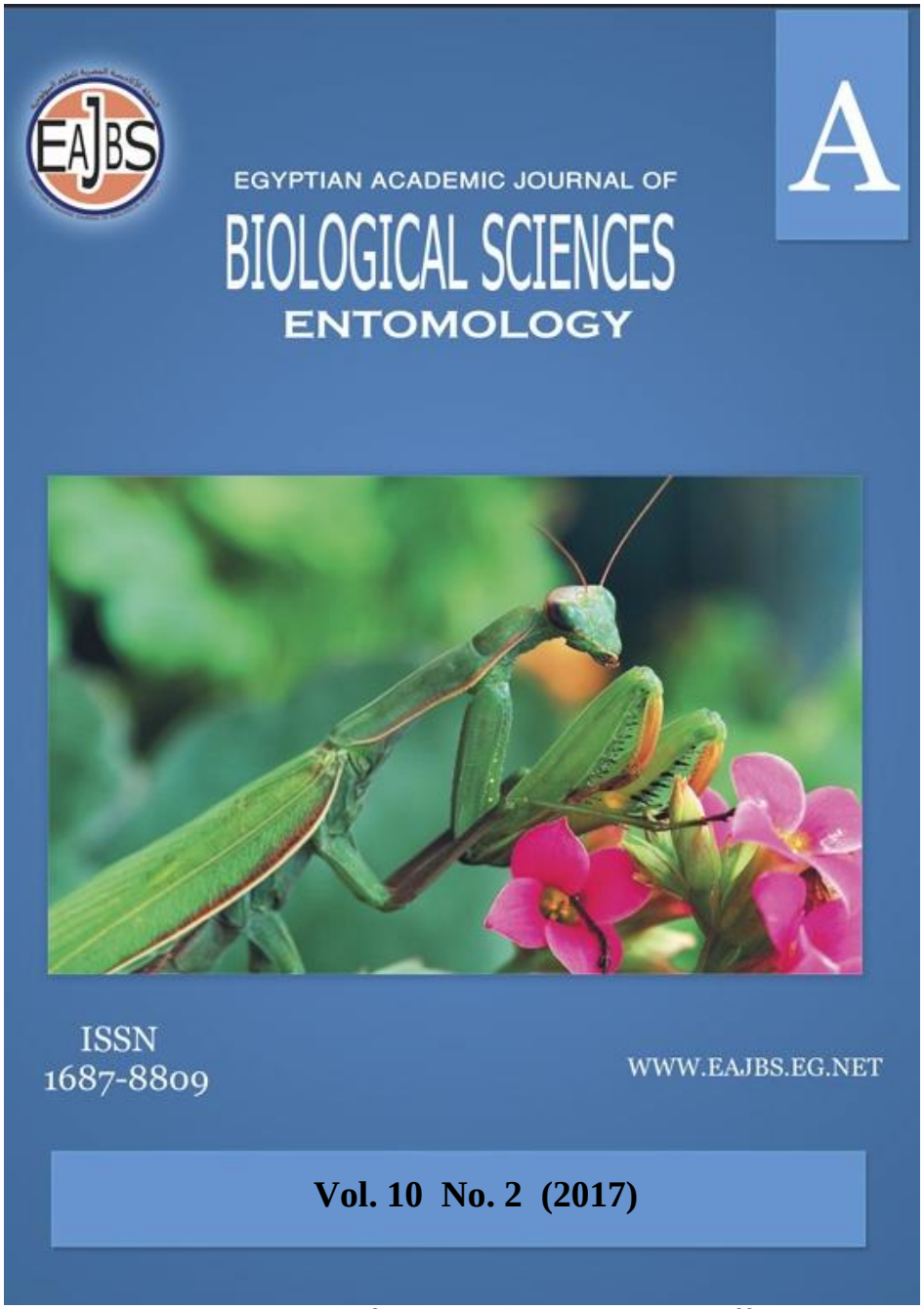

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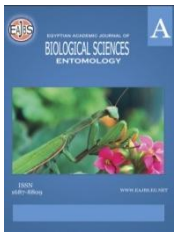
www.eajbs.eg.net

Citation: *Egypt. Acad. J. Biolog. Sci. (A. Entomology) Vol. 10(2)pp: 81- 87 (2017)*

Egypt. Acad. J. Biolog. Sci., 10(2): 81–87 (2017)

Egyptian Academic Journal of Biological Sciences
A. Entomology

ISSN 1687- 8809
www.eajbs.eg.net



Using New Cage Type for Keeping Virgin Honey Bee Queens with Investigating Impacts of Feeding Type and Number of Attendant Workers

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ARTICLE INFO
Article History
Received:1/3/2017
Accepted:9/4/2017

Keywords:
honey bees
queens
cages
feeding
survival

ABSTRACT

Under normal conditions, each honey bee colony contains one queen. Queenless colonies are mostly supplied with new queens using commercial caged queens. In some countries, caged virgin queens are commonly sold for such purpose. Keeping virgin queens for a long period is essential for marketing and shipping purposes. In this study, a new cage type (round plastic cages) was compared with traditional wooden cages to identify impacts of cage type on queens' survival. Also, the impacts of solid diets as well as number of workers in a retinue on queens' survival period were investigated. The results showed that round plastic cages can enhance queens' survival slightly higher than wooden ones. Honey candy was the best feeding type followed by creamed honey which can be used as a potential alternative especially for short caging period. Queens survived longer when five or seven attendant workers were added better than using three workers. It could be said that using plastic cages provided with honey candy and 5 or 7 attendant workers can enhance the survival of caged virgin queens.

INTRODUCTION

Honey bee queens are sold mainly as mated queens. But in some countries like Egypt, queens are also sold whilst still virgin. The virgin queens are usually sold in wooden cages and provided with three attendant workers and some honey candy. Increasing queens' survival period is very important to enhance their selling possibility. Also for instrumental insemination purpose, usually queens are inseminated at age of 5 to 10 days (Cobey, 2007). Cage type can affect the survival of honey bees. Jay (1965) found that few queens were lost when queen cages of large-mesh wire gauze were used. Bigio *et al*. (2012) found that wooden cages with three holes (traditional cages) were better than plastic cages (with approximately similar dimensions to wooden cages) in keeping virgin queens. Also, cage type can affect workers survival and health (Huang *et al*., 2014). However, traditional wooden cages have some disadvantages, including the hardness of inspecting the caged queen, queens could be injured during insertion inside cages, and these cages allow the insertion of small number of attendant bees as well as small amounts of food due to their small dimensions. Thus, developing a new cage type to overcome such disadvantages is required.

In a study by Bigio *et al*. (2012), they found that workers presence with caged queens enhanced queens' survival ability.

But they tested only five workers in their study. In Egypt, virgin queen and

three attendant workers are placed in wooden cage. Therefore, studying the impact of the number of attending workers on queens' survival period is required to recommend which number is the best. It is not suitable to use liquid diets during queens shipping because queens could drown and die in such diets. Usually, solid diets like honey candy is preferred. Other alternatives to honey candy could be used including; mixing sugar with light corn syrup or using a piece of marshmallow to plug the cage for a short period (Zawislak and Burns, 2012). Recently, Abd Elhamid and Abou-Shaara (2016) presented a simple method to produce creamed honey and they found that bees can consume creamed honey without apparent problems. Finding natural alternative to honey candy with potential positive impact on queens' survival ability is important. Therefore, comparisons between the survival rates of caged queens using different solid feeding types derived from bee honey were investigated. Here, the impacts of cage type, (traditional wooden cages in comparison with a new plastic cage type), diet type (honey candy in comparison with different types of solid diets), and number of workers on the survival rates of virgin honey bee queens were studied.

MATERIALS AND METHODS

The Experimental Conditions

All the experiments were performed during the spring of 2016. Hybrids of Carniolan honey bee queens and workers were used. Virgin queens were obtained from commercial apiary (queen breeder) at the next day of their emergence. The queens, in all the experiments, were kept at room temperature (about 20±1°C for the first two experiments and 24±1°C for the last experiment) in a dark and well ventilated place through the experiment period. Also, they were inspected every 12 hours. The weight of queens and attendant bees were recorded at the beginning of the experiments (W1) and at the time of queens' death (W2) using digital balance (Diamond, model 200, China). The percentage of weight loss was recorded as: (W1 – W2/W1) X100. Also, the number of surviving workers at the time of queens' death was recorded.

Survival of the Queens in New and in Traditional Cages.

Two queen cages were tested in this experiment (Figure 1). The first one is the commonly used wooden cages with three holes (one for candy and two holes for queen and attendant workers). The second one is round plastic cage (developed cage). This cage is a normal petri dish provided with wire mesh for ventilation and two small round holes; one for inserting bees and one for introducing queens back to the colonies. The dimensions of each cage are presented in Figure 1.

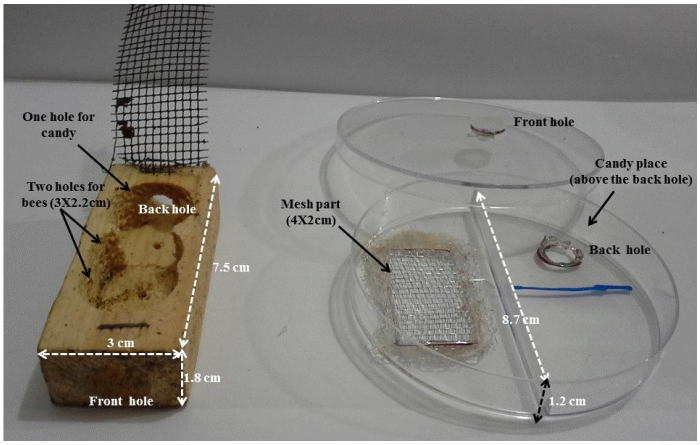


Fig. 1: Dimensions of traditional wooden cage and those cages made from modified plastic Petri dish.

Ten virgin queens and 30 workers (one queen and 3 workers per cage/ 5 wooden cages and 5 round cages) were used in this experiment. Honey candy (8 g) was placed in each cage as feeding to caged bees. The honey candy was prepared by saturating honey with powdered sugar. The survival of queens in each cage type was then compared to identify the best cage type in keeping queens for a longer period of time.

Impacts of Solid Diets on Queens' Survival

Three types of solid diets were compared in this experiment: 1) honey candy, made by saturating bee honey with powdered sugar on water bath, 2) Creamed honey, made by adding 2.4% (w/w) powdered glucose to liquid cotton honey and using storing temperature of 4°C according to Abd Elhamid and Abou-Shaara (2016), and 3) honey jelly, produced by mixing 200 grams of honey with 10 grams of powdered gelatine dissolved in 100 ml of water. Twelve round cages were used, in each cage a virgin queen and three workers. Per each feeding type, 4 cages were used. Ten grams of feeding type was added to each cage. The survival rates of queens and workers were compared at the end of the experiment to determine the best diet that enhanced the survival of the queens.

Impacts of Attendant Workers Number

To access this, different numbers of attendant workers (3, 5, and 7 workers) with virgin queens were tested. Twelve round cages were prepared, and in each cage one virgin queen was placed with 8 grams of honey candy as feed. Per each group of workers (treatment), 4 round cages were used. The survival of workers and queens was compared, after which the group with longest queens' survival was then identified.

Statistical Analysis

A complete randomized design with 4 or 5 replicates (cages) per each treatment (cage type, diet type or number of attendant workers) was used. Means and S.E. were calculated for all treatments. The data were converted using arcsine transformation prior to the analysis. ANOVA was performed and then means were compared using $L.S.D._{0.05}$ or Duncan's multiple range test ($DMRT_{0.05}$). SAS 9.1.3 (SAS Institute, Cary NC, USA, 2004) was used to perform the analysis.

RESULTS

Survival of the Queens in New and in Traditional Cages.

The death of queens kept in plastic cages started at 120 hours (5 days) and in wooden cages at 84 hours (3.5 days). The time at which 50% of queens were able to survive (ST_{50}) was 138 and 114 hours for plastic and wooden cages, respectively, with difference of 24 hours (Fig. 2).

All the queens had died after 216 hours (9 days) and 144 hours (6 days) for plastic and wooden cages, respectively, with difference of 72 hours. The mean of queens survival period (up to 100% queens death) was 160.8±18.4 hours (6.6 days) and 120.0±11.3 hours (5 days) in plastic and wooden cages, respectively, with insignificant difference ($L.S.D._{0.05}$= 49.96). It could be said that queens survived insignificantly longer in round plastic cages over traditional wooden ones.

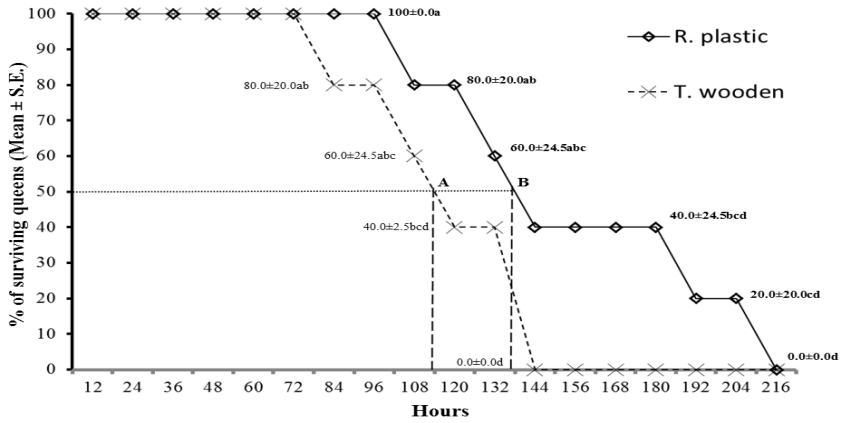


Fig. 2: Survival of queens over 216 hours (9 days) for the two cage types (traditional wooden cages (dashed line) and round plastic cages (continuous line). Means followed by the same letter are not significantly different ($DMRT_{0.05}$). The letters A and B denote to the time at which 50% of queens were able to survive (ST_{50}) in wooden and plastic cages, respectively. (No. of queens= 5 per cage type, and 3 attendant workers with each queen).

Impacts of Solid Diets on Queens' Survival.

The death of queens started at 84, 108, and 108 hours (3.5, 4.5, and 4.5 days) for treatments with honey jelly, creamed honey and honey candy, respectively (Fig. 3). The time at which 50% of the queens died was about 95, 103, and 192 hours (3.9, 4.3, and 8 days) while the death of 100% of them was at 108, 144, and 228 hours (4.5, 6, and 9.5 days), for honey jelly, creamed honey, and honey candy, respectively. The impact of feeding type on queens survival was significant (DF= 2, F= 14.92, P=0.0001<0.05). Death rate of queens was high in honey jelly group followed by creamed honey group. Queens fed on honey candy survived significantly (P<0.05) longer than those fed on creamed honey and honey jelly. Queens provided with honey jelly survived insignificantly (P>0.05) less than those fed with creamed honey. Means of queens survival period (up to 100% death) were189±28.3hours (7.87 days), 117±9.0 hours (4.87 days), and 99±5.7 hours (4.12 days) for honey candy, creamed honey and honey jelly, respectively, with significant differences ($L.S.D._{0.05}$=55.87).

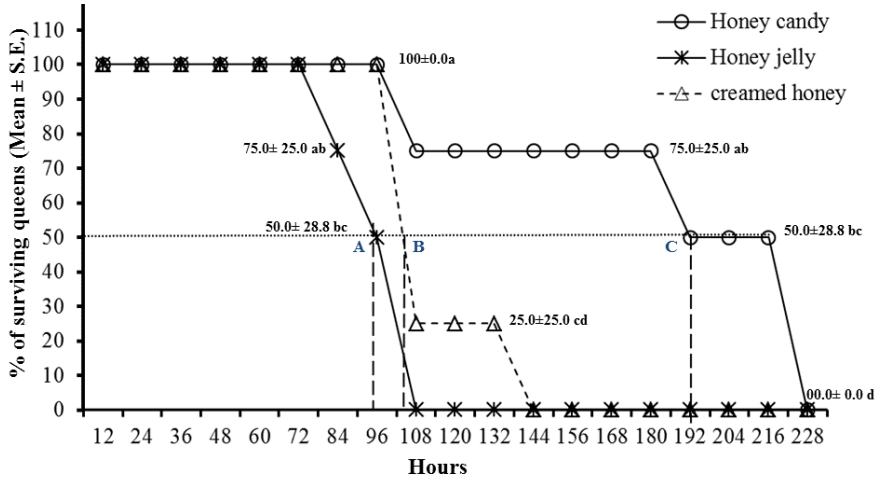
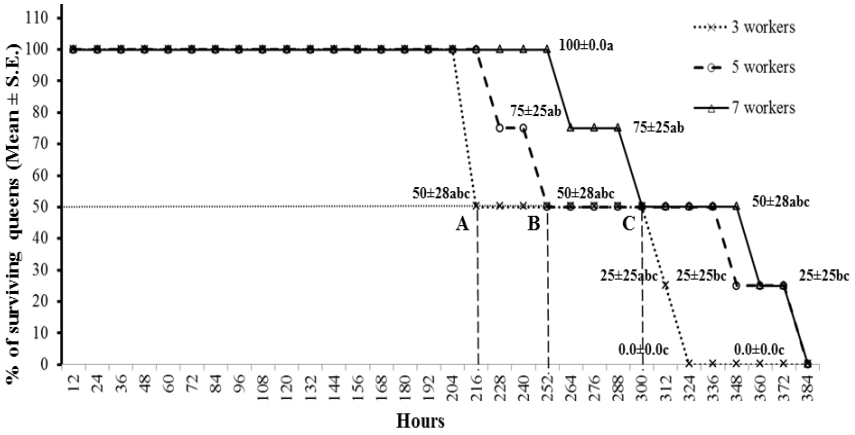


Fig. 3: Survival of queens over 228 hours (9.5 days) for three feeding types; honey candy, honey jelly, and creamed honey. Means followed by the same letter are not significantly different according to $DMRT_{0.05}$. The letters A, B, and C denote to the time at which 50% of queens were able to survive (ST_{50}) in group of honey jelly, creamed honey, and honey candy, respectively. (No. of queens = 4 per feeding type, and three attendant workers with each queen).

Impacts of Attendant Workers Number

Death of the queens was observed as from 216, 228, and 264 hours (9, 9.5, and 11 days) while all the queens had died (100% death) at 324, 352, and 360 hours (13.5, 14.6, and 15 days) for queens with 3, 5, and 7 attendant workers, respectively. The ST_{50} values were 216, 252, and 300 hours (9, 10.5, and 12.5 days) for queens with 3, 5, and 7 attendant workers, respectively (Fig. 4). The mean time at which all the queens had died was 267±29.5, 303±37.4, and 327±27.4 hours (11.1, 12.6, and 13.6 days) for queens with 3, 5, and 7 attendant workers, respectively, without significant differences ($L.S.D._{0.05}$=101.62). Queens provided with 7 or 5 attendant workers were able to survive insignificantly longer than those with 3 workers. Approximately, queens with 5 attendant workers showed similar survival trend to those with 7 workers.



Fig, 4: Survival of queens over 384 hours (16 days) for different number of attendant workers. Means followed by the same letter are not significantly different according to $DMRT_{0.05}$. The letters A, B, and C denote to the time at which 50% of queens were able to survive (ST_{50}) in group of 3 workers, 5 workers, and 7 workers, respectively. (No. of queens= 4 per each treatment).

DISCUSSION

Survival of the Queens in New and in Traditional Cages

Queens in round plastic cages survived longer than those in wooden cages. It is clear that queens in plastic cages had more ability to move and to feed freely due to the wide area of the cages. While the crowdedness of the queen and attendant workers in the narrow area of the wooden cages may impact queens feeding ability and passive movements. This might explain the reduction in queens' survival rate in such cages as recorded here. The absence of significant differences between queens survival period in both cage types indicates the possibility of using round plastic cages as alternative to the traditional wooden ones. In a similar way, Bigio *et al*. (2012) found that cage type can impact queens' survival ability. But they found that wooden cages were better than plastic cages with approximately similar dimensions to wooden ones.

Impacts of Solid Diets on Queens' Survival

Queens fed on honey candy survived significantly longer than those fed on creamed honey or honey jelly. LT_{50} was higher by 104 and 93 hours while LT_{100} was higher by 120 and 84 hours in honey candy group than honey jelly and creamed honey. It is clear that the form of feeding can impact queens' survival. Similarly, Bigio *et al*. (2012) found that honey was better than sugar candy or mix of honey and sugar candy in regard to queens' survival. In the present study, honey jelly showed

less ability to enhance the survival of the queens. This could be explained by the nature of jelly (i.e., high viscosity) which may prevent queens from absorbing nutrients from it. Also, maybe queens were not able to utilize nutrients from creamed honey greatly as well. Honey candy was the best and queens were able to feed on it without any clear problems. Absorbing honey from honey saturated with sugar was easier to virgin queens over absorbing it from jelly or creamed honey.

Impacts of Attendant Workers Number

The results showed that queens provided with 7 or 5 attendant workers were able to survive insignificantly longer. Thus, increasing the number of attendant workers can slightly increase the survival of the queens. According to the study of Bigio *et al*. (2012), the presence of attendant workers (5 workers) enhanced the survival of the caged queens over caged queens without attendant workers. Basically, virgin queens can survive without attendant bees for some time but the attendant bees have an important role in thermoregulation of caged queens. Gontarz *et al*. (2005) recorded increase in temperature to about 35°C within few hours when attendant bees were added to caged queens. Also, they recorded increase in the temperature within the cages than the ambient temperature of the incubators. The role of the attendant workers in the thermoregulation within cages is the most potential explanation for their role in enhancing the survival of caged virgin queens.

Queens's survival period differed from 9 to 16 days. In the first two experiments, queens survived for about the same period (9 and 9.5 days, in respect for first and second experiments) while in the last experiment queens survived up to 16 days (difference of about 7 days). This could be explained by the role of the ambient temperature during the experiments. It was about 20±1°C for the first two experiments and 24±1°C for the last one. It could be said that the relatively low ambient temperature during the first two experiment impacted queens' survival passively and vice versa with the last experiment. According to Woyke (1988), storing temperature of caged queens with few numbers of bees is 28°C. Thus, temperature of 20°C is lower than the suitable one which might have pushed the queen and attendant bees to perform more efforts towards appropriate thermoregulation within cages. The experiments showed that queens can be kept in round plastic cages for relatively long period of 10 days or more. But it is recommended to introduce queens to the queenless colonies within the first week of their emergence. According to Perez-Sato and Ratnieks (2006), queens need about 12.9 days from emergence to start egg lying. This time includes pre-nuptial and nuptial period. While Tibor et al. (1987) found the oviposition started after queen emergence by 4 to 22 days with a mean of 10.6 days.

CONCLUSION

Using round plastic cages showed slightly better ability over traditionally used wooden cages in regard to keeping virgin queens. The study showed that using honey candy is better over creamed honey or honey jelly in regard to enhancing the survival of the queens. Creamed honey could be considered as a potential alternative to honey candy, especially if queens will be kept for a short period of time. Using 5 or 7 attendant workers is advisable to keep caged virgin queens for longer period. It could be said that using plastic cages provided with honey candy and 5 or 7 attendant workers can enhance the survival of caged virgin queens.

ACKNOWLEDGEMENTS

Authors would like to thank Professor Jerzy Woyke (Agricultural University, Poland) for his comments on the first draft of the manuscript.

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ARABIC SUMMERY

حفظ ملكات نحل العسل غير الملقحة بإستخدام نوع جديد من الأقفاص مع دراسة تأثير نوع التغذية وعدد الشغالات المصاحبة

حسام فرج أبوشعرة – منى إبراهيم البانوبى
قسم وقاية النبات، كلية الزراعة، جامعة دمنهور، مصر

تحت الظروف الطبيعية، يوجد بكل خلية نحل ملكة واحدة. الطوائف عديمة الملكات فى أغلب الأحيان يتم إمدادها بملكات جديدة يتم شرائها فى أقفاص. فى بعض الدول، الملكات غير الملقحة يتم تسويقها فى أقفاص لهذا الغرض. حفظ الملكات العذارى لفترة طويلة نسبياً أمر ضرورى لغرض التسويق والشحن. فى هذة الدراسة، نوع قفص جديد (قفص دائرى بلاستيك) تم مقارنة مع القفص الخشبى التقليدى لمعرفة تأثير نوع القفص على بقاء الملكات. كذلك، تم دراسة تأثير نوع التغذية الصلبة وعدد الشغالات المصاحبة على مدة بقاء الملكة. أظهرت النتائج أن الأقفاص البلاستيكية يمكنها تحسين بقاء الملكات عن الأقفاص التقليدية. كاندى العسل كان أفضل تغذية متبوعاً بالعسل الكريمى واللذى يمكن إعتبارهبديل مناسب وخاصة لحفظ الملكات لفترة قصيرة.كان بقاء الملكات أفضلعند وضع خمس أو سبع شغالات نحل مع الملكة عن وضع ثلاث شغالات فقط.ويمكن القول أن إستخدام الأقفاص البلاستيكية المزودة بكاندى العسل وعدد 5 أو 7 شغالات يمكنه تحسين بقاء الملكات غير الملقحة.