J. Plant Prot. and Path., Mansoura Univ., Vol.6 (10): 1357 – 1368, 2015

INCUBATION METHOD OF HONEYBEE QUEEN CELLS IN INCUBATOR AFTER QUEEN CELLS SEALING DIRECTLY.

Zohairy, A. M.
Beekeeping Research Dept., Plant Protection Res. Inst. Agric. Res. Center, Egypt .

ABSTRACT

This study was carried out to evaluate the following aims : 1 - What honeybee workers do to queen cells ready to successful caging, comparison between caging queen cells inside the normal cage and queen-excluder-cage, after sealing directly, in the colony . 2 – Evaluation of the best day for caging inside the normal cage after queen cells sealing directly . 3 – Patterning which honeybee workers do to wax layer removal of queen cells from on queen emergence slot and evaluation this in the incubator by comparison between incubation in the colony and incubator with handling removal of this new way.

The results showed that caging inside queen-excluder-cage was better than the caging inside the normal cage, after queen cells sealing directly, in the colony. Also, the results appeared that the best day for the caging was after four days from sealing of queen cells. The experiments clearing that the honeybee workers removed wax layer of queen cell from on queen emergence slot with 6 – 9 mm circular. Using handling method in removing this layer on the queen cells inside incubator, after sealing directly, gave the same. Thereby the colonies could be used for queen rearing cells only. This way gave two batch instead of one batch in the same time, the production become duple comparing with all normal ways.

INTRODUCTION

It is known that the economic characteristics of the honeybee colonies are dependent mainly on the quality of its queens. The queen quality, in turn depends on both genetic and environmental factors (Hoopingarner & Farrar 1959). The rearing conditions that offered by nursery colonies are the most important requirement among the ecological factors to obtain good queens, (Johansson & Johansson, 1973; Chang 1977 ; Skowronek and Skubida 1988 ; Abou El-Enain, (2000) ; Zohairy, 2001,2007 ; Mohammad 2002 ; Mustafa *et. al.* 2002 and Abd Al- Fattah *et al.* 2003). Many researches considered the weight of newly emerged queen as reliable criterion in appreciating their quality (Weaver, 1957, Szabo, 1975, Salem *et al*., 1976 and Eid *et al*., 1980). It is well known that there are several methods of queen rearing such as punch method (the cutting may was outside of worker cell, or may was inside of worker cell) Snelgrove, 1946, Richard Smailes, 1977 and (Suhayda & Nichols, 1995) and grafting methods Doolittle, 1888, Pellett, 1929 and Snelgrove, 1946.

Eskov & Toroptsov, (1979) mentioned that 33 – 34 ºC. are optimum temperature for producing with high quality queens.

Commercial propagation of queen honeybees is a laborious and time-consuming process that would benefit greatly from the maximization of

queen-cell acceptance in larval transplantation procedures or grafting (Laidlaw and Page, 1997). The design of queen cups can significantly affect both acceptance of grafting larvae and characteristics of the queens subsequently produced (Weiss, 1967a and b; Johansson & Johansson, 1978; Ebadi & Gary, 1980). For characterizes of brood pheromones and larvae presence into queen rearing colonies it help for increasing the acceptance of the queen cells, enhanced the amounts of royal jelly deposited by the worker, improved the weight of the larvae. Also act as a primer pheromone in the regulation of division of labour among adult workers, hypopharyngeal gland development , protein biosynthesis compound and variable inhibition of worker bee ovary development. In addition these pheromones affect on attractant – induces mild retinue-like response. Foraging ontogeny and forage choice behavior. Modulation of worker sucrose response thresholds,(Le Conte *et al*. 1995 and 2001**,** Pankiw *et al.* 2004).

(Laidlaw, H. H. Jr. 1981) and (Abou El-Enain, 2000) used hive with large enough to care and finish off the queen cells. On day 10, after the queen cells are sealed (queen cells ripe), then moved them into the incubator.

Many problems were faces beekeepers when they used incubator for the commercial production of queens, when the queen cells are caged after queen cells sealing directly or when it was transferred into the incubator. Thus, this study was carried out to evaluate the following aims: 1 –Evaluation of the best day for caging inside the normal cage after queen cell sealing directly. 2 – What do honeybee workers do to queen cells ready to successful caging. 3 – Patterning which honeybee workers do to wax layer removal of queen cells from on queen emergence slot and evaluation this in the incubator by comparison between incubation in the colony and incubator with handling removal of this new way.

MATERIALS AND METHODS

Experiments were carried out in a private apiary, El-Satayta Village, El-Manzala Center disterect, El-Dakahlia Governorate, Egypt, at the period from 2011 till 2014.

1 – Honeybee race and queen rearing method: -

This study was used Apis mellifera carnica El-Manzala Carniolan race and used grafting method (Doolittle) for queen rearing.

2 –Percentages of the hatching and weights for the normal virgin queens: -

The hatching percentage was accounted and the weights of the normal newly emerged queens were recorded within about 5 five hours after emergence using electrical balance to nearest 0.01 gram (exclusion each of abnormal emerged virgin queens and the queens which had curly wings).

3 –Experimental colonies preparation: -

Every colonies were equal in strength(8 combs) were provided daily with sugar syrup (approximately 66 %) and pollen supplement for three days before and within the period of experiments. The protein supplements

consisted of a mixture of dried brewer's yeast, Soya flour and sugar powder (1 : 2 : 3), which were mixed with concentrated sugar syrup (4 sugar : 1.4 water).

4 –Incubator preparation : -

Two hour before transfer queen cells into the incubator , degree of temperature was 33ºC and relative humidity was 70% (it prepare at 33ºC and 70% R.H).

This study included the following points:-

A –First experiment was started in 15/8/2011, and repeated in 24/8/2011, 2/3/2012, 10/3/2012 and 18/3/2013 to knowing, what bee workers do to queen cell ready to successful caging by comparison between caging inside the normal cage and queen-excluder-cage, after queen cells sealing directly, in the colony.

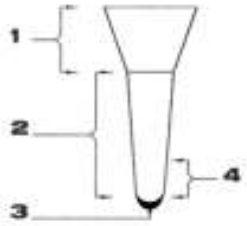
B – Second experiment was started in 25/3/2013 and repeated in 5/4/2013 to evaluation of the best day – after queen cells sealing directly – by caging inside the normal cage (after queen cells sealing directly and after one , two , three , four and five days) . Four replicates of colonies were used for this experiment. The abnormal (curly wing) emerged virgin queens were exempt from the hatching percentage and the weight.

C – Third experiment was started in 6/5/2011, and repeated in 14/5/2014 and 22/5/2014. In the colony, the queen cells were caged inside normal cage, two days before hatching. While in the incubator the queen cells were transferred after its sealing directly to patterning which bee workers do to queen cell is ready to successful caging. The wax layer of queen cells was removed from on queen emergence slot till 6 – 9 mm circular. The removal handling over carefully, with deference not cutting cocoon filaments and not overturn queen cell. In incubator this removal was done handling after three days from incubating this cells in incubator, taking into consideration that this removal must begin from air room of queen emergence slot to easy the handling removal, (when the queen larvae spins the cocoon leave space in the end of queen cell between the wax and cocoon filaments this space makes this the air room.) (fig.1)

Evaluation was done comparison between incubation in the colony and incubator by this new way.

Also, may putting these queen cells in queenless colony even removal this wax layer by worker, to one day term.

Or, putting the queen cells under (half-ball-cage with queen excluder) in any normal colony from apiary even wax layer removal by worker, too.



(**Fig. 1) Queen cell with holder consists of (1) wooden or plastic holder (2) queen cell (3) queen emergence slot and air room (4) layer of removal till 6 – 9 mm. circular.**

5 – Statistical analysis of data:

All data were statistically analyzed by Duncan's Multiple Range Test as described by Duncan (1955).Test and L.S.D. value at 0.05. All the obtained results were statistically analyzed according to analysis of data variance. The proper "F" and L.S.D. values were calculated according to Snedecor and Cochran (1967). The computer program for that was Cohort 2 (Mstatc. Exe).

RESULTS AND DISCUSSION

1 – Comparison between caging with the normal cage and queen-excluder-cage, after queen cells sealing directly, in the colony.

The statistical analysis of data obtained in Table (1) throughout successive years (2011, 2012 and 2012) showed that: -

A – Mean of hatching percentages: -

The mean of hatching percentages were 68.93% and 98.66% by caging with the normal cage and the queen-excluder-cage, respectively. The hatching percentage of caging with queen-excluder-cage was higher than the hatching percentage of caging with normal cage. LSD value at 0.05 was 19.481.

These results appeared that honeybee workers make something with queen cells caged with queen-excluder-cage to become ready of successful caging , it could be noticed that the workers make wax layer removal of queen cells from on queen emergence slot.

Table (1) :Comparison between caging with the normal cage and queen-excluder-cage, after queen cells sealing directly, in the colony.

Type of the cage	% Hatching (Mean± SE)	Weight (Mean± SE) (mg)
Normal cage	68.93 ±!6.8 b	149.29 ± 0.60 b
Queen-ex cluder-cage	98.66 ± 2.66 a	159.62 ± 0.38 a
LSD	19.481	0.8241

Means followed by the same small letter in a column are not significantly differences at the 5% level of probability (Duncan's Multiple Range Test)

B – The weight of emerged queens: -

The mean of the weight of emerged queens were 149.29 mg and 159.62 mg for caging with the normal cage and the queen-excluder-cage, respectively. Results obtained clearly indicated that the mean of the weight of emerged queens by caging with queen-excluder-cage was higher than the mean of the weight of emerged queens by caging with normal cage. LSD value at 0.05 was 0.8241.

These results agreed with Abou El-Enain (2000) who mentioned that the highest percentage and weight of emerged virgin queens, obtained by incubation in queenless colony followed by that incubated in queen right colony, while the latter was incubated by incubator (queen cells were ripe queen cells). This mane that the honeybee workers make something with queen cells till it become ready of successful incubation resulting in highest weight of emerged queens. But these results disagree with her too (Abou El-Enain 2000) for the time of the caging of the queen cells, he caged on ripe queen cells (after 5 five days from queen cells sealing), in these experiments, queen cells were incubated directly after sealing with good results.

2 - Evaluation of the best day – after queen cell sealing directly – to caging with the normal cage.

A – Percentage of the normal virgin queens hatching: -

The statistical analysis of data obtained in Table (2 - 1) from 25/3/2013, 5/4/2013 and the mean of this dates for hatching showed that:-

On 25/3/2013, there were insignificant differences for hatching percentage between the caging after four days and after five days, also between the caging after one day and after two days. There were insignificant differences between the caging after queen cell sealing directly and each of (the caging after one day and after two days), too between the caging after three days and each of (the caging after one day and after two days).

But, there were significant differences for hatching percentage between each of (the caging after four days and after five days) and the caging after three days. There were highly significant differences between each of (the caging after four days and after five days) and the caging after queen cell sealing directly. There were significant differences between the caging after three days and the caging after queen cell sealing directly.

The highest percentage was (100 ± 0.0 %) for each of (the caging after four days and after five days). Then, (75 ± 0.0 %) for the caging after three days, then, (56.25 ± 20.72 %) for each of (the caging after one day and after two days). The lowest percentage was (50 ± 0.0 %) for the caging after queen cell sealing directly. LSD value at 0.05 was 20.529.

On 5/4/2013, there were not significant differences for hatching percentage between the caging after four days and after five days, also between the caging after queen cell sealing directly and caging after one day. There were insignificant differences between the caging after two days and after three days, also, between the caging after two days and each of (the caging after queen cell sealing directly and after one day).

But, on the other hand, there were significant differences for hatching percentage between each of (the caging after four days and after five days) and each of (the caging after two days and after three days). But, there were

highly significant differences between each of (the caging after four days and after five days) and each of (the caging after queen cell sealing directly and after one day). There were significant differences between the caging after three days and each of (the caging after queen cell sealing directly and after one day).

The highest percentage was (100 ± 0.0 %) for each of (the caging after four days and after five days), then, (81.5 ± 10.82 %) for the caging after three days, then, (68.75 ± 10.82 %) for the caging after two days, while the lowest percentage was (62.5 ± 12.5 %) for each (the caging
three days. But, there were highly significant differences among each after queen cell sealing directly and after one day). LSD value at 0.05 was 16.376.

The mean of hatching percentage, there were not significant differences for hatching percentage between the caging after four days and after five days. There were insignificant differences among each of (the caging after queen cell sealing directly, the caging after one day and after two days).

There were significant differences for the mean of the hatching percentage between each of (the caging after four days and after five days) and the caging after of (the caging after four days and after five days) and each of (the caging after queen cell sealing directly, the caging after one day and after two days). There were significant differences among the caging after three days and each of (the caging after queen cell sealing directly, the caging after one day and after two days).

The highest mean of the hatching percentage was (100 ± 0.0 %) for each of (the caging after four days and after five days). Then, (78. 25 ± 8.26 %) for the caging after three days, then, (62.5 ± 17.67 %) for the caging after two days, then, (59.37 ± 17.39 %) for the caging after one day. The lowest mean of the hatching percentage was (56.25 ± 10.82 %) for the caging after queen cell sealing directly. LSD value at 0.05 was 12.462.

Table (2 - 1): Evaluation of the best day – after queen cell sealing directly – to caging with the normal cage.

Caging time	% Hatching (Mean± SE)		
	25/3/2013	**5/4/2013**	**Mean**
After queen cell sealing directly	50 ± 0.0 c	62.5 ± 12.5 c	56.25 ± 10.82 c
After 1 day	56.25 ± 20.72 bc	62.5 ± 12.5 c	59.37 ± 17.39 c
After 2 days	56.25 ± 20.72 bc	68.75 ± 10.82 bc	62.5 ± 17.67 c
After 3 days	75 ± 0.0 b	81.5 ± 10.82 b	78. 25 ± 8.26 b
After 4 days	100 ± 0.0 a	100 ± 0.0 a	100 ± 0.0 a
After 5 days	100 ± 0.0 a	100 ± 0.0 a	100 ± 0.0 a
LSD	20.529	16.376	12.462

Means followed by the same small letter in a column are not significantly differences at the 5% level of probability (Duncan's Multiple Range Test).

Table (2 - 2) : Evaluation of the best day – after queen cell sealing directly – to caging with the normal cage.

Caging time	Weight (mg)		
	25/3/2013	5/4/2013	Mean
After queen cell sealing directly	147.5 ± 5.59 b	149.57 ± 5.83 b	148 ± 5.79 b
After 1 day	150.4 ± 2.99 b	147.9 ± 3.93 b	149.15 ± 3.73 b
After 2 days	150.82 ± 5.96 b	150.8 ± 2.77 b	150.81 ± 4.64 b
After 3 days	153.3 ± 0.0 b	153.1 ± 0.34 b	153.2 ± 0.26 b
After 4 days	162.5± 5.86 a	163.75 ± 2.79 a	163.12 ± 4.63 a
After 5 days	163.12 ± 3.24 a	162.5 ± 3.06 a	162.81 ± 3.17 a
LSD	7.693	6.0478	4.421

Means followed by the same small letter in a column are not significantly differences at the 5% level of probability (Duncan's Multiple Range Test)

B – Weight of the normal virgin queens hatching: -

From the forementioned statistical analysis results of data in Table (2 - 2) through 25/3/2013 , 5/4/2013 and the mean of this dates for weight of the normal virgin queens hatching proved that:-

On 25/3/2013, there were insignificant differences between the weight of the normal virgin queens hatching after four and five days caged, also among each of (after queen cell sealing directly, after one day, after two days and after three days).

But, there were significant differences among each of virgin queens hatching from cells caged (after four days and after five days) and each of (after queen cell sealing directly, caging after one day, after two days and after three days).

The highest weight of virgin queens was (163.12 ± 3.24 mg) for caging after five days, then, (162.5 ± 5.86mg) for caging after four days, then, (153.3 ± 0.0 mg) for caging after three days, then, (150.82 ± 5.96 mg) for the caging after two days, then, (150.4 ± 2.99) for caging after one day. The lowest weight was (147.5 ± 5.59 mg) for caging after queen cell sealing directly. LSD value at 0.05 was 7.693.

On 5/4/2013, there were insignificant differences between for the weight of the normal virgin queens hatching after four days caging and after five days, also among virgin queens hatching (after queen cell sealing directly, caging after one day, after two days and after three days).

But, there were significant differences among each of (the caging after four days and after five days) and each of (after queen cell sealing directly, the caging after one day, after two days and after three days).

The highest weight of virgin queens obtained was (163.75 ± 2.79 mg) for the caging after four days, then, (162.5 ± 3.06 mg) after five days, then, (153.1 ± 0.34 mg) for the caging after three days, then, (150.8 ± 2.77 mg) for the caging after two days), then, (147.9 ± 3.93 mg) the caging after one day. The lowest weight was (149.57 ± 5.83 mg) for the caging after queen cell sealing directly. LSD value at 0.05 was 6.0478.

The mean of the weight, there were insignificant differences for the weight of the normal virgin queens hatching between the caging after four days and after five days, also among each of (after queen cell sealing directly, the caging after one day, after two days and after three days).

But, there were significant differences for among each of (the caging after four days and after five days) and each of (after queen cell sealing directly, the caging after one day, after two days and after three days).

The highest weight was (163.12 ± 4.63 mg) for the caging after four days, then, (162.81 ± 3.17 mg) after five days, then, (153.2 ± 0.26 mg) for the caging after three days, then, (150.81 ± 4.64 mg) for the caging after two days), then, (149.15 ± 3.73 mg) the caging after one day. The lowest weight was (148 ± 5.79mg) for the caging after queen cell sealing directly. LSD value at 0.05 was 4.421.

These results presented in tables (2-1) and (2-2) proved that: -

The honeybee workers play grand role to ripe the queen cells, and the virgin queens are able hatching powerfully. The workers remove the wax layer of queen cells from on queen emergence slot till 6 – 9 mm. circular, this removal are necessary for increasing transfer and change the gases (oxygen and carbon dioxides), because meanwhile the development of larvae into prepupa, pupa stage and adult (before hatching) need big oxygen quantity.

Nota bene (N.B.):-

- Ripe brood means that the workers remove the wax layer from on the emergence slot, and the colour become light brown (pallid) or yellowish.
- This removal occurs for each of queen cells, workers brood and drones brood after the cocoon spinning.
- When the queen larvae spins the cocoon, it leave space in the end of queen cell between the wax and cocoon filaments this space makes an air room.
- When used the handling removal, must removal beginning from the air room this firstly for ease removal carefully.
- The lesser weights were abnormal and curly wings when the caging was after queen cell sealing directly, the caging after one day, after two days and after three days).

3 - Evaluation was comparison between the incubation in the colony (the caging was two days before hatching) and the incubation in the incubator by this new way (after queen cells sealing directly and the removal was handling).

A – Percentage of the normal virgin queens hatching: -

The statistical analysis of data obtained in Table (3) from the mean of hatching percentage showed that:-

There were not significant differences for hatching percentage between the incubation in the colony (the caging was two days before hatching) and the incubation in the incubator by this new way (after queen cells sealing directly and the removal was handling).

The hatching percentage was (100 ± 0.0 %) for each of the incubation in the colony and the incubation in the incubator after queen cells sealing directly. LSD value at 0.05 was 0.0

The results of the incubation in the incubator by this new way (after queen cells sealing directly and the removal was handling) was equal with the results of the incubation in the colony (the caging was two days before hatching).

Table (3) : Comparison between incubation in the colony and the incubator after queen cells sealing directly.

Incubation	% Hatching (Mean± SE)	Weight (Mean± SE) Mg.
Colony	100± 0.0 a	164 ± 0.41 a
Incubator	100± 0.0 a	164.8 ± 0.23 a
LSD	0	0.925

Means followed by the same small letter in a column are not significantly differences at the 5% level of probability (Duncan's Multiple Range Test.

B – Weight of the normal virgin queens hatching: -

From the forementioned statistical analysis results of data showed in Table (3) from the mean of this dates for weight of the normal virgin queens hatching proved that:-

There were insignificant differences for the weight of the normal virgin queens hatching between the incubation in the colony (the caging was two days before hatching) and the incubation in the incubator by this new way (after queen cells sealing directly and the removal was handling).

The mean of the weight was (164 ± 0.41 mg) for the incubation in the colony and (164.8 ± 0.23 mg) for the incubation in the incubator. LSD value at 0.05 was 0.925

The results of the incubation in the incubator by this new way (after queen cells sealing directly and the removal was handling) was equal approximately with the results of the incubation in the colony (the caging was two days before hatching).

The results of hatching percentage and the weight of the normal virgin queens hatching for the table (3) are equal. Then the incubation in the incubator by this new way (after queen cells sealing directly and the removal was handling) are better than the incubation in the colony (the caging was two days before hatching). Thereby may get two batches from queen cells for a rearing colony and the incubation in the incubator by this new way instead of the rearing and incubation in the same colony.

These results agreed with Laidlaw, H. H. Jr. (1981) and Abou El-Enain (2000) they moved the ripe queen cells into incubator with good results, and inagreement with them in the time of transfering the queen cells into incubator.

These results conflict with Abou El-Enain (2000) who mentioned that the incubated by incubator was the lowest hatching percentage than incubated in queenless colony or queenright colony, and all measurements of emerged virgin queens declined in the all characteristics when incubation was in incubator, despite of transferring the ripe queen cells to the incubator (three days before the hatching), also, these results conflict the results and the time of transfer.

It is recommended with using the incubation in the incubator by this new way (after queen cells sealing directly and the handling removal), instead of using the incubation in the colony, therefore for get two batches from queen cells for a rearing colony and the incubation in the incubator by this new way instead of the rearing and incubation in the same colony.

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طريقة تحضين بيوت ملكات نحل العسل في الحضانة بعد قفل بيوت الملكات مباشرةً

أحمد محمد زهيري
قسم بحوث النحل - معهد بحوث وقاية النباتات – مركز البحوث الزراعية – مصر

أجري هذا البحث في منحل خاص بقرية الستايتة مركز المنزلة دقهلية خلال الأعوام من ٢٠١١ إلى٢٠١٤ لدراسة ما تقوم به شغالات نحل العسل لكي يصبح البيت الملكي ناضج وجاهز للتقفيص بالقفص العادي – وتحديد أفضل يوم للتقفيص بالقفص العادي بعد قفل البيت الملكي مباشرةً – محاكات ما تقوم به شغالات نحل العسل لبيوت الملكات وتقييم ذلك في الحضانة ، بالمقارنة بين التحضين في الطائفة والتحضين بهذه الطريقة في الحضانة – وتم استبعاد الملكات العذارى المشوهة وذات الأجنحة المجعدة وكانت النتائج كالآتي :-

- التقفيص بقفص حاجز الملكات أفضل لما تقوم به الشغالات تجاه بيوت الملكات المقفولة (من حيث فقس الملكات السليمة – والوزن) من التقفيص بالقفص العادي بعد قفل البيت الملكي مباشرةً .
- أفضل يوم (في فقس الملكات السليمة – وفي الوزن) للتقفيص بالقفص العادي هو بعد اليوم الرابع من قفل البيت الملكي مباشرةً ، حيث تلاحظ أن الشغالات تقوم بإزالة طبقة الشمع من على فتحة خروج الملكة ولمسافة من ٦ – ٩ مم دائرياً حول هذه الفتحة - وهذا يحدث في بيوت الملكات وحضنة الشغالات وحضنة الذكور وهو ما يسمى بالحضنة الناضجة - إزالة طبقة الشمع ضرورية للتقفيص الجيد لأنها تعمل على زيادة تبادل الغازات عند تطور اليرقة إلى طور ما قبل العذراء ثم طور العذراء ثم طور الحشرة البالغة (قبل الفقس)واحتياجها إلى كمية كبيرة من الأوكسجين والتخلص من ثاني أكسيد الكربون (تبادل الغازات).
- وبتطبيق ذلك عند نقل بيوت الملكات إلى الحضانة بعد قفل البيت الملكي مباشرةً مع إجراء هذه الإزالة يدوياً بعد ٣ أيام من وضعها في الحضانة أبتداءً من غرفة الهواء (الموجودة في قمة البيت الملكي) لتسهيل عملية الإزالة مع الحرص لعدم قطع خيوط الشرنقة – كانت النتائج متساوية (من حيث فقس الملكات السليمة – والوزن) مع التحضين في الطائفة والتقفيص بعد ٤ أربعة أيام من قفل البيت الملكي بالقفص العادي.
- يمكن إجراء هذه الإزالة يدوياً – أو توضع بيوت الملكات في طائفة يتيمة لكي تقوم الشغالات بهذه الإزالة – أو توضع بيوت الملكات تحت قفص نصف كرة بحاجز ملكات وتوضع في أي طائفة من طوائف المنحل العادية لكي تقوم الشغالات بهذه الإزالة .
- لذلك نوصي باستخدام هذه الطريقة في الحضانة لكي تتفرغ طوائف النحل لتربية بيوت الملكات حتى القفل فقط ثم تنقل إلى الحضانة – وبذلك نحصل على دفعتين من بيوت الملكات بدلاً من دفعة واحدة عند استخدام الحضانة بالطريقة العادية (وهو نقل البيت الملكي قبل الفقس بثلاثة أيام) وبذلك يكون الإنتاج ضعف الطريقة العادية .