall mean for the three years indicated an improvement in the emergence percentage on the top, (90.7 %) and middle, (92.0 %) bars over the lower, (87.0 % ) , ones.

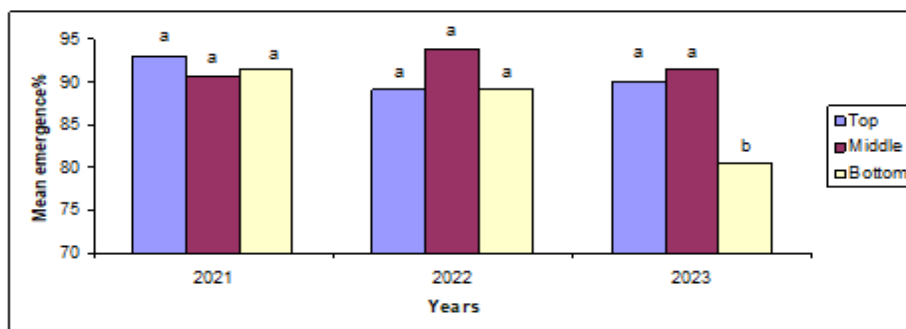
**Table 2. Effect of number of grafted larval cups and their bar's level within grafting frame on the acceptance and emergence percentages and weight of newly emerged queens in starter-finisher queen-less rearing colonies during 2021, 2022 and 2023 years.**

Year	No. of grafted cups	Acceptance %				Emergence%				Weight(mg)			
		Top	Middle	Bottom	Mean	Top	Middle	Bottom	Mean	Top	Middle	Bottom	Mean
2021	24	100	96.9	93.8	96.9 A	96.9	93.8	93.8	94.8 A	184.5	186.3	176.8	182.5 A
	48	93.8	89.1	89.1	90.7 B	89.1	87.5	89.1	88.6 B	154.0	156.8	143.8	151.5 B
	Mean	96.9 a	93.0 ab	91.5 b	93.8	93.0 a	90.7 a	91.5 a	91.7	169.3 a	171.6 a	160.3 b	167
2022	24	96.9	100	93.8	96.9 A	90.6	100	90.6	93.8 A	179.0	183.8	170.5	177.8 A
	48	92.2	89.1	87.5	89.5 B	87.5	87.5	87.5	87.5 B	149.8	154.8	142.5	149.0 B
	Mean	94.6 a	94.6 a	90.7 a	93.2	89.1 a	93.8 a	89.1 a	90.7	164.4 a	169.3 a	156.5 b	163.4
2023	24	93.8	93.8	90.6	92.7 A	93.8	93.8	84.4	90.6 A	195.8	200.8	181.8	192.8 A
	48	89.1	89.1	81.3	86.5 B	85.9	89.1	76.6	83.9 B	173.8	178.8	161.3	171.3 B
	Mean	91.5 a	91.5 a	86.0 b	89.6	89.9 a	91.5 a	80.5 b	87.3	184.8 a	189.8 a	171.6 b	182.1
General mean		94.3 A	93.0 A	89.4 B	92.2	90.7 AB	92.0 A	87.0 B	90.0	172.8 AB	176.9 A	162.8 B	170.8

Means in the same column followed by the same capital letter are not significant at 0.05 level according to Duncan's Multiple Range test. Means in the same row followed by the same small letter are not significant at 0.05 level according to Duncan's Multiple Range test.



**Fig. 6. Effect of queen cell level within grafting frame on acceptance percentages.**



**Fig. 7. Effect of queen cell level within grafting frame on emergence percentages**

These variations between the three evaluated bar levels during the experimental years agreed with Macicka,(1985) who found that the acceptance percentage of grafted larvae did not follow a consistent pattern through different years. Also,Visscher (1986) showed that larvae in cells near the top of the frame are more readily to be queens in the upper than in the lower part of the brood nest. Therefore, the present results are agreement with the findings of Fathy, *et al.*, (2019) at Manzala province, Dakahlia governorate, where the acceptance and percentage of queen cells did not affected by the level bar within grafting frame. Also, El-Kady, *et al.*, (2021) pointed out that there was no significant difference between the top,(81.3 %) and medium, (83.3 %) bars concerned the acceptance percentage. On the other hand, the present results are contrary to those found by Zeedan (2002) where the acceptance percentages of grafted larvae were significantly differed between the top, (75.8%), middle, (84.5%) and bottom, (84.7%) bar levels within rearing frame.

Regarding the percentage of virgin queens emerging, Abd Al-Fattah, *et al.*, (2011) noticed that queen cells located

on the upper bar attained a significantly higher emergence percentage than the other bars in summer while there was not any pronouncing difference between the three bar's levels in spring. Many investigators such as Fathy, *et al.*, (2019) and El- Kady, *et al.*, (2021) have agreed and confirmed that the rates of emerged queens from queen cells that raised on different levels within rearing frame approximately similar.

**2- Fresh weight of virgin queens at emergence**

The mean weights of newly emerged virgin queens that reared on the top and middle bars within rearing frame were significantly heavier than those reared on the bottom bar as appear in Table (2). Queens emerged on the middle bar gained the heaviest weight, (171.6, 169.3 and 189.8 mg), followed by top bar, (169.3, 164.4 and 184.8 mg) then bottom bar, (160.3, 156.5 and 171.7 mg) during the three successive years, respectively, (Fig. 8 ).

These findings are coincided with that obtained by El-Barbary, (2007) who recoded that heavy weight queens appeared significantly in a higher percentages on the middle bar than those located on the upper or lower bars. Also, Abd



Al-Fattah, et al., (2007) registered that the lightest mean of queen's weight was for those raised on the bottom bar level, (167.3 mg) which was significantly less than the both means recorded for the middle, (175.8 mg) and top, (173.8 mg)

bars. In the same respect, Hamada, (2019) mentioned that the newly virgin queens weighed similar insignificant values, (160.7, 158.9 and 160.8 mg), when reared on the top, medium and bottom bars, respectively.

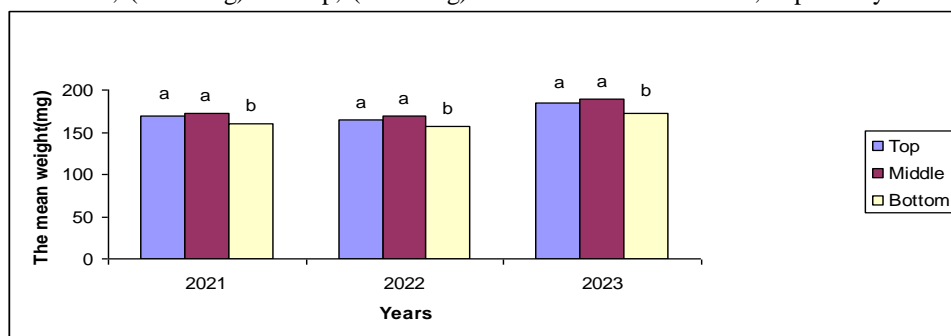


Fig. 8. Effect of queen cell level within grafting frame on fresh weight of virgin queen at emergence

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## كيفية وجودة الملكات المرباة تجاريا وتأثرها بعدد وتوزيع الكؤوس الملكية المطعمة صناعيا داخل طوائف نحل العسل الميتمة في منطقة وسط الدلتا، مصر

محمد عبد الوهاب عبد الفتاح<sup>١</sup>، عبد البديع عبد الحميد غانم<sup>٢</sup>، أسماء انور عيسى<sup>٣</sup> و محمد عبدالله على<sup>٤</sup>

<sup>١</sup>قسم الحشرات الاقتصادية والمبيدات- كلية الزراعة- جامعة القاهرة- الجيزة- مصر

<sup>٢</sup>قسم الحشرات الاقتصادية - كلية الزراعة- جامعة المنصورة- الجيزة- مصر

<sup>٣</sup>قسم بحوث النحل- معهد بحوث وقاية النباتات- مركز البحوث الزراعية- الدقى- الجيزة

### الملخص

تناول هذا البحث تأثير كل من عدد الكؤوس المطعمة (٢٤ و ٤٨ كلاس مطعوم مقدم/ طائفة)، ومستوى السداية (العليا والوسطى والسفلى)، وموضع الكؤوس على السداية (المحيطى والوسطى) داخل اطار التطعيم. على محمل كل من الصفات (الكمية والنوعية) المدروسة (القبول،الفقس،الوزن الملكات العنارى حديثة الفقس) خلال ثلاث سنوات متتالية (٢٠٢١ إلى ٢٠٢٣). باستخدام طوائف بيتمة بلنة ومتممة في آن واحد.. تأثرت الصفات المدروسة معنوياً بعدد كؤوس الملكات المطعومة. وبشكل عام بمستوى السداية وموضع الكؤوس. كان محمل المتوسط العام الذى تم الحصول عليه أعلى في المستويين العلوى (٩٤,٣٪ و ٩٠,٧٪) والوسطى (٩٣,٠٪ و ٩٢,٠٪) مقارنة بالمستوى السفلى (٨٩,٤٪ و ٨٧,٠٪) على التوالي، للصفيتين (القبول،الفقس). وكان محمل المتوسط العام لصفة القبول التى تم الحصول عليها أعلى معنوياً في الموضع الوسطى (٩٣,٦٪) مقارنة بالموضع المحيطى (٩٠,٨٪). ولصفة الفقس، كان الموضع الوسطى (٩٠,٨٪) أعلى بشكل غير معنوى من الموضع المحيطى (٨٨,٩٪). وكان المتوسط العام لوزن الملكات حديثة الفقس أقل معنوياً في المستوى السفلى عن المستوى العلوى وبشكل غير معنوى عن المستوى الوسطى، وكان أقل معنوياً في الموضع المحيطى عنه في الموضع الوسطى. مسجلا في المستوى الوسطى والعلوى والسفلى ١٧٦,٩ و ١٧٢,٨ و ١٦٢,٨ ملجم/ملكة؛ وبالنسبة للموضع الوسطى والمحيطى كان ١٧٤,٦ و ١٦٧,٠ ملجم/ملكة، على التوالي.