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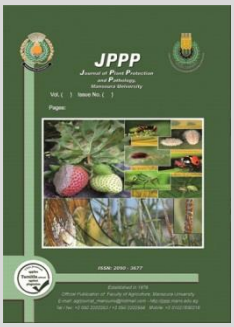
Effect of Genotype of Grafted Larvae and Rearing Bar Level on some Economic Traits in Commercial Production of Honeybee Queens under Damietta Governorate Conditions, North Egypt

El-Kady, H. A.[1]; M. A. Abd Al-Fattah[2] and Noura Abd Al-Hady[1*]

[1] Dept. of Economic Entomology, Fac. Agric., Damietta Univ., Damietta, Egypt
[2] Dept. of Economic Entomology & Pesticides, Fac. Agric., Cairo Univ., Giza, Egypt



ABSTRACT

The present study was conducted in the privet apiary established at Kafr Saad distances, Damietta Governorate, North Egypt, during summer season of 2019, (July and August). The experiments were carried out to determined the effect of genetic origin of the grafted larvae, (*Apis mellifera carnica* and *Apis mellifera bukfast*) and level of bar at which the queen cells hung within rearing frame, (top, medium and bottom levels) and the position of these cells on the rearing bar (middle and peripheral positions) on some output parameters concerned in commercial queens production. The genetic origin of larvae used in grafting process has a positive and significant effect on their acceptance percentage and the live weight of the produced queens when nursed by related starter and finisher building colonies. The percentages of acceptance, sealing and queen emergence as well as the weight of newly emerged virgin queens slightly influenced by the level and position at which the cells presented. In general, queen cells on the bottom bar and middle position were attained a little performance than the others in the commercial queen production during summer season of North Egypt.

Keywords: Carnica race - Bukfast race - Origin of grafted larvae - level of bars - Middle and peripheral queen cell positions - Starter colonies

INTRODUCTION

It is known that the economic characteristics of the honeybee colony are dependent mainly on the quality of its queen. The queen quality, in turn, depends on both genetic and environmental factors, (Hoopingarner and Farrar, 1959). The rearing conditions that offered by nursery colonies are the most important requirement among the environmental factors to obtain good queens, (Johansson & Johansson, 1973; Morse, 1979; De Grandi-Hoffman, *et.al.*, 1993, Abd Al-Fattah & El Shemy 1996, and Abd Al-Fattah *et.al.*, 2003). Various procedures were artificially followed to rear potential honeybee queens in the commercial queen rearing process. Queen-less builder colony was the first and the common design in this aspect upon which different modifications of deepening have been developed, (Laidlaw, 1979; El-Sarrag & Nagi, 1985 and Ahmed, 2016).

Introducing queen cell cups grafted with larvae into queenless starter colony for one day then the accepted larvae were transferred to a finishing colony until many authors and commercial beekeepers, (Kefuss, 1984; Laidlaw and page 1997; Braunstein & Braunstein, 1993 and El Barbary, 2007), followed emergence. Many researchers considered the weight of newly emerged queens as reliable criterion in appreciation their quality, (Weaver, 1957; Szabo, 1975; Salem *et. al.*, 1976 and Eid *et. al.*, 1980). Tarpy *et al.*, (2000, 2004) and Rangel, *et. al.* (2013) considered the weight at emergence is the major indicator in the queen reproductive potential. Genetically, different subfamilies within a group of bee larvae can also contribute in to the variability of emerged queens, (Page & Erikson, 1986 and Moritz *et. al*, 2005).

So, the weight of emerged queens shows a wide range because it is affecting by various factors during rearing process such as the race of grafted larvae, or the race of nursery workers, (Mirza *et. al.*, 1967 and Koç and Karacaoglu, 2004), and the level and position at which the queen cells presented within rearing colony, (Woyke, 1971; Tarpy, *et. al.*, 2000; El Barbary,2007; Helaly, 2012, Kumar and Mall, 2016 and Abou yahia, 2020).

The aim of the present work is to evaluate the influence of biotype larvae which select to introduce into different nursery queen rearing colonies and the level and position at which the queen cells present within the rearing frame during summer season on the percentages of accepting the grafted larvae, sealing queen cells, queen emergence and weight of newly emerged virgin queens, under the environment of Damietta governorate.

MATERIALS AND METHODS

These studies were undertaken at private apiary established in Kafr Saad distances, Damietta governorate , during summer season (July, 15 and August, 25) of 2019. The effect of the origin grafted larvae (*Carniolan* and *Bukfast races*), the level of bar carrying cells within rearing frame, (Top, Medium and Bott) and position at which the queen cells was presented on the cells bar (middle or peripheral) on the percentages of accepted grafted larvae by starter colonies, sealed and emerged queen cells by the finisher colonies were evaluated through this work. The live weight of newly emerged virgin queens was recorded as well.

Preparing the starter and finisher honeybee colonies:

Eighteen honeybee colonies of the local Carniolan F1, were weekly provided with a lot of young nurse bees, (by

* **Corresponding author.**
E-mail address: nourhesham419@gmail.com
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adding 2 capped worker brood combs just before emergence) and one pollen comb. This procedure occurs 1-3 weeks early before starting the experiment. Daily feeding with 1:1 sugar syrup, (w:v) for two weeks before and during the period of queen rearing was done, (Abd Al-Fattah, *et.al*., 2003). These colonies were divided into two groups, the first contained 12 colonies used as starter colonies where six of them received frames of grafted Carniolan larvae (one grafted frame / colony) and the other six received frames of grafted Bukfast larvae as well. The second group contained six colonies used as finisher units as three of them received the successful larval queen cells of pure Carniolan race and the other three received the successful cells of pure Bukfast race as well to complete their development. 24 hours before grafting, (or introducing the defined larval queen cells of each race), queen and all combs containing unsealed brood, except one, were removed from the experimental colonies and workers of each queenless colony were condensed on six combs in the brood chamber. Therefore, the combs of each prepared queenless rearing colony were arranged from the hive wall beside the entrance as follow, one food store, one capped brood, one uncapped brood, one capped brood, two food stores then the feeder.

The grafted frame was inserted between the unsealed and sealed brood. The accepted queen cells were checked 24 hours after introduction, then they gently collected and each larval queen cell was fixed on another bar (as in its position on the grafting frame). Three rearing frames, each carried 48 larval queen cells of Carniolan biotype distributed on three bars (16 cells each) were inserted in the prepared three finisher colonies to complete their development. The three bars were hung under the head of the rearing frame at 5cm.apart between each other, so, they represent three horizontal levels within the frame (Top, Medium and Bottom levels). Another three frames, each carried 48 larval queen cells of Bukfast race were also distributed on three bars (16 cells each) hung as previously mentioned in three bar levels within rearing frame were inserted in another group of the prepared finisher colonies to complete their development. The queen cells on bars of each rearing frame were imaginably divided into three sets according to position of each cell on the bar. One of these sets was considered as middle position, (contained eight queen cells) and the other two sets as peripheral positions (each of them contained four queen cells).

The mature queen cells (after 9-10 days of grafting), were removed and each queen cell was separated under semi-ball screen cage on unripe honeycomb in the same finisher colony until emerged.

Plastic cups were used in this work where each cup was provided with a young worker larva less than 24 h. old on a small droplet of 1:1 aqueous solution of royal jelly according to the commercial queen rearing technique (Laidlaw, 1979). The obtained data were collected as means for statistical analysis. The following parameters were applied to evaluate the importance of the pervious factors in the field of queen rearing and commercial queen production:

1-Number and percentages of accepted larvae, sealed queen cells and emerged queens, 2-Weight of newly emerged virgin queens (not more than 12 hours) using an electrical balance to the nearest 0.01 mg.

Statistical analysis:

The percentage of acceptance, sealed cells and emerged queens were firstly transformed to angular (arc sine) according to the rules of Gomez & Gomez (1976). The split split complete block design was followed for data analysis and the means were compared by Duncan's Multiple Range Test. The program of MSTAT version. 6.4 was applied for this analysis.

RESULTS AND DISCUSSION

The potential of F1 hybrid Carniolan queen-less starter colony in accepting the artificially grafted queen cups and the same race as finisher colony in sealing the introduced larval queen cells and incubating (nursing) these cells until the new queens emerged as well as the quality of the resulted queens (dependent on their weight at emergence) had been investigated under the change expression of the origin of grafted larvae and some internal impulses in rearing frame during summer season of year 2019.

- Effect of the larval genetic origin on queen rearing output

The genetic origin of larvae that used in grafting process to be rearing as queens has various effects on the different considered parameters in queens production as shown in Tables (1-4).

Data presented in Table (1) revealed that the accepting percentage of the grafted larvae from Carniolan biotype and received by starter colonies from the same genotype attained 88.9% compared with those grafted from Bukfast race and received in Carniolan starter colonies where it was recorded 80.6% (Fig. 1).

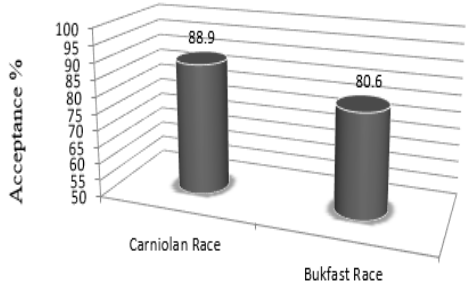

Fig. (1): Effect of honey bee race on the acceptance percentage of grafted larvae during summer season (July & August) of 2019

Table 1. Effect of honey bee race, bar level within grafting frame and position of queen cell on rearing frame on the acceptance percentage of grafted larvae during Summer season (July & August) of 2019

Bar level & position	Carniolan Race			Bukfast Race			Mean / position		Mean/ level ± SE
	Middle	Peripheral	Mean	Middle	Peripheral	Mean	Middle	Peripheral	
Top Bar	95.8	79.2	87.5	70.8	79.2	75.0	83.3 b*	79.2 b*	81.3 B ± 5.239
Medium Bar	91.7	79.2	85.4	70.8	91.7	81.3	81.3 b*	85.4 ab*	83.3 AB ± 5.117
Bottom Bar	91.7	95.8	93.8	91.7	79.2	85.4	91.7 a*	87.5 a*	89.6 A ± 3.599
Mean / Race	93.1 a*	84.7 a*	88.9 A	77.8 b*	83.3 ab*	80.6 B	85.4 a	84.0 a	84.7 ± 2.683
± SE		± 3.157			± 3.840		± 4.668	± 3.427	

Means in the same column or row with the same capital letter, (s) do not significantly differed according to Duncan's Multiple Range test at o.o5 probability of 5% .

***Means in the same column or row with the same small latter with asters do not significantly differ according to Duncan's Multiple Range test of probability of 5%.**

The difference between them was significant. These findings are agreement with many authors such as Mohammedi and Le Conte (2000) where they stated that colonies of *Apis mellifera mellifera* accepted more sisters (77.5%) than Italian colonies (20 %). Ahmed, (2016) found that 66.2% of the grafted *Apis mellfera carnica* larvae compared with 50.41% of *Apis mellifera bukfast* larvae were accepted by the Carniolan building colonies. This difference between the donor and the hostel bees means that the honeybee workers have the ability to discriminate between the related and unrelated larvae, (Krol, 1974 and Rangel, *et. al.*, 2013).

Results in Table (2) indicate to insignificant influence of the origin of the grafted larvae on the sealing percentage of queen cells by the Carniolan finisher colonies.

These sealing percentages were 97.9% and 95.9% for *Apis mellifera carnica* and *Apis mellifera bukfast* grafted larvae, respectively (Fig. 2).

However, there were highly significant interaction between the genotype of grafted larvae and the level of bars carrying queen cells within rearing frame. This interaction appeared in the superior of sealing queen cells of the two races on the top (100% and 97. 9%) and bottom (100% and 95.8%) bars than the medium (93.8% and 93.8%) ones, respectively as shown in Fig. (3).

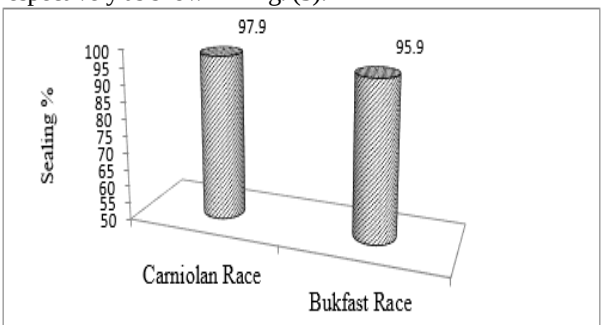

Fig. (2): Effect of honey bee race on the sealing percentage of grafted larvae during summer season (July & August) of 2019

Table 2. Effect of honey bee race, bar level within grafting frame and position of queen cell on rearing frame on the sealing percentage of queen cell larvae during summer season (July & August) of 2019

Bar level & position	Carniolan Race			Bukfast Race			Mean / position		Mean/ level ± SE
	Middle	Peripheral	Mean	Middle	Peripheral	Mean	Middle	Peripheral	
Top Bar	100	100	100 a*	100	95.9	97.9 a*	100	97.9	99.0 A ± 1.989
Medium Bar	95.8	91.7	93.8 b*	91.7	95.8	93.8 b*	93.8	93.8	93.8 B ± 1.184
Bottom Bar	100	100	100 a*	91.7	100	95.8 ab*	95.9	100	97.9 A ± 2.075
Mean / Race	98.6	97.2	97.9 A	94.5	97.2	95.9 A	96.6 a	97.2 a	96. ± 1.079
± SE			±1.420			±1.380	±1.515	±1.667	

Means in the same column or row with the same capital letter, (s) do not significantly differed according to Duncan's Multiple Range test at o.o5 probability of 5% .

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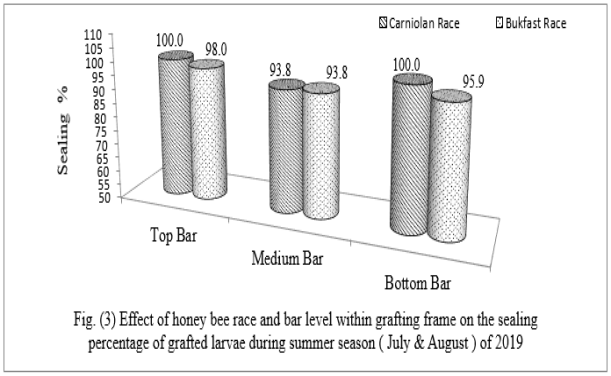

Fig. (3) Effect of honey bee race and bar level within grafting frame on the sealing percentage of grafted larvae during summer season (July & August) of 2019

This variation in the sealing percentage of queen cells may be due to the thermal distribution within brood nest during summer season where the workers employing in regulating the brood nest temperature, especially the middle area of nest, than in other places, (Seeley and Hierich, 1981). Tarpy *et.al.*, (2004) mentioned that during queen rearing, there may a small conflict over which individuals to promote as queens based on genetic relatedness, but the workers mostly cooperate in constructing queen cells so that the emerged queens are of high reproductive potential.

The related and unrelated grafted larvae had not any effect on the percentage of emerged queens. These percentages were 96.5% and 97.8% for the Carniolan and Bukfast races, respectively, (Table 3).

Table 3. Effect of honey bee race, bar level within grafting frame and position of queen cell on rearing frame on the emerging percentage of sealed queen cell larvae during summer season (July & August) of 2019

Bar level & position	Carniolan Race			Bukfast Race			Mean / position		Mean/ level ± SE
	Middle	Peripheral	Mean	Middle	Peripheral	Mean	Middle	Peripheral	
Top Bar	100	95.8	97.9	95.2	100	97.6	97.6	97.9	97.8 A± 1.305
Medium Bar	95.8	91.7	93.8	100	100	100	97.9	95.9	96.9 A± 1.989
Bottom Bar	95.8	100	97.9	100	91.7	95.9	97.9	95.9	96.9 A± 1.989
Mean / Race	97.2	95.8	96.5 A	98.4	97.2	97.8 A	97.8 a	96.5 a	97.2 ± 0.943
± SE			±1.278			±1.453	±0.988	±1.667	

Means in the same column or row with the same capital letter, (s) do not significantly differed according to Duncan's Multiple Range test at o.o5 probability of 5% .

***Means in the same column or row with the same small latter with asters do not significantly differ according to Duncan's Multiple Range test of probability of 5%.**

Data in Table (4) obvious that the biotype origin of larvae had a pronouncing impact in the commercial queen rearing production. Rearing the grafted larvae from *Carniolan* origin resulted significantly heavier queens (mean value of 175.8mg./queen) than those grafted from *Bukfast* origin (mean value of 168.8mg./queen) when they complete their development within building colonies of Carniolan genotype, (Fig.4).

These results agreed with those found by Masry (2010) where the queens reared from ligustica larvae raised by its own colonies recorded heavier weight (185.97mg). The ligustica nursing colonies produced queens with weight of 142.87mg,

when reared from Carnica larvae. However, Ahmed (2016) found that queens reared from Bukfast larvae were insignificantly lighter (172.97mg) than those reared from Carniolan larvae (174.58mg.) when they produced by Carniolan building colonies during the period from April to August.

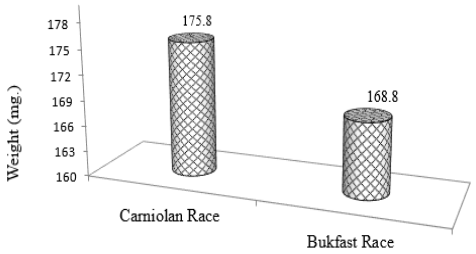

Fig.(4): Effect of honey bee race on the weight of newly emerged virgin queens during summer season (July & August) of 2019

- Effect of cell bar level on queen rearing output

The level at which the introduced queen cell larvae were hung within queen-less starter building colony had various influences on the percentages of accepting the grafted larvae by the starter colony, sealed queen cells, emerged queens, and their weights by the finisher building colony as registered in Tables (4)

Results in Table (1) revealed that the percentages of accepting the grafted larvae were significantly differed according to bar is level, which is carrying those cells. The bottom bar was owned the highest significant percentage, (89.6 %) than the top (81.3%) bar, but it was not with the medium (83. 3%) ones. On the other hand, no significant difference was found between the medium and top bars in this parameter as shown in Fig. (5).

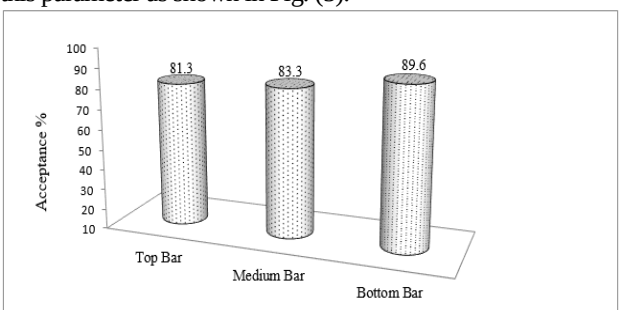

Fig. (5) Effect of bar level within grafting frame on the acceptance percentage of grafted larvae during summer season (July & August) of 2019

These findings are agreed with Ibrahim (1977) who found a significant difference between the lower and upper bars in accepted grafted larvae, while no differences were found between each of (upper and middle bars) or (middle and lower bars), regarding the acceptance percentages of queen cells. The lower bar gave the best result of acceptance (77.70%) followed by middle (64.44%) then upper bar which gave (51.10%).Similar results were recorded by Zeedan (2002) where the acceptance percentages of grafted larvae were 75.8%,84.5% and 84.7% for the first, second and third bar levels within rearing frame with significant differences between them. Therefore, Macicka (1985) stated that there was no consistent pattern from year to year when he found the percentage of accepted larvae varied between the top, middle and bottom bars. However, Visscher (1986) showed that eggs or 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.

The ability of finisher colony in sealing the accepted larval queen cells, for the three bar levels were differed significantly as appeared in Table (2). The highest percentage of sealed queen cells was recorded on the top bar, followed by the bottom then the medium bars. The corresponding percentages were 99.0%, 97.9 % and 93.8% for the top, bottom and medium bars, respectively, (Fig.6). These results are agreement with those found by El-Barbary (2007) during summer season where the sealing percentages of queen cells where the top bar was significantly owned the highest rate, (94.33 %) While the down, (86.30%) bar came next then the middle, (85. 51%) bar.

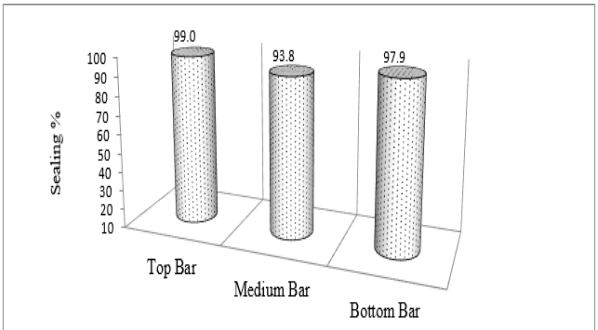

Fig. (6) Effect of bar level within grafting frame on the sealing percentage of grafted larvae during summer season (July & August) of 2019

It is clear from data presented in Table (3) that during summer, the emerged queen percentages from queen cells that hung on different bar's levels within rearing frame were similar and not significantly differed. These percentages of emerged queens were 97.8%, 96.9% and 96.9% for the top, medium and bottom bars, respectively. Fathy *et.al.,* (2019) under the same circumstance, at El-Manzala region, found insignificant differences in the emergence percentages of queen cells presented on top, (93.1%), medium (92.5%) and bottom (91.4%) bars within rearing frame.

Table 4. Effect of honey bee race, bar level within grafting frame and position of queen cell on rearing frame on the weight of newly emerged virgin queens during summer season (July & August) of 2019

Bar level & position	Carniolan Race			Bukfast Race			Mean / position		Mean/ level ± SE
	Middle	Peripheral	Mean	Middle	Peripheral	Mean	Middle	Peripheral	
Top Bar	174.2	173.6	173.9	169.4	165.2	167.3	171.8	169.4	170.6 B ± 2.086
Medium Bar	176.4	176.9	176.6	167.9	170.5	169.2	172.2	173.7	172.9 AB ± 2.208
Bottom Bar	177.4	176.5	176.9	169.9	169.6	169.8	173.7	173.0	173.4 A ± 2.074
Mean / Race	176.9	176.7	175.8 A	168.9	170.1	168.8 B	172.5 a	172.0 a	172.3 ±1.155
± SE		± 0.632			± 0.791		± 1.629	± 1.822	

Means in the same column or row with the same capital letter, (s) do not significantly differed according to Duncan's Multiple Range test at o.o5 probability of 5% .

***Means in the same column or row with the same small latter with asters do not significantly differ according to Duncan's Multiple Range test of probability of 5%.**

The weight of newly emerged virgin queens was not affected by the level within brood nest at which queen cells were hung during summer season (Table 4). The weight of queens resulted on the bottom bar was insignificantly heavier (173.4 mg./queen) than queen weights that reared and produced on the medium, (172.9 mg./queen) and top (170.6 mg./queen) as shown in (Fig.7). The present results are similar with those obtained by Abd Al-Fattah *et. al.* (2007) where they registered 175.77 mg./queen as a mean value for the middle bar level, followed by 173.84 mg.)/queen for those resulted from the top bar level. However, the lightest weight of queens was found with queen reared on the down bar level, (167.34 mg. /queen). The last queen weight was significantly less than those obtained for the formers were. Hamada, (2019) found that no significant difference in the weight of virgin queens (mg./queen) between the different bar levels of the tested colonies .The mean weights of virgin queens was 160.69±2.3827 mg./queen, 158.88±2.9025 mg./queen and 160.75±2.9396 mg./queen for the top, medium and bottom bars, respectively.

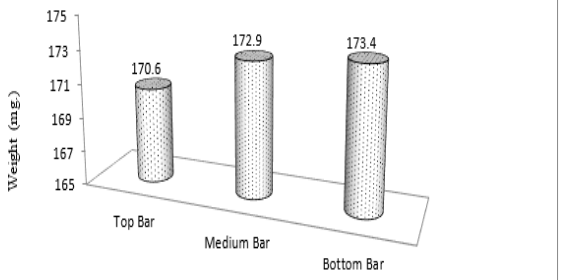

Fig. (7) Effect of bar level within grafting frame on the weight of emerged virgin queens during summer season (July & August) of 2019

- Effect of queen cell position on queen rearing output

The queen cell position within rearing frame had not impact on any of the quality parameters of the issued queens during this period of study (summer season) as shown in Tables (1-4).

The percentages of accepted grafted larvae that presented in the middle, (85.4%) and peripheral, (84%) positions were not significantly differed (Table1). These result are agreement with Zeedan(2002) who found insignificant difference between the middle (84.0%) and edge (80.0%) positions as a mean of two summer seasons of years 2000 and 2001.

There were insignificant differences between the percentages of sealed queen cells that positioned in the middle, (96.9%) of rearing frame and those positioned on the peripheral, (97.2%) of it, (Table 2). Abd Al-Fattah *et.al.*, (2011) registered similar insignificant percentages in this manner for the middle (87.11%) and peripheral (90.32%) positions during summer season.

Similar influence observed for the impact of queen cell position on the emerged percentage of queens during summer season. Results in Table (3) appeared that the mean percentages of queen emergence were 97.8% and 96.5% for the middle and peripheral positions, respectively (Fig. 8). Similar findings were found by Abd Al-Fattah *et.al.*, (2011) in this respect where the mean rate of queen emergence, during summer season, were 84.96% and 87.73% for the middle and edge positions, with non-significant differences between them.

Results in Table (4) appeared that, during summer season, the mean weight of queens emerged from cells located on the middle (172.5mg./ queen) position of rearing frame was not significantly differed from those produced on the peripheral (172.0 mg. /queen), ones Ahmed, (2016) recorded that the mean weights of virgin queens which recently emerged on the center, (172.52mg.), near edge (171.97mg.) and edge (171.59mg.) positions within rearing frame were not significantly differed from each other when the grafted larvae from different genotypes were introduced to raise queens into Carniolan building colonies.

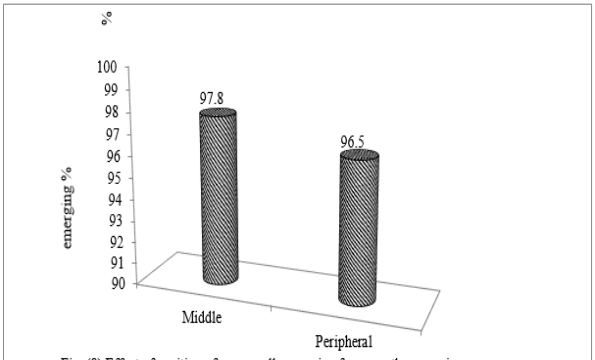

Fig. (8) Effect of position of queen cell on rearing frame on the emerging percentage of queen cell larvae during summer season (July & August) of 2019

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تأثير الأصل الوراثى ليرقات التطعيم ومستوى السدابة على بعض الصفات الاقتصادية لملكات نحل العسل المنتجة تجاريا تحت ظروف محافظة دمياط ، شمال مصر .

حافظ عبد الرحمن القاضى [1]، محمد عبد الفتاح [2] و نورا عبد الهادى[1]*

[1]قسم الحشرات الاقتصادية – كلية الزراعة - جامعه دمياط

[2]قسم الحشرات الاقتصادية و المبيدات – كلية الزراعة - جامعه القاهرة

أجريت هذه الدراسة فى منحل خاص بمنطقة كفر سعد – محافظة دمياط – شمال مصر خلال فصل الصيف لعام 2019 (يوليو و أغسطس) حيث تم دراسة تأثير الأصل الوراثى لليرقات المطعومة من سلالتى النحل الكرنيولى ونحل البيكفاست) ، ومستوى السدابة التى تعلق عليها البيوت الملكية و كذلك موضع هذة البيوت داخل اطار التطعيم على بعض الصفات المرتبطة بالملكات المنتجة تجاريا .أوضحت الدراسة أن الأصل الوراثى لليرقات المطعومة أظهر تأثيرا فعالا وإيجابيا على كلٍ من نسبة القبول لليرقات المطعومة و الوزن الحى للملكات الناتجة عندما أجريت التربية فى طوائف بادئة ثم مكملة من نفس السلالة . كما تأثرت النسبة المئوية لقبول وغلق و فقس الملكات العذارى الناتجة و كذلك وزنها بموضع البيت الملكي على سدابة التطعيم . وبصفة عامة فان البيوت الملكية المرباة على السدابة السفلية و كذلك الموضع الوسطى للبيوت الملكية فى اطار التطعيم قد حققت معدلات أعلى من النجاح عن المواضع الأخرى عند تربية الملكات تجاريا فى تلك المنطقة .