

## **EFFECT OF ACCESS OF WORKER BEES TO SEALED QUEEN CELLS ON SOME MORPHOLOGICAL CHARACTERS OF THE PRODUCED VIRGIN QUEENS**

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### **ABSTRACT**

In this work the effect of the exposure time of the sealed queen cells to the worker bees was investigated. For this purpose two Carniolan bee colonies were chosen, one being the mother of the queen larvae and the other as rearing colony. After they had been sealed, the queen cells were divided randomly into five groups according to the period they have been staying in the rearing colony (48, 72, 96, 120 and 144 hour), then they were transferred to an incubator at 34 C until their emerge. For each group the rate of emerge was calculated, then the virgin queens were weighted, and dissected for measuring the length and width of the right fore wing and for counting the ovarioles of the right ovary. The results indicated that only the number of ovarioles was correlated positively with the different exposure periods times to the worker bees in rearing colony.

### **INTRODUCTION**

Honeybee queen is the reproductive female in honeybee. Her ovaries are very large, occupying most of the abdominal cavity. The queen larvae as well as drone and worker larvae are fed by nurse bees in order to complete their development, and the queen cells are sealed around the end of the fifth day after the eggs hatch (Morse, 1979). During the sealed stage the larvae are advanced to the pupal stage, in which the larvae structures are remodeled into those of the adult queen. The characters of virgin queens are affected by the conditions under which it grows as larvae (Delaplane, 1988 ). Both of number of ovarioles and weight of newly emerged virgin queens are affected by the dimensions of the wax cups in which the queen larvae were grafted and the number of the transplanted cups provided to the rearing colonies (Hassan and Mazeed, 2003). Bodolanova,( 1974) stated that the exposure time of the queen cups to the bees before grafting can help in producing heavier queens.

As well as the unsealed stage, the worker bees take care with sealed queen cells. They are emitting a pheromone which attract the worker bees to warm them (Heimken et al, 2006).

Many of beekeepers maintain the sealed royal cells in finisher colonies and then transfer them to an incubator on the ninth day after grafting and still there till their emerge, others, specially French beekeepers, prefer to collect the queen cells six days after grafting and letting the queens complete their developing cycle there (Fert, 1997). The importance of the contact between worker bees and the sealed queen cells was investigated in terms of some morphological characters of the resulted virgin queens.

## MATERIALS AND METHODS

One strong Carniolan colony was used for obtaining the queen larvae in this study. The queens were produced by the simple transfer method using larvae aged about 24 h. Having been sealed, the queen cells were divided randomly to five groups, each of 20 virgin queens, and these groups were transferred periodically to an incubator at 34 C (Moretto *et al* 2004) as follows:

- The first group were transferred after 48 hours,
- The second group were transferred after 72 hours,
- The third group were transferred after 96 hours,
- The fourth group were transferred after 120 hours
- The fifth group were transferred after 144 hours

The queen cells were remained in the incubator until they emerged as adults.

The rate of emerge was calculated and the weight of the produced virgin queens was estimated to the nearest mg.

The queens of each group were prepared for dissection for measuring the fore wing size as indicator of the body size , and for counting the ovarioles of the ovary..

For that purpose the right fore wing of each queen was pulled, spread between two glass-slides and by using a dissecting Binocular-microscope supplied with a micrometer lens both length and width were measured.

For ensuring that all body measurements are taken from the same queen, each queen was marked by removing one part of their body, which was not to be determined: For instance, No. 1. removal of right antenna; No.2. Removal of left antenna; No.3. Removal of both antennae. As soon as the queens were marked they were preserved in Boan solution. By this method, series of extra-Examination could be made including the interdependence between various parts of the bee s body.

The number of ovarioles was determined directly under a dissecting microscope (Eckert, 1934). The right ovary of each queen was spread on a glass-slide with the adding of puri s medium to the preparation (El-Helaly *et al*, 1980), divided longitudinally to many parts to facilitate the count, and with the aid of two dissecting needles the ovarioles number was determined.

### **Statistical analysis:**

The data were analyzed by Kruskal and Willy method for comparing the different exposure times and by correlation to examine the relationship between the different exposure times with the parameters under study. The analysis was undertaken by Almo-Statistic-System (Holm, 2003)

## RESULTS

The percentage of emerge, average values of the weight of the virgin queens, length and width of the forewing, as well as the number of ovarioles are listed in table(1) The percentage of emerge was 100 % in 96h. 120 h. and 144h treatment but was lower in 48 h. ( 15 % ) and in 72 h.( 5 % ).

With regard to the weight of the virgin queens, and although there was differences between some treatments, these differences were not correlated with the exposure time of the sealed queen cells to worker bees ( $r = -0.07$ ,  $P > 0.05$ )

As well, both of length and width of the fore wing did not show a significant relationship with treatments, ( $r = 0.72$  and  $P > 0.05$ )

**Table (1):Percentage of emerge, Average values of weight, Fore wing length and width and number of ovarioles of the virgin queens under different exposure periods**

Periods	Emerge (%) (N=20)	Weight (mg)	Forewing Length (mm)	Forewing width (mm)	No. Ovarioles
48 h.	3(20)	156.15 a*	9.53 a	3.13 a	123.33 a
72 h.	1(20)	159.16 a	9.59 a	3.25 a	130.5 a
96 h.	0(20)	134.27 b	9.54 a	3.24a	142.76 ab
120 h.	0(20)	147.02 ab	9.56 a	3.13 a	154.66 b
144 h.	0(20)	159.68 a	9.7 a	3.24 a	153.63 b

\* Kruskal willy test at  $P < 0.05$

Concerning number of ovarioles, statistical tests showed a significant differences among the 5 means, and these were between both 120 , 144 hour and 48, 72 hour, but there was no difference between the 120 s and 144 s hour treatments. The number of ovarioles in the queens of 96h. treatment was not significantly different from there of shorter or longer exposure time.

When Correlation coefficient was applied to the data, it was clearly established that there was a linear relationship between number of ovarioles and exposure periods to the bees ( $r = 0.96$ ,  $p < 0.05$ ).

As the result indicate, incubating queen cells in artificial incubator after they have been sealed lead to a degenerating in ovarioles number of the resulted virgin queens, and the longer the queen cells had been exposed to the bees , the better result was obtained concerning the number of ovarioles.

The results suggest that there may be a pheromonal effect on queen pupae in their sealed cell, which accelerate their ovary development. The important role of the honeybee pheromones in many activities in- and outside the colony had been established (Free, 1987). The effect of worker sealed brood on many biological activities of the queens has been established (Hassan et al, 2004). They stated that the sealed brood affect positively mating success, pre-oviposition period and the number of spermatozoa of honeybee queens. Both mating success and pre-oviposition period may related more or less to ovary development of the queens. This could be attributed to the effect of sealed brood pheromones, being emitted from worker brood and releasing different behaviour patterns and so different functions (Free, 1987)

**Fig ( 1): the relationship between five exposure periods ( 48, 72, 96, 120 and 140 hour) of sealed queen cells to worker bees and the number of ovarioles (A), Forewing length (B), forewing width (C), and weight (D) of the resulted virgin queens**

The results may be related also to the temperature inside the colonies. The queen pupae produce enough of a pheromone that attracts workers bees to make them cluster over the cells and thus keep it warm (Free, 1987). Queen pupae have been found to contain 30 µg of a pheromone, which attracts the workers, in comparison to drone and worker pupae which contain ten and 2-5 µg pheromone, respectively (Crane, 1990). The constant temperature in the incubator may be unuseful for the best development of the ovary, since every pupal age may require a defined temperature. The quantity of the pheromones released by the pupae may vary with pupal age, and thus attract different numbers of worker bees around the queen cells, which generate different temperature grades. Also, the optimal temperature for queen development may vary with their genotype.

If the number of ovarioles was taken as an important criterion for the quality of the queen, the sealed queen sells must be incubated in the rearing colony until at least the 8<sup>th</sup> day from grafting.

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### تأثير تلامس الشغالات للبيوت الملكية المغلقة على بعض الصفات المورفولوجية للملكات الناتجة

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في هذا البحث تم دراسة تأثير طول فترة بقاء البيوت الملكية المغلقة داخل خلية التربية وبين وزن العذارى الناتجة، طول وعرض الجناح الأمامي، وعدد فروع المبيض لها، بالإضافة إلى نسبة فقس تلك البيوت، وقد خصص لذلك الغرض طائفتان من طوائف النحل الكرنيولي أحدهما كمصدر ليرقات التطعيم والأخرى لتربية وتحضين تلك اليرقات. بعد غلق البيوت الملكية تم تقسيمها إلى ٥ مجموعات حسب فترة بقائها داخل طائفة التربية (٤٨، ٧٢، ٩٦، ١٢٠ و ١٤٤ ساعة) ثم نقلت بعد ذلك إلى حضان على درجة حرارة ٣٤ م. بعد خروج الملكات تم حساب نسبة الفقس ثم وزنت الملكات الناتجة وجهزت بعد ذلك للتشريح بغرض قياس طول وعرض الجناح الأمامي وحساب عدد فروع المبيض، وقد أظهرت النتائج وجود علاقة خطية بين طول فترة بقاء البيوت الملكية وسط النحل في طائفة التربية وبين عدد فروع المبيض، بينما لم تظهر بقية الصفات وجود تلك العلاقة.