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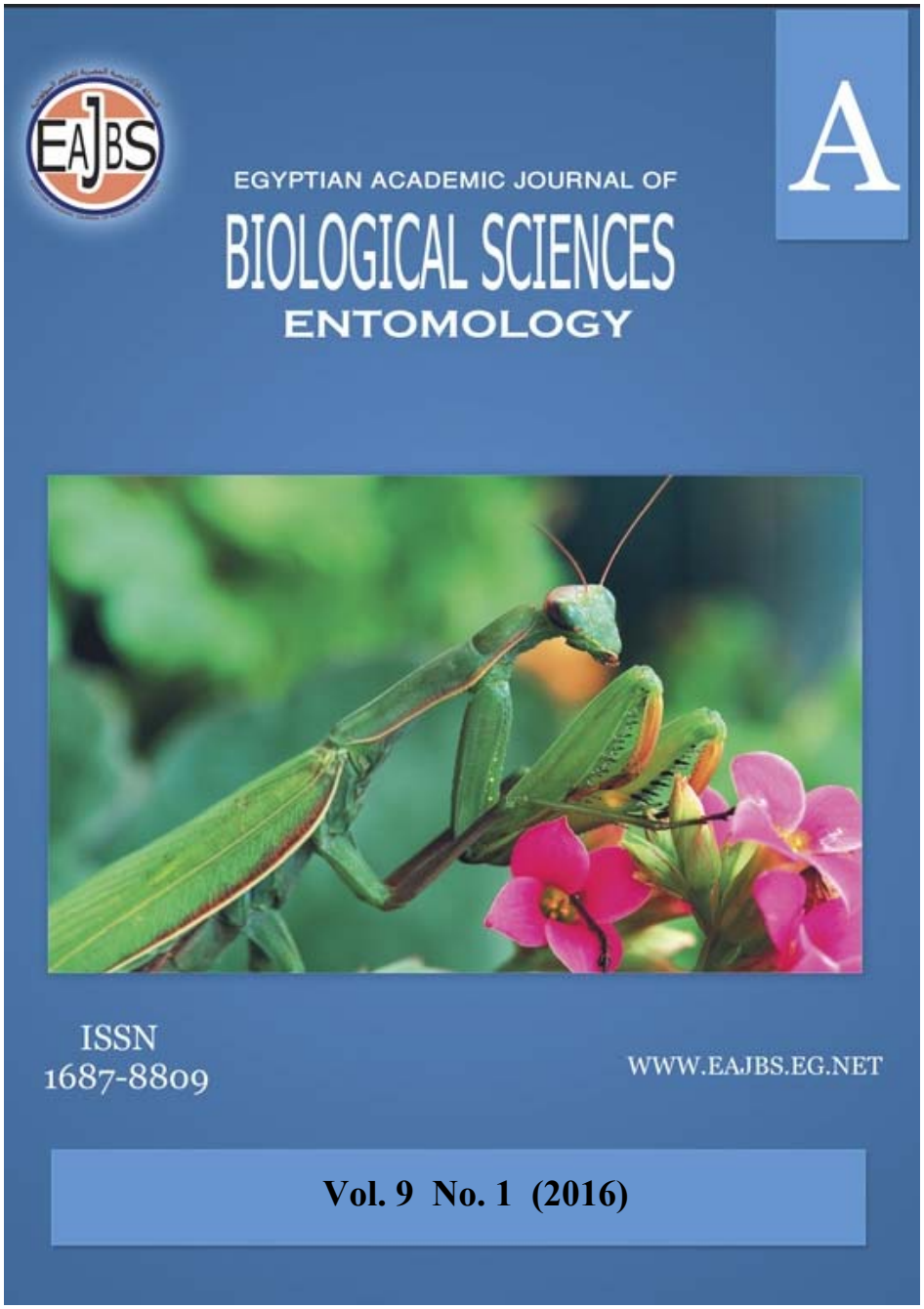

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Effect of Queens Density, Cage Level and Position of Honeybee Mated Queens Stored for Different Periods in Queen-Right Bank Colonies on Their Supersedure Rate

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ABSTRACT

This study was carried out in the apiary yard of Agricultural Experimental Station, Faculty of Agriculture, Cairo University, Giza Governorate, Egypt, during the active season of 2014, (from March to October). The work determined the effect of stored queens densities (20, 30 and 40 queen / colony), cage level (upper and lower levels) and it's position (peripheral and middle positions) on the supersedure rate of queens stored in queen-right colony for 45 and 75 days. Storing mated queens in the mentioned densities had a significant effect on their replacement rate, as the lowest significant replacement rate was for storing 20 and 30 mated queens (20.0% and 30.0%, respectively), while density of 40 queens had the highest significant percentage (45.0%). Concerning the upper and lower levels of storing cages, the mean percentages of supersedure reached it's highest rank when the cages contained queens stored in the lower level (35.0%) of the holding frame, while the upper level had a less significant mean of replacement recording 25.0%. Queens stored at the peripheral position had a higher significant supersedure rate (45.0%), while only 20.0% of the queens stored in the middle position have been replaced. Supersedure rate was influenced positively with increase of storage period, as queens stored for 75 days had the highest significant replacement rate (35.6%) comparing to the 45 days storage period (27.2%).The overall supersedure rate ranged from 30.0% to 32.5% throughout the experiments, which encourage and give another importance to of storing mated honeybee queens.

INTRODUCTION

Naturally, there is only one queen in honeybee colony that laying eggs and control various activities of her nest. The presence of more than one queen can be occurred in honeybee colony for short periods at the time of swarming or queen replacement (Michener, 1974 and Winston, 1987). However, the existence of more than one queen in the same colony for a long period can be achieved in the two-queens colony system (Moeller, 1976), or in a reservoir colony system (Reid, 1975) by using a physical barrier such as a queen excluder or wire screen to separate individual queens.

According to Woyke (1984), honeybee colonies headed by 1 year old queens harvested 27% more honey than those with 2 years old queens. Akyol *et al.* (2008) reported that about 71% less honey was produced from colonies with 3 years old queens than colonies with 1 and 2 years old.

Since queens can't be reared during every season, beekeepers are often unable to obtain queens at critical times of the year, especially in early spring or before wintering (Levinsohn and Lensky, 1981; Fries, 1982; Wilde, 2006 and Siuda *et al.*, 2014). Thus, the reliable storage of queens until they are needed for requeening is of great importance.

Storage of queens on a short-term basis in the spring and summer is commonly practiced by commercial queen breeders and beekeepers (Mackensen & Tucker, 1970; Morse, 1979 and Siuda *et al.*, 2011).

It was noticed that the level and position of the confined queen in the nest is of a great importance (Orosi Pal, 1957; Fell and Morse, 1984 and Macika 1985), as it influence the workers care to it. Visscher (1986) noticed that queens are reared more readily in the upper than in the lower part of the brood nest. So, this may be suitable circumstances for storage.

On the same trend Zeedan (2002) cleared that the percentage of accepted larvae, the rates of sealed queen cells, the rates of emerged queen from cells and the weight of reared queens from cells on the 3rd and 2nd strip levels were significantly higher than those reared on the 1st and 4th strip bar levels.

On the other hand, the acceptance of grafted larvae, sealing of queen cells and virgin queens emergence in the upper and lower levels were studied and revealed a superiority for the lower level over the upper one (Shah, 2000 and Sharaf El-Din *et al*., 2000).

Concerning the position of stored queen in the hive, Visscher (1986) found that the probability that an egg in a queen cup will be reared as a potential queen in a honeybee colony depends on its position in the rearing colony. The probability of a queen being reared is low at the periphery of the hive,

So as Wyborn (1991) stored approximately 500 *Apis mellifera* queens and revealed that mortality of queens at the outer edges of the queen holding frame was greater than mortality of central queens when the distance of the outer cage to the hive wall was 13 cm. or less.

Zeedan (2002) and El-Barbary (2007) cleared that the acceptance percentage of grafted larvae, the rates of capped queen cells, percentage of queens emerged and the weight of newly emerged queens were higher in the middle of bar than on the edge.

Post storage performance is the determining factor that evaluate stored queens quality, upon that this work investigated the impact of the density of the stored queens as well as queen's level and position on their supersedure rate after different periods of storage during honeybee active season (March to October) within queen-right colonies. This long time of storage could be a solution to beekeepers wishing to initiate new nuclei through artificial swarming to replace the old queens before main nectar flow, and at the same time to compensate for the lost queens and to offer queens required for the bee packages produced during Spring and late Summer.

MATERIAL AND METHODS

This work was carried out in the apiary yard of Faculty of Agriculture, Cairo University, Egypt, during the active season of 2014.

The queen bank colonies (QBC)

Nine honeybee strong colonies with no apparent sings of diseases, having adequate pollen and honey stores and high number of young workers were selected (Harp, 1969) and divided into three groups, (three colonies each) where used as queen banks. Twenty mated queens were stored in each queen bank (QBC) of the

first group, while 30 mated queens were stored in each bank of the second group, and 40 mated queens were stored in each bank of the third group. The preparation of QBC started 2 days before the beginning of experiments by caging the colony's queen and removing all uncapped brood to increase the acceptance of the stored mated queens and prevent rearing a new one (Gencer, 2003). After one week of inserting the stored queens, the colony's queen was released in the colony for egg laying and it was observed after three days of releasing to ensure it's acceptance. The unaccepted original queens were replaced by releasing one from the stored queens in order to keep the queen bank in a state of queen-right colony, (Szabo, 1974).

The queen bank colonies were exposed to a routine work such as feeding with sugar syrup (1:1 sugar: water) 2 times weekly. Varroa mites were controlled by using Apistan strips.

The storing cages and the holding frame

All storing cages were of Benton mailing type (8×3×2 cm.) supplied with candy (Shehata, 1982 and Gencer, 2003). All queens were individually stored without attendant workers (Szabo, 1974; 1975a & 1977a).

A Hoffman frame with 2 wooden horizontal trays was used for holding the queen cages. The first tray (upper level) was fixed at 9 cm below the upper bar of the frame, while the second one (lower level) at 10 cm. under the first tray (19 cm from the upper bar of the frame). The cages were put vertically with the candy chamber towards the bottom of the try. Each tray could contain two rows of 20 Benton cages back to back, so each frame could hold up to 40 cages in total (Mohamed *et al.*, 2002).

The frame carrying the cages of the confined mated queens was inserted between two capped brood combs in the center of the brood nest, in a way that where the screen mesh of cages were facing the bees (Johansson and Johansson, 1971; Szabo, 1974 & 1977a).

Determining the supersedure of mated queens stored for 45 and 75 days

All tested queens (including the control ones) were introduced to five frames queenless colonies in Benton cages and released 2 days after that. The original queens of these colonies were removed 2 days before introducing. Replacement percentages were followed and recorded weekly.

Effect of stored queens density on their replacement

Thirty stored mated queens represent the different storage densities (20, 30 and 40 queens) were chosen from queen bank colonies after 45 days of storage. Another 30 mated queens represent the mentioned densities were removed after 75 days of storage. For each storage period, the queens were divided equally into three groups, (10 each).

Effect of stored queens level on their replacement

Twenty stored mated queens represent two storage levels (upper and lower) were chosen from queen bank colonies after 45 days of storage. Another 20 mated queens represent the mentioned levels were removed after 75 days of storage. For each storage period, twenty queens were divided equally into two groups. These queens were chosen from different storage densities as three queens from each of the 20 and 30 queens densities and four queens from the density of 40 queens were chosen.

Effect of stored queens position on their replacement

Twenty stored mated queens represent two storage positions (peripheral and middle) were chosen from queen bank colonies after 45 days of storage. Another 20 mated queens represent the mentioned densities were removed after 75 days of

storage. For each storage period, twenty queens were divided equally into two groups. These queens were chosen from different storage densities as previously mentioned.

Control treatment for the three experiments was prepared by removing ten mated queens (in the same age of stored queens) from their normal colonies and introduced to the experimental queenless colonies in comparison with the stored queens.

Statistical analysis

Data related to replacement percentage were analyzed by using Analysis of Variance (ANOVA) in a split-split randomized complete block design with observation dates, density of stored queens (or stored queen level or position) and storage period as factors followed by Duncan's Multiple Range Test to determine the differences between the obtained means using MSTAT-C (Version 2.10) computer program. Percentages of replacement were arc-sin transformed before analysis.

RESULTS AND DISCUSSION

Effect of stored queens density on their replacement

Data presented in Table (1) revealed that, storing mated honeybee queens in three densities within queen-right bank colonies had a significant effect on their supersedure during the honeybee active season. The mean replacement percentage of stored queens in the highest density (40 queens) was significantly higher (45.0%) than the replacement means of 30 and 20 queens stored together (30.0% and 20.0%, respectively). The non-stored queens had the same rank for supersedure percentage (20.0%) as shown in Fig. (1).

Table 1: Supersedure rate of introduced mated queens stored in different densities for 45 and 75 days.

Colony inspected after	Queens density									Control			Mean / Storage period		Mean Inspection
	20			30			40								
	45 d.	75 d.	Mean	45 d.	75 d.	Mean	45 d.	75 d.	Mean	45 d.	75 d.	Mean	45 d.	75 d.	
2 d.	0.0	0.0	**0.0**	0.0	0.0	**0.0**	0.0	0.0	**0.0**	0.0	0.0	**0.0**	0.0	0.0	**0.0**
2 w.	0.0	0.0	**0.0**	0.0	0.0	**0.0**	0.0	0.0	**0.0**	0.0	0.0	**0.0**	0.0	0.0	**0.0**
4 w.	0.0	0.0	**0.0**	0.0	0.0	**0.0**	0.0	0.0	**0.0**	0.0	0.0	**0.0**	0.0	0.0	**0.0**
6 w.	0.0	0.0	**0.0**	0.0	0.0	**0.0**	20.0	0.0	**10.0**	0.0	0.0	**0.0**	5.0	0.0	**2.5**
8 w.	10.0	0.0	**5.0**	10.0	0.0	**5.0**	0.0	20.0	**10.0**	0.0	0.0	**5.0**	7.5	5.0	**6.3**
10 w.	0.0	20.0	**10.0**	10.0	0.0	**5.0**	20.0	10.0	**15.0**	10.0	0.0	**10.0**	10.0	10.0	**10.0**
12 w.	10.0	0.0	**5.0**	0.0	20.0	**10.0**	0.0	20.0	**10.0**	10.0	10.0	**5.0**	2.5	12.5	**7.5**
14 w.	0.0	0.0	**0.0**	0.0	20.0	**10.0**	0.0	0.0	**0.0**	0.0	10.0	**0.0**	0.0	5.0	**2.5**
16 w.	0.0	0.0	**0.0**	0.0	0.0	**0.0**	0.0	0.0	**0.0**	0.0	0.0	**0.0**	0.0	0.0	**0.0**
18 w.	0.0	0.0	**0.0**	0.0	0.0	**0.0**	0.0	0.0	**0.0**	0.0	0.0	**0.0**	0.0	0.0	**0.0**
Tot. Rep.	**20.0**	**20.0**	**20.0 B**	**20.0**	**40.0**	**30.0 B**	**40.0**	**50.0**	**45.0 A**	**20.0**	**20.0**	**20.0 B**	**25.0 a**	**32.5 a**	**28.8**

Rep.: Replacement

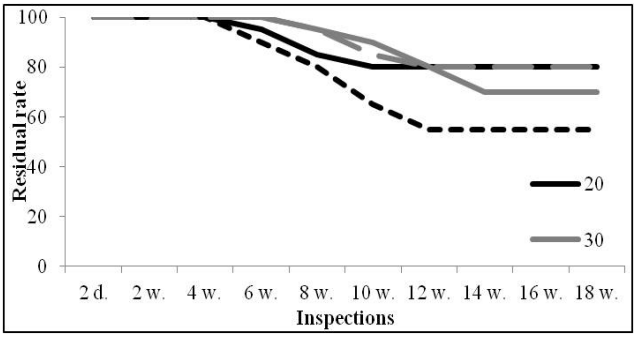

Fig. 1: Residual rate of stored mated queens in different densities after eighteen weeks of introducing into queenlees colonies.

The non-stored queens and density of 20 stored queens had the same trend as 5.0% of queens were replaced after 8 weeks and another 10.0% replaced at the inspection after ten weeks. After twelve weeks of observation, the total replacement reached 20.0% of queens and no other replacement happened after that till the end of experiment. Supersedure in 30 stored queens density happened at the 8, 10, 12 and 14 weeks with percentages of 5.0%, 5.0%, 10.0% and 10.0%, respectively with a total replacement of 30.0%. Forty stored queens density resulted in 10.0%, 10.0%, 15.0% and 10.0% replacement at the observation of 6, 8, 10 and 12 weeks, respectively.

The first replacement observed after six weeks as 2.5% of queens were replaced. Replacement percentage was increased after that achieving 6.3% and 10.0% after eight and ten weeks, respectively. However, at 12 and 14 weeks of observation the replacement percentages were to attain 7.5% and 2.5%, respectively. No more replacement was happened after that till the eighteenth week of observations with total replacements of 28.8%.

The density of the queens in a bank seems to be an important parameter for their survival where the lowest number of queens per bank having the lowest supersedure rate. However, not much of work on the density of queens in queen banks have been previously performed. Abd Al-Fattah *et al.* (forthcoming, 2016) noticed that storing up to 20 honey bee mated queens in queen-right banks maintained queens quality and survival rate to high levels till at least 45 days compared to higher number of queens in each bank.

Effect of stored queens level on their replacement

Data presented in Table (2) revealed that, storing mated honeybee queens in two levels within queen-right bank colonies had a significant effect on their supersedure. The highest significant replacement percentage was in the lower level (35.0%) followed by these of higher level (25.0%), while the non-stored queens had the lowest significant supersedure percentage (20.0%) as shown in Fig. (2).

Supersedure for queens stored in the upper level was firstly observed after eight weeks as 10.0% of introduced queens were replaced, then after ten weeks another 10.0% of queens were replaced. At the observation of fourteen weeks, another 5.0% were replaced.

Table 2: Supersedure rate of introduced mated queens stored in different levels for 45 and 75 days

Colony inspected after	Queens level						Control			Mean / Storage period		Mean / Inspection
	Upper level			Lower level								
	45 d.	75 d.	Mean	45 d.	75 d.	Mean	45 d.	75 d.	Mean	45 d.	75 d.	
2 d.	0.0	0.0	**0.0**	0.0	0.0	**0.0**	0	0	**0.0**	0.0	0.0	**0.0**
2 w.	0.0	0.0	**0.0**	0.0	0.0	**0.0**	0	0	**0.0**	0.0	0.0	**0.0**
4 w.	0.0	0.0	**0.0**	0.0	0.0	**0.0**	0	0	**0.0**	0.0	0.0	**0.0**
6 w.	0.0	0.0	**0.0**	10.0	20.0	**15.0**	0	0	**0.0**	3.3	6.7	**5.0**
8 w.	10.0	10.0	**10.0**	20.0	10.0	**15.0**	10	0	**5.0**	13.3	6.7	**10.0**
10 w.	0.0	20.0	**10.0**	0.0	10.0	**5.0**	10	10	**10.0**	3.3	13.3	**8.3**
12 w.	0.0	0.0	**0.0**	0.0	0.0	**0.0**	0	10	**5.0**	0.0	3.3	**1.7**
14 w.	10.0	0.0	**5.0**	0.0	0.0	**0.0**	0	0	**0.0**	3.3	0.0	**1.7**
16 w.	0.0	0.0	**0.0**	0.0	0.0	**0.0**	0	0	**0.0**	0.0	0.0	**0.0**
18 w.	0.0	0.0	**0.0**	0.0	0.0	**0.0**	0	0	**0.0**	0.0	0.0	**0.0**
Tot. Rep.	**20.0**	**30.0**	**25.0 AB**	**30.0**	**40.0**	**35.0 A**	**20.0**	**20.0**	**20.0 B**	**23.3 a**	**30.0 a**	**26.7**

Rep.: Replacement

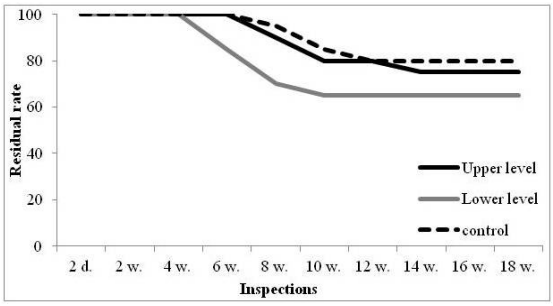

Fig. 2: Residual rate of stored mated queens in different levels after eighteen weeks of introducing into queenlees colonies.

Concerning queens stored in the lowest level, they were replaced with a percentage of 15.0% after 6 weeks of introduction. Another 15.0% of queens were replaced after eight weeks of observation then, only 5.0% of queens replaced after ten weeks giving a total replacement of 35.0%. Non stored queens replacement percentages were 5.0%, 10.0% and 5.0% after eight, ten and twelve weeks, respectively.

No significant differences in rate of replacement were observed between queens stored for either 45 or 75 days with slight increase for the longest period (6.7%).

Requeening honeybee queenless colonies with previously stored and non stored queens resulted in 5.0% and 10.0% of them replaced after 6 and 8 weeks, respectively. After ten weeks of introduction 8.3% of queens were replaced. The replacement was sharply decline recording 1.7% after both of twelve and fourteen weeks. No other replacements were noticed till the end of experiment.

Many researchers suggested that the upper level of holding frame is surrounding by numerous workers, which give a more care than the lower one. Visscher (1986) stated that queens are reared more readily in the upper than in the lower part of the brood nest. Also, Zeedan (2002) cleared that the mean weight of reared and newly emerged queens on strips beneath the frame top bar by 10 cm. was higher than the mean weight of queens reared on a strip 15 cm. under the frame top bar.

Likewise, Abd A1-Fattah *et al.* (2011) indicated that the percentage of queen emergence, development time and weight of emerged queens were significantly affected by the strip level. Queens emerged from cells on the middle rearing bars (10 cm. from top bar) had a high frequency of heavy weight in comparison with those reared on lower bars (15 cm.).

Effect of stored queens position on their replacement

Data presented in Table (3) revealed that, the storage position of mated queens had a significant effect on it's supersedure after 45 and 75 days. The highest significant mean percentage of replacement was noticed in the peripheral position (45.0%), while the mean of middle position and the non-stored queens was the same (20.0%) as shown in Fig. (3).

Replacement of queens stored in the peripheral position began strongly after 6 weeks of introduction (25.0%), and then decreased to 10.0% after eight and ten weeks of observation. Queens stored in the middle position started to be replaced after eight weeks with 10.0%. Five percent of queens were replaced after 12 weeks and another 5.0% after fourteen weeks. Non-stored queens replacement percentages were 5.0%, 10.0% and 5.0% after 8, 10 and 12 weeks of introduction.

Table 3: Supersedure rate of introduced mated queens stored in different positions for 45 and 75 days

Colony inspected after	Queens position						Control			Mean		Mean / Inspection
	Peripheral			Middle								
	45 d.	75 d.	Mean	45 d.	75 d.	Mean	45 d.	75 d.	Mean	45 d.	75 d.	
2 d.	0.0	0.0	**0.0**	0.0	0.0	**0.0**	0	0	**0.0**	0.0	0.0	**0.0**
2 w.	0.0	0.0	**0.0**	0.0	0.0	**0.0**	0	0	**0.0**	0.0	0.0	**0.0**
4 w.	0.0	0.0	**0.0**	0.0	0.0	**0.0**	0	0	**0.0**	0.0	0.0	**0.0**
6 w.	30.0	20.0	**25.0**	0.0	0.0	**0.0**	0	0	**0.0**	10.0	6.7	**8.3**
8 w.	10.0	10.0	**10.0**	10.0	10.0	**10.0**	10	0	**5.0**	10.0	6.7	**8.3**
10 w.	0.0	20.0	**10.0**	0.0	0.0	**0.0**	10	10	**10.0**	3.3	10.0	**6.7**
12 w.	0.0	0.0	**0.0**	0.0	10.0	**5.0**	0	10	**5.0**	0.0	6.7	**3.3**
14 w.	0.0	0.0	**0.0**	10.0	0.0	**5.0**	0	0	**0.0**	3.3	0.0	**1.7**
16 w.	0.0	0.0	**0.0**	0.0	0.0	**0.0**	0	0	**0.0**	0.0	0.0	**0.0**
18 w.	0.0	0.0	**0.0**	0.0	0.0	**0.0**	0	0	**0.0**	0.0	0.0	**0.0**
Tot. Rep.	**40.0**	**50.0**	**45.0 A**	**20.0**	**20.0**	**20.0 B**	**20.0**	**20.0**	**20.0 B**	**26.7 a**	**30.0 a**	**28.3**

Ob: Observation, Rep.: Replacement

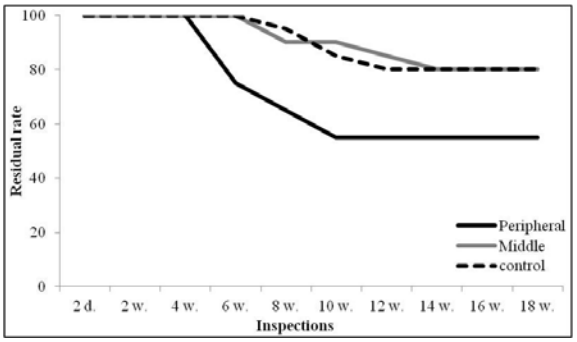

Fig. 3: Residual rate of stored mated queens in different positions after eighteen weeks of introducing into queenlees colonies.

No significant differences were observed between queens stored for 45 and 75 days. The replacement was firstly appeared after 6 weeks (8.3%) and it was the same of next observation (8 weeks). As for 10, 12 and 14 weeks of observations, the replacement percentages were 6.7%, 3.3% and 1.7%, respectively, which achieving 28.3% in total. These results were in the same trend with Visscher (1986) who stated that queens in cells near the centre are reared more frequently than those on the edges. The extensive care and the consistency of brood nest temperature in the middle area than on the peripheral ones may be the reason of harvesting high weights of honeybee queens (Visscher, 1986 and Hoffman *et al.*,1993).

Wyborn (1991) revealed that, mortality of queens at the outer edges of the queen holding frame was greater than mortality of central queens when the distance of the outer cage to the hive wall was 13 cm. or less. Moreover, Zeedan (2002) cleared that the weight of newly emerged queens was higher in the middle than on the edge position, which could be referred to workers extensive care to these queens, consequently the low supersedure rate was attained. He showed that the acceptance percentage of grafted larvae, rates of capped queen cells and queens emergence in the middle position were higher (81.4%, 78.6% and 76.5%, respectively) than those of the peripheral one (74.1%, 69.4% and 66.3%, respectively).

In 2003 Gencer reported that queens close to the hive wall or to the edge of the frame were more susceptible being abandoned by the workers of the colony than queens near the centre of the frame. Queen position on the frame contributes towards the isolation and starvation of queens in colonies.

El-Barbary (2007) revealed that during the honeybee active season (February – October) the mean weight of queens emerged from cells located in the middle position of rearing frame was significantly heavier (179.2 mg.) than those produced

on the edge position (164.3 mg.). In addition, 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 . Abd Al-Fattah *et al.* (2011) found that the percentages of heavy queen weight were 69.7% & 44.8% for middle position and were 25.8% & 31.5% for edge position during summer and spring seasons, respectively.

The overall supersedure (Table 4) for stored queens was ranged from 30.0% to 32.5% with a mean of 31.4%, while for the non-stored ones was 20.0%, Meanwhile supersedure rate was influenced positively with increase of storage period, as queens stored for 75 days had the highest significant replacement rate (35.6%) comparing to the 45 days storage period (27.2%).

Table 4: Supersedure rate of introduced mated queens stored for 45 and 75 days

Parameters	45 days	75 days	Mean
Densities	26.7	36.7	**31.7**
Levels	25.0	35.0	**30.0**
Positions	30.0	35.0	**32.5**
Mean	**27.2** **B**	**35.6** **A**	**31.4**

These results are in agreement with Rangel *et al.*, (2013) who revealed that 25.0% of high quality queens were superseded after 82 days of introduction. Bigio, *et al.*, (2012) had the same trend and reported that storing virgin queens for 7 days resulted in 80% acceptance in queenless nucleus hives.

Hendriksma, *et al.*, (2004) revealed that about 20% of normal queens are superseded in the overwintering colonies, Meanwhile in 2003 Gencer found that 11.2% of stored queens in queenless colonies were superseded after five months of storage, and did not differ significantly from the control ones. Similar results were reported in other studies (Poole *et al.*, 1973; Levinsohn and Lensky, 1981 and Wyborn *et al.*, 1993). Al-Qami (2002) revealed that 25% of naturally mated queens were superseded after 1 year.

On the other hand, Dankaa *et al.*, (1992) reported that in Africanized bees 48% of tested colonies superseded their queens once, three (5%) had two supersedures and one (2%) had three supersedures after 15 months of observation

Wybron (1991) found that replacement percentage ranged from 33.3% to 41.7% for stored queens and 16% for non-stored ones, in addition the forty five days storage for queens resulted in 27.2% supersedure, while 35.6% of queens stored for 75 days replaced.

So as results revealed by Mitchell *et al.* (1985) who found that in the control or non-banked queens 20% of them were replaced and Szabo (1977b) who reported that 33% of them were replaced. Queens stored under laboratory condition were also superseded at 63% (Poole *et al.*, 1973) and 50% as mentioned by Szabo (1975b).

The consistently close levels of supersedure for both stored and non-stored queens in our study suggests that stored queens perform as well as normal colony queens and both queen types were of good quality

In conclusion the obtained results suggest that the best technique to store mated honeybee queens would be storing them in low density (20 queens / bank) within the middle position of the upper level of holding frame for 45 days.

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ARABIC SUMMERY

تأثير كثافة الملكات ومستوى القفص وموقعه للملكات المُلقحة المُخَزنة لفترات مختلفة في طوائف ذات ملكات على معدل إحلالها

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تم إجراء هذا البحث في منحل محطة التجارب الزراعية - كلية الزراعة – جامعة القاهرة خلال موسم نشاط النحل لعام 2014، وذلك لتحديد تأثير عدد الملكات المُخزنة (20 و 30 و 40 ملكة) و المستوى الموجود عنده قفص التخزين (علوي – سفلي) وكذلك موضع القفص في إطار التخزين (على الحافة أو الوسط) على معدل استبدال النحل لهذه الملكات المُخَزنة لمدة 45 يوم و75 يوم. أتضح من الدراسة وجود تأثير معنوي للكثافات المختلفة من الملكات الملقحة المُخَزنة على معدل إحلالها حيث كان معدل الإحلال الخاص بتعداد 20 و30 ملكة / طائفة هو 20% و 30% ، على الترتيب،وكان ذلك منخفضاً بدرجة معنوية عن معدل الإحلال في حالة تخزين 40 ملكة (45%). كما تبين أن تخزين الملكات الملقحة في المستوي السفلي لإطار التخزين أدى إلى ارتفاع معدل الإحلال بدرجة معنوية (35%) عنه عند التخزين في المستوى العلوي للإطار (25%).كما تبين أيضاً أن الملكات المُلقحة المُخَزنة عند حافة الإطار قد تعرضت لحدوث عملية إحلال لها بدرجة معنوية بلغت 45%، في حين كان معدل الاحلال عند التخزين في وسط اطار 20%. كما لوحظ وجود تناسب طردي بين معدل الإحلال ومدة التخزين، حيث أنه عند تخزين الملكات لمدة 45 يوم كان معدل الإحلال الكلي أقل معنوياً (27,2%) عنه عند التخزين لمدة 75 يوم (35,6%). وبصفة عامة فإن معدل الاحلال للملكات المُلقحة المُخَزنة في هذه الدراسة كان 31,4% مما يؤكد أهمية تخزين الملكات المُلقحة خلال موسم نشاط النحل للاستفادة منها في النواحي الاقتصادية الهامة في تربية النحل خلال موسم النشاط الذي يمتد ما بين شهري مارس الى أكتوبر.