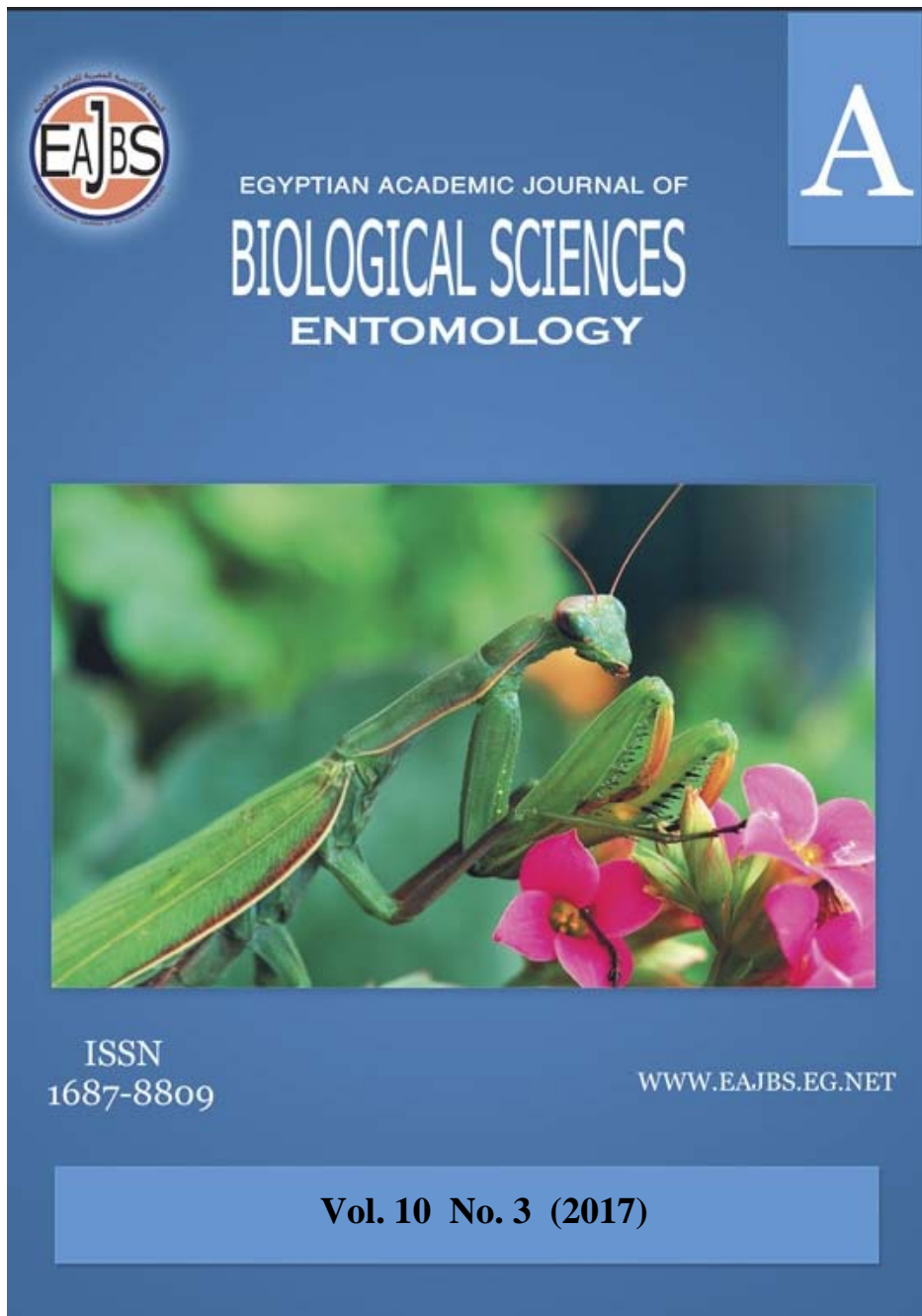
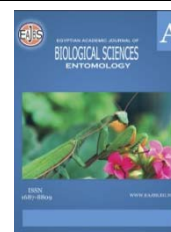


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Relationship Between Number of Grafted Queen Cell Cups and Amount of Produced Royal Jelly in Honey Bee Colonies *Apis mellifera* L.

Ali M. A. M.

Department of Plant Protection, Faculty of Agriculture, Ain Shams University, Cairo, Egypt.

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ABSTRACT

The experiment carried out to study the effect of number of grafted cell cups on number and percentage of accepted queen cells and the amount of royal jelly produced. 45 queen cell cups fixed on three alternated rows (or 15 X 3) on one wide cell-bar (3.5 X 42 cm) were used. The experimental colonies were divided into four different groups; each group was received 45, 90, 135 and 180 queen cells/colony on one, two, three and four cell bars, respectively. The experimental colonies were grafted with 24hrs old-larvae and the royal jelly was collected after 2.5 days after grafting (i.e. when the larvae became 3.5 days-old) and three successive cycles (batches) of royal jelly production were processed at 2.5 days intervals. The data indicated that the amount of produced royal jelly increased by increasing the number of grafted queen cell cups to reach the maximum (49, 88 g/colony) when 180 queen cells were used. These amounts decreased to 17.50, 38, 84 and 45.29 (g/colony) when 45, 90 and 135 queen cell cups were used, with significant differences between them. Regarding the amount of produced royal jelly/queen cell, the honey bee colonies grafted with 180 queen cells on four wide cell bars significantly obtained less amount of royal jelly per cell (132.26 mg/cell), followed by those grafted with 135 queen cells in three wide bars (150.76 mg/cell). Whereas the honey bee colonies grafted with 90 queen cells in two wide bars significantly obtained the highest amount of royal jelly per cell (196.48 mg/cell). Irrespective the number of queen cells, the first graft (batch) produced signify.

INTRODUCTION

Royal jelly (RJ), which is secreted from the hypopharyngeal gland and mandibular gland of the worker honeybee, is the exclusive food for the queen honeybee and larvae (Satomi, *et al.*, 2004). RJ has an important commercial appeal and nowadays it is used in many industries like food and pharmaceutical characteristics (Sabatini *et al.* 2009) as anti-tumor (Tamura and Kuboyama, 1987) anti-bacterial (Sauerwald *et al.*, 1998) anti-hypercholesterolemic (Nakajin *et al.*, 1982) anti-allergic (Kataoka *et al.*, 2001 and Oka *et al.*, 2001) anti-fatigue (Kamakura *et al.*, 2001) insulin-like (Okuda *et al.*, 1998) and wound-healing properties (Fujii, *et al.*, 1990).

There are several factors affecting royal jelly production (Ali, 1994 and Pahinler, 2005 and Sharaf El-Din, 2010). The most important of them are the age of transferred larvae (Sahinler and Kaftanoğlu 1997), feeding (Fuhai *et al.*, 1993), number of transferred queen cell cups (Van-Toor *et al.*, 1994, Kutluca *et al.*, 1998 and Pahinler and Pahinler 2002), harvesting interval (Ali 1994 and Sharaf El-Din, 2010), whether the colony is queenless or queenright (Van-Toor *et al.*, 1994), age of

nurse workers (Ali, 1994) and bee race (Shibi *et al.*, 1993 and Jianke *et al.* 2003, Lian-Fei 2016).

Some countries, like Switzerland (Bogdanov *et al.*, 2004), Bulgaria, Brazil (Brasil Leis e decretos, 2001) and Uruguay have defined national standards for this product. A group of the International Honey Commission is dealing presently with royal jelly standardization (Sabatini *et al.*, 2009).

MATERIALS AND METHODS

Honey bee colonies

To study the relationship between number of grafted larvae and number of accepted queen cell cups and the amount of produced royal jelly, 16 honey bee colonies headed with f1 carniolian (*Apis mellifera carnica*) open-mated queens were used. The colonies were equal in bee population; each had 9-10 combs covered with adult bees. They were divided into four different groups; each group had four colonies (replicates) as follows:

First group: grafted with 45 queen cell cups/colony fixed on three alternated rows on one wide cell-bar measured (3.5 X 42 cm), and the queen cell bar fixed underneath the top-bar of grafting frame (the highest level of queen cell-bar in the grafting frame).

Second group: grafted with 90 queen cell cups/colony fixed on three alternated rows on two wide queen cell-bars (3.5 X 42 cm), each had 45 queen cell cups, and the two cell bars fixed underneath the top-bar of grafting frame and the next lower level of cell bar in the same grafting frame.

Third group: grafted with 135 queen cell cups/colony fixed on three alternated rows on three wide queen cell-bars (3.5 X 42 cm), each had 45 queen cell cups, and the three bars fixed underneath the top-bar of grafting frame, and the next two lower levels of cell bar in the same grafting frame.

Fourth group: grafted with 180 queen cell cups/colony fixed on four alternated rows on four wide cell-bars (3.5 X 42 cm), each had 45 queen cell cups, and the four bars fixed underneath the top-bar of grafting frame, and the next three lower levels of cell bar in the same grafting frame.

Preparation of queenless colonies:

For preparing queen less colonies, strong honey bee colonies, each contained 9-10 frames covered with adult workers were selected as nurse colonies (queenless colonies). The queen was taken with one frame of brood covered with adult bees from each colony and transferred into nucleic box, the box was closed with some green grasses. The boxes were transferred 50 m away of their original hives until finish the cycles of jelly production. After that the adult bees of the queenless colonies were shaken from all the brood and honey frames inside their colonies and crowded in the hive box which provided with two honey and pollen combs with a space between them to insert the grafted frame. A division board feeder was put beside the outer comb for bees feeding. The brood and extra honey combs were inserted inside queen right colonies. After 3-4 hrs of preparing the queenless colonies, the grafted frames with 24 hrs old-larvae were inserted inside the queenless colonies in the space between the two honey and pollen frames, they were left 2.5 days (when the larvae became 3.5 days old), at then they were removed from the queenless colonies and the accepted queen cells were counted and their larvae were removed and royal jelly was collected and weighted. The same technique was repeated three times (batches) at three days-intervals for producing three successive

cycles of royal jelly production (Ali, 1994).

After finishing the experiment, the same queens with bee-workers and relatively the same numbers of sealed brood combs which removed before were returned to their same original colonies for colony build up.

Experimental Design and Analysis:

The experimental design was a completely a randomized design. Results were analyzed, using SAS (SAS Institute, 2006). The general linear modules procedure to test for differences ($\alpha = 0.05$) and the application of the least significant differences as a mean separation test were used.

RESULTS AND DISCUSSION

Number of accepted queen cell cups:

The accepted numbers of queen cell cups were significantly higher when the number of grafted queen cell cups increased, but the percentages of acceptance did not differ (Table 1). The mean numbers of accepted queen cell cups in three successive batches increased as the number of queen cell cups introduced in honey bee colonies increased to reach the maximum (121.67 cell/colony) when 180 queen cell cups were used in four queen cell bars. Where the mean numbers of accepted queen cell cups were 34.92, 63.92, 98.50 and 121.67 cell/colony when 45, 90, 135 and 180 wax queen cells on one, two, three and four grafting frames were grafted. The differences between these means proved to be highly significant. The L.S.D. value (7.948 cells) emphasizes the obtained results.

Irrespective the number of grafted queen cell cups, the first graft (batch) produced the highest numbers of accepted queen cells (90.75 cell/colony), followed by second and third graft (81.63 and 66.88 cell/colony, respectively). The differences between these means proved to be highly significant. The L.S.D. value (6.017 cells) proved these results (Table 1).

Table 1: Mean numbers and percentages of accepted queen cell cups /colony in honey bee colonies grafted with different numbers of queen cell cups, grafted three times at three days-intervals.

Arrangement of cell-bars	Number of queen cell cups	Successive grafts			Mean \pm S.E
		1 st	2 nd	3 rd	
1 st (The upper)	45	35.75 \pm 1.65 (79.44%)	38.25 \pm 1.55 (85.00%)	30.75 \pm 0.75 (68.30%)	34.92 \pm 2.21 d (77.59%)
1 st and 2 nd	90	73.25 \pm 1.38 (81.39%)	64.25 \pm 2.66 (71.39%)	54.25 \pm 2.56 (60.28%)	63.92 \pm 0.89 c (71.02%)
1 st , 2 nd and 3 rd	135	110.75 \pm 4.89 (82.04%)	93.25 \pm 3.33 (69.07)	91.50 \pm 2.10 (67.78%)	98.50 \pm 3.07 b (72.96%)
1 st , 2 nd , 3 rd and 4 th	180	143.25 \pm 8.18 (79.58%)	130.75 \pm 9.06 (72.64%)	91.00 \pm 4.60 (50.56%)	121.67 \pm 6.94 a (67.59%)
Mean \pm S.E		90.75 \pm 23.25 a	81.63 \pm 19.86 b	66.88 \pm 14.87 c	79.75 \pm 19.08

L.S.D. between treatments = 7.948

L.S.D. between grafts = 6.017

Quantity of royal jelly produced:

As shown in Table (2) the amount of produced royal jelly increased by increasing the number of grafted queen cell cups to reach the maximum 49,88 (g/colony) when 180 queen cells were used. These amounts decreased to 17.50, 38,84 and 45.29 (g/colony) when 45, 90 and 135 queen cell cups were used, with significant differences between them (L.S.D. 1.443).

Irrespective the number of grafted queen cells, the first graft (batch) produced the highest amount of royal jelly (18.12 g/colony), followed by second and third graft (13.28 and 6.47 g/colony, respectively). The differences between these means proved to be highly significant. The L.S.D. value (1.25 g) proved these results (Table 2).

Table 2: Mean weights of royal jelly (g/colony) produced in honey bee colonies grafted with different numbers of queen cell cups, grafted three times at three days-intervals.

Arrangement of cell-bars	Number of queen cell cups	Successive graft			Mean \pm S.E
		1 st	2 nd	3 rd	
1 st (The upper)	45	7.85 \pm 0.53	6.40 \pm 0.27	3.25 \pm 0.24	17.50 \pm 1.36 d
1 st and 2 nd	90	19.31 \pm 0.99	12.73 \pm 1.10	6.79 \pm 0.41	38.84 \pm 0.28 c
1 st , 2 nd and 3 rd	135	20.74 \pm 0.98	16.75 \pm 1.43	7.80 \pm 1.26	45.29 \pm 1.37 b
1 st , 2 nd , 3 rd and 4 th	180	24.60 \pm 0.70	17.25 \pm 0.94	8.03 \pm 0.65	49.88 \pm 1.34 a
Mean \pm S.E		18.12 \pm 3.60 a	13.28 \pm 2.51 b	6.47 \pm 1.11 c	37.87 \pm 7.16

L.S.D. between treatments = 1.443

L.S.D. between grafts = 1.250

Quantity of royal jelly produced per cell:

Table (3) shows that the honey bee colonies grafted with 180 queen cell cups on four wide queen cell bars significantly obtained less amount of royal jelly per cell (132.26 mg/cell), followed by those grafted with 135 queen cells in three wide bars (150.76 mg/cell). Whereas the honey bee colonies grafted with 90 queen cells in two wide bars significantly obtained the highest amount of royal jelly per cell (196.48 mg/cell).

Irrespective the number of grafted queen cells, the first graft significantly gave the highest amount of royal jelly per cell (233.51 mg/cell), followed by second and third graft (204.54 and 114.39 mg/cell, respectively), with significant differences between them (Table 3).

Table 3: Average weights of royal jelly (mg/cell) of produced royal jelly in honey bee colonies grafted with different numbers of queen cell cups, grafted three times at three days-intervals.

Arrangement of cell-bars	Number of queen cell cups	Successive graft			Mean \pm S.E
		1 st	2 nd	3 rd	
1 st (The upper)	45	220.08 \pm 14.67	167.70 \pm 6.42	105.98 \pm 8.66	164.59 \pm 32.98 b
1 st and 2 nd	90	264.22 \pm 16.10	199.75 \pm 21.69	125.46 \pm 6.83	196.48 \pm 4.48 a
1 st , 2 nd and 3 rd	135	187.61 \pm 7.00	180.36 \pm 16.75	84.47 \pm 11.53	150.76 \pm 5.24 c
1 st , 2 nd , 3 rd and 4 th	180	172.77 \pm 6.39	134.79 \pm 15.82	89.20 \pm 10.06	132.26 \pm 10.58 d
Mean \pm S.E		233.51 \pm 32.28 a	204.54 \pm 35.61b	114.39 \pm 17.30c	161.02 \pm 13.55

L.S.D. between treatments = 17.323

L.S.D. between grafts = 20.05

From the previously mentioned results, it could be concluded that, after two to three successive grafts, honey bee colonies which received 180 grafted wax queen cell cups in four wide queen cell bars in each graft accepted more queen cells (121.67 cell/colony) and significantly produced more quantities of royal jelly (49.88 g/colony) than those received 45, 90 or 135 queen cells/colony in one, two or three

wide bars in each graft. Irrespective the number of grafted queen cells, the first graft (batch) produced the highest numbers of accepted queen cells and highest amount of royal jelly (90.75 cell/colony and 18.12 g/colony), followed by second and third graft (81.63 and 66.88 cell/colony, and 13.28 and 6.47 g/colony, respectively).

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