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# BIOLOGICAL AND BIOMETRICAL CHARACTERS OF QUEEN ( *APIS MELLIFERA* L.) ARTIFICIALLY REARED BY DIFFERENT GRAFTING TECHNIQUES IN NORTH SINAI, EGYPT

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## ABSTRACT

Study was carried out to investigate the effect of worker larval age which used in grafting process and the technique of grafting on some biological and biometrical characters of queen cells and virgin queens that reared in Al-Arish, North Sinai. The results appeared that percentage acceptance, sealing cells and emerging queens were significantly higher when the grafted larvae were 24 h. old, followed by 36 h. old then 12 h. old. On the other hand, heaviest queens, diameter of queen abdomen, depth, width and volume of queen cells were significantly larger with 12 h. old larvae, followed by 24h.old, then 36 h. old larvae. Double grafting technique attained the highest percentages of acceptance, sealing queen cells and emergence of queens. Wet grafting method came next for the previous parameters, then dry grafting technique. No significant differences were found between double and wet grafting methods for body weight of virgin queens and measurements of both diameter of queen abdomen, depth, width and volume of queen cells. All parameters were in lowest measure when dry grafting technique was followed.

## INTRODUCTION

The methods of getting queen cells built and getting the subsequent queens meted were improved with the development of hive equipment and as additional knowledge of bee behaviort became available. It is known that queen rearing to be considered an important and vital factor to the beekeeping industry all over the world, (Evans *et al.*,2000). The strength of any colony is determined by the ability of her queen in egg laying, secretion of pheromones that strongly influence and control the worker behaviour and physiology as wall as many colony activities. So, beekeepers are always in need of queens for many purposes in Apiculture such as compensation of the lost queens and establishing of new colonies,(Laidlaw, 1979). For the previous reasons, many attempts have been made, especially in commercial queen production, to increase the acceptance of grafted worker larvae into artificial queen cups and to increase the produced queen quality, which depend, in turn, on its weight at emergence. There are various factors affecting the quality of virgin queens that produced in artificial rearing, (Laidlaw, 1992). Among those factors, 1-age of worker larva which choose to be rear as a queens, 2- the grafting technique followed for transplanting the selected larvae within artificial queen cups. The best age of worker larvae to be used for producing queens had given rise to much discussion. Many researchers preferred newly hatched to 12 hours worker larvae such as,(

Weiss,1974, Ahmed,1995 and Mickiewicz & prabucki,1999). Highest rate acceptance of transplanted larvae was attained using 48 h. old grated larvae followed by 24 h. larvae. (Abd- Afattah, 1996). Others found that the best queens resulted using one day old larvae, ( Diab, 1986 and El-Hanafy, 1991 ). On the other hand, ( Komarov, 1934) reared queens from worker larvae not more than 3 days old being 178 mg. in average weight. Hozberlein (1958) suggested that the best age for queen production was from 36 to 48 h. old. Weiss,(1974) found no difference in body weight between the queens that developed from larvae at ages up to 2 days.

Grafting technique has also, significant influence on the quality artificially of reared queen. Hozberlein, (1958); Marza (1965); Taber (1981) and Dedei,(1994), recommended double grafting for queen production. Wet or dry grafting techniques were also, used by several authors such as Sultanov, (1976); Shawer(1981) and Ibrahim, (1997).

The aim of the prsent study is to investigate the effect of larval age and grafting technique on some biological and biometrical features of newly emerged virgin queens which reared under North Sinai conditions.

## MATERIAL AND METHODS

The study was conducted at apiary of honeybee Research Center, Faculty of Environmental Agricultural Sciences at Al-Arish, North Sinai, during seasons of two successive years,(2000 and 2001).

**1-Honeybee strain :**

First hybrid of carniolan race was used in this study.

**2-Determination of larval age for grafting :**

To obtain worker larvae of determined ages, twelve honeybee colonies were used as mothers colonies. Queen of each mother colony was restricted in a queen excluder cage containing empty comb for eggs laying, then these cages were returned back to their colonies for one day to allow queen to lay suitable numbers of eggs. Then, each comb was removed, dated and replaced by another empty comb. These dated combs was preserved in their colonies and observed after the eggs hatching till reaching to the critical larval age for grafting followed a schedule time. The examined larval age were12, 24 and 36 hours old larvae.

**3-The grafting techniques** :

Worker larvae less than 24 hours old were used. Three types of grafting were carried out as followes: 1- Dry grafting. 2- Wet grafting, where a small droplet of royal jelly solution (1part of R.J.: 1 part of water w/w) was putted in each queen cup before transplanting the larva. 3- Double grafting, where the previous wet grafting was done and the grafted frame was introduced to building colony for 24 hours, then, this grafted frame was removed and the accepted larvae were replaced by another young worker larvae.

**4-Preparation of queen rearing colonies :**

Eighteen strong honeybee colonies, each contained 5- 8 brood combs, were chosen and provided daily with about 66% sugar syrup for two weeks

before and during the queen rearing period. These colonies were divided into six groups, each of 3 colonies. One day before starting the process of grafting, queen and young brood combs of each colony were removed. Therefore, each prepared queenless colony contained about four sealed brood combs, three combs of honey and pollen beside lateral feeder. Each queenless rearing colony received one grafted frame, with two bars that inserted between two combs of brood and pollen. Fifteen artificial wax cups were fixed on each bar before grafting with the tested larval age.

**5-Biological and biometrical parameters :**

Numbers and percentages of acceptance of grafted larvae, sealed queen cells and emerged virgin queen were recorded. Each virgin queen was weighed within 4-5 hours after emergence using electrical balance to the nearest 0.01 gram. Abdomen diameter in Cm. of virgin queen was measured by using a hard clipper at widest point of the abdomen. Depth, width and volume of each queen cell were also measured. The data obtained were analyzed by F test and the means (+SE) were separated by L.S.D.

## RESULTS AND DISCUSSION

The honeybee queens were artificially reared in starting queenless colonies to investigate the effect of worker larval age which used in grafting process and the type of grafting technique on the number and percentages of accepted larvae, sealed queen cells, emerged virgin queens, queen cell measurements, (depth, width and volume),as well as weight of emerged virgin queens and diameter of their abdomens throughout two successive years, (2000 and 2001). The data obtained were recorded in Table (1&2).

**1- Effect of larval age on some biological and biometrical characters of reared queens**

**1.1-Effect on percentages of acceptance, sealed cells and queen emergence**

Data presented in Table (1) showed that the mean acceptance percentage were 51.7%, 92.2% and 83.3% for the larvae aged 12, 24 and 36 hours, respectively. The differences between the three larval ages were significant. The highest rate of acceptance was occurred when 24h. old larvae were grafted, followed by 36h. old then 12h. old larvae. The same trend was done for sealing (mature) the queen cells where their percentages were 89.4% for 24h. old larvae, 80.6% for 36h. old and 48.3 % for 12h. old larvae. The rate of virgin queen emergence was significantly higher ( 87.2 %) by grafting 24 h. old larvae. These rates were 77.2 % for 36h. old larvae and 45.0 % for the smallest age with significant difference between them. These results were agreed with many researchers. Shingaeva, (1953) found that bees preferred one to two days old larvae for raising queens in emergence cases. El-kordy (1979) reported that the amount of royal jelly was higher when 1 day old larvae were used, then 2 days old larvae.

**Table (1): Effect of grafted larval age on some biological and biometrical characters of the produced virgin queen during two successive years under North Sinai conditions**

| Parameter | | LARVAL AGE (h.) | | | | | | | | | L.S.D. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 12 h. | | | 24 h. | | | 36 h. | | | |
| | | 2000 | 2001 | Mean | 2000 | 2001 | Mean | 2000 | 2001 | Mean | |
| Queen cell acceptance | Mean | 17.5 | 14.0 | 15.5 | 28.0 | 27.3 | 27.7 | 24.7 | 25.3 | 25.0 | |
| | + SE | 0.58 | 0.58 | 0.58 | 0.01 | 0.33 | 0.33 | 0.33 | 0.33 | 0.33 | |
| | % | 56.7 | 46.7 | 51.7 c | 93.3 | 91.1 | 92.2 a | 82.2 | 84.4 | 83.3 b | 0.726 |
| Sealed queen cell | Mean | 16.0 | 13.0 | 14.3 | 27.3 | 26.3 | 26.8 | 24.0 | 24.3 | 24.2 | |
| | + SE | 0.58 | 0.67 | 0.05 | 0.33 | 0.33 | 0.01 | 0.58 | 0.33 | 0.13 | |
| | % | 53.3 | 43.3 | 48.3 c | 91.1 | 87.8 | 89.4 a | 80.0 | 81.1 | 80.6 b | 0.873 |
| Emerged virgin queen | Mean | 12.0 | 15.0 | 13.5 | 26.0 | 26.3 | 26.2 | 23.3 | 23.0 | 23.2 | |
| | + SE | 0.58 | 0.58 | 0.50 | 0.58 | 0.33 | 0.16 | 0.33 | 0.58 | 0.16 | |
| | % | 40.0 | 50.0 | 45.0 c | 86.7 | 87.8 | 87.2 a | 77.8 | 76.7 | 77.2 b | 0.906 |
| Depth of queen cell (Cm.) | Mean | 2.10 | 2.11 | 2.11 | 2.05 | 2.05 | 2.05 | 2.01 | 2.01 | 2.01 | |
| | + SE | 0.01 | 0.01 | 0.01 a | | 0.01 | 0.01 b | 0.01 | 0.01 | 0.01 c | 0.0171 |
| Width of queen cell (Cm.) | Mean | 1.12 | 1.16 | 1.14 | 1.10 | 1.11 | 1.11 | 1.08 | 1.08 | 1.08 | |
| | + SE | 0.01 | 0.01 | 0.01 a | 0.01 | 0.01 | 0.01 b | 0.01 | 0.01 | 0.01 c | 0.0188 |
| Volume of queen cell (Cm)3 | Mean | 1.13 | 1.13 | 1.13 | 0.82 | 0.83 | 0.83 | 0.81 | 0.81 | 0.81 | |
| | + SE | 0.01 | 0.01 | 0.01 a | 0.01 | 0.01 | 0.01 b | 0.01 | 0.01 | 0.01 c | 0.0067 |
| Weight of virgin queen (mg.) | Mean | 180.0 | 175.0 | 177.5 | 152.0 | 151.0 | 151.5 | 143.0 | 142.0 | 142.5 | |
| | + SE | 0.01 | 0.01 | 0.02 a | 0.01 | 0.01 | 0.01 b | 0.01 | 0.01 | 0.01 c | 0.0029 |
| Diameter of queen abdomen (Cm.) | Mean | 0.55 | 0.56 | 0.56 | 0.47 | 0.48 | 0.48 | 0.47 | 0.47 | 0.47 | |
| | + SE | 0.01 | 0.01 | 0.01 a | 0.01 | 0.01 | 0.01 b | 0.01 | 0.01 | 0.01 b | 0.0055 |

**Means in the same row followed by the same letters do not different significantly according to LSD. ,value, at 0.05.**

### 1.2- Effect on the characters of queen cells and virgin queens

Data in Table (1) appeared that the depth, width and volume of queen cell produced form grafted 12 h. old larvae were significantly larger than those obtained with 24 h. and 36 h. old larvae. The figures were 2.11 Cm., 1.14 Cm. and 1.13 Cm., respectively for the preceding age. These means were, (2.05 & 2.01 Cm.), (1.11 & 1.08 Cm.) and (0.83 & 0.81 Cm.) for the depth, width and volume of queen cells which produced from the ages of 24 h. old and 36 h. old, respectively. Body weight of virgin queens and their abdomen's diameter were, also, significantly affected by the age of larvae that selected for grafting as shown in Table (1). The selected larvae at 12 h. old for grafting attained heaviest queen weight, (177.5 mg.) followed by those produced from 24 h. old larvae, (151.5 mg.) and finally the queens resulted from 36 h. old larvae, (142.5 mg.). It is clear from data presented in Table (1) that the mean diameter of abdomen for emerged queens reared from 12 h. old larvae was significantly wider, (0.56 Cm.) than those obtained from both 24 h. old, (0.48 Cm.) and 36 h. old, (0.47 Cm.) larvae.

It is obvious from these data that the biometrical characters of queen cells, (depth, width & volume), weight and diameter of virgin queen abdomen

were dependent on the age of larvae that used in grafting process. Besides, there are relationships between these traits as reported by several authors such as, (Rawash *et.al.*,1983;Abd Al-Fattah & El-Shemy, 1996 and Sharaf El-Din *et. al.*,2000)

Therefore, it could be concluded that to produce high quality of queens under North Sinai conditions the youngest worker larvae were preferred for grafting process. This conclusion was agreed with the finding of (Diab, 1986 and Laidlaw, 1992).

**2- Effect of grafting technique on some biological and biometrical characters of reared queens.**

**2.1- Effect on percentages of acceptance, sealed cells and queen emergence**

Results obtained in Table (2) indicate that the double grafting technique attained the highest percentages of acceptance, sealing of queen cells, and emerging queens. These rates were 95.6 %, 92.8 % and 89.4 % for the previous parameters, respectively. Significant difference was found between double graft method and other two techniques. The wet grafting technique came next in their importance in queen production. The rates of acceptance, (81.1 %), sealing queen cells, (77.8 %) and emerged queens, (74.4%) were significantly higher than those obtained when the dry grafting was followed, where these percentages were 41.7 %, 38.3 % and 35.0 %, respectively. These finding were in agreement with those obtained by many authors. Macicka, (1985) found that mean acceptance percentage of larvae transferred into queen - cups using wet grafting technique was 75.6 % compared with 41.3 % of those obtained from dry grafting technique. Also, each of Shawer, (1981), El-Hanafy, (1991) and Ibrahim, (1997) proved that the best queens resulted from young larvae which grafted into queen –cups provided with diluted royal jelly than those without royal jelly. On the other hand, when the double grafting technique was followed in the commercial production, Taber, (1981), Diab, (1986), Dedei, (1994) and Ibrahim, (1997) found that this technique produced superior queens, in their biological characters, than those obtained by wet or dry grafting ones.

**2.2- Effect on the characters of queen cells and emerged queens**

Data recorded in Table (2) appeared that the depth, width and volume of queen cells as well as the diameter of queen abdomen were significantly higher when the queen rearing programme occurred by double and wet grafting techniques than used dry grafting ones. Means of those characters were, (1.98, 1.09, 0.95 & 0.52 Cm.3), (1.97, 1.08, 0.95 & 0.51 Cm.3) and (1.77, 1.01, 0.77 & 0.45 Cm. 3) for the previous grafting techniques respectively.

**Table (2): Effect of grafting technique on some biological and biometrical characters of the reared virgin queen throughout two successive years under North Sinai conditions**

| Parameter | | Grafting Technique | | | | | | | | | L.S.D. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Dry grafting | | | Wet grafting | | | Double | | | |
| | | 2000 | 2001 | Mean | 2000 | 2001 | Mean | 2000 | 2001 | Mean | |
| Queen cell acceptance | Mean | 12.0 | 13.0 | 12.5 | 24.7 | 24.0 | 24.3 | 29.0 | 28.3 | 28.7 | |
| | + SE | 0.58 | 0.58 | 0.01 | 0.33 | 0.58 | 0.13 | 0.01 | 0.33 | 0.13 | |
| | % | 40.0 | 43.3 | 41.7 c | 82.0 | 80.0 | 81.1 b | 96.7 | 94.4 | 95.6 a | 0.803 |
| Sealed queen cell | Mean | 11.0 | 12.0 | 11.5 | 23.7 | 23.0 | 23.3 | 28.3 | 27.3 | 27.8 | |
| | + SE | 0.58 | 0.58 | 0.01 | 0.58 | 0.58 | 0.01 | 0.33 | 0.33 | 0.33 | |
| | % | 36.7 | 40.0 | 38.3 c | 78.9 | 76.7 | 77.8 b | 94.4 | 91.1 | 92.8 a | 0.839 |
| Emerged virgin queen | Mean | 10.0 | 11.0 | 10.5 | 22.7 | 22.0 | 22.3 | 27.3 | 26.3 | 26.8 | |
| | + SE | 0.58 | 0.58 | 0.50 | 0.33 | 0.58 | 0.33 | 0.33 | 0.33 | 0.50 | |
| | % | 33.3 | 36.7 | 35.0 c | 75.5 | 73.3 | 74.4 b | 91.1 | 87.8 | 89.4 a | 0.839 |
| Depth of queen cell (Cm.) | Mean | 1.74 | 1.80 | 1.77 | 1.97 | 1.97 | 1.97 | 1.99 | 1.98 | 1.98 | |
| | + SE | 0.03 | 0.03 | 0.03 b | 0.02 | 0.02 | 0.01 a | 0.02 | 0.02 | 0.04 a | 0.0287 |
| Width of queen cell (Cm.) | Mean | 1.0 | 1.02 | 1.01 | 1.08 | 1.08 | 1.08 | 1.09 | 1.08 | 1.09 | |
| | + SE | 0.01 | 0.01 | 0.01 b | 0.01 | 0.01 | 0.01 a | 0.01 | 0.01 | 0.03 a | 0.0152 |
| Volume of queen cell (Cm)3 | Mean | 0.74 | 0.80 | 0.72 | 0.99 | 0.88 | 0.95 | 1.01 | 0.88 | 0.95 | |
| | + SE | 0.02 | 0.02 | 0.03 b | 0.01 | 0.01 | 0.12 a | 0.01 | 0.01 | 0.10 a | 0.0195 |
| Weight of virgin queen (mg.) | Mean | 139.0 | 142.7 | 140.9 | 153.4 | 151.7 | 152.5 | 163.8 | 160.4 | 162.1 | |
| | + SE | 0.01 | 0.01 | 0.01 c | 0.01 | 0.01 | 0.01 b | 0.01 | 0.01 | 0.01 a | 0.0029 |
| Diameter of queen abdomen (Cm.) | Mean | 0.45 | 0.45 | 0.45 | 0.52 | 0.50 | 0.51 | 0.54 | 0.50 | 0.52 | |
| | + SE | 0.01 | 0.01 | 0.01 b | 0.01 | 0.01 | 0.01 a | 0.01 | 0.01 | 0.01 a | 0.017 |

**Means in the same row followed by the same letters do not different significantly according to LSD., value**

In spite of there were similar measurements for the characters of queen cells produced by double and wet grafting techniques, the first one had a special discriminate than the second ones (Table 2). This trait was increasing the body weight of emerged queens, (162.1 mg. / queen) for double grafting technique than those obtained (152.5 mg. / queen) for the wet grafting ones. This difference may by attributed to the explain of both Hozberlein, (1958) and Taber, (1981). They found that, in double grafting technique, the purpose of the first graft was get as many of the cells accepted as possible and conditioned, so, when the second graft was made the bees will go forward the process of rearing queens from these tiny-larvae with the least possible interruption in their feeding and development.

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## الصفات البيولوجية و البيومترية لملكات نحل العسل المرباة صناعيا بطرق تطعيم مختلفة في شمال سيناء, مصر

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**** قسم الإنتاج النباتى ووقايته – كلية العلوم الزراعية البيئية بالعريش – جامعة قناة السويس**

أجرى هذا البحث في منحل كلية العلوم البيئية الزراعية بالعريش, شمال سيناء, لدراسة تاثير العمر اليرقى المستخدم في عملية التطعيم و كذلك نوع التطعيم على بعض الصفات البيولوجية و البيومترية للبيوت الملكية و الملكات العذراى الناتجة تحت ظروف منطقة شمال سيناء. وكرر هذا العمل مرتين خلال عامين متتالين ٢٠٠٠ , ٢٠٠١ . وتتلخص النتائج المتحصل عليها فيما يلى :-

١- تأثير العمر اليرقى المستخدم في التطعيم :-

- كانت نسبة القبول لليرقات المطعومة عمر ٢٤ ساعة و كذلك ختم البيوت الملكية و نسبة الفقس للملكات (٩٢,٢ %, ٨٩,٥ % و ٨٧,٢ % على الترتيب) أعلى من النتائج المتحصل عليها عند استخدام يرقات عمر ٣٦ ساعة (٨٣,٣ %, ٨٠,٦ % و ٧٧,٢ %). و سجلت أقل نسبة عند استخدام يرقات عمر ١٢ ساعة ( ٥١,٧ %, ٤٨,٣ % و ٤٥,٠ % على الترتيب)
- بلغ عمق و قطر و كذلك حجم البيوت الملكية أقصاه عند استخدام يرقات عمر ١٢ ساعة (٢,١١ سم, ١,١٤ سم , ١,١٣ سم ) ثم تلك ذات عمر ٢٤ ساعة حيث كانت النتائج ( ٢,٠٥ سم , ١,١١ سم, ٠,٨٣ سم ) وأخيرا الناتجة من يرقات عمر ٣٦ ساعة ( ٢,٠١ سم , ١,٠٨ سم , ٠,٨١ سم ).
- ارتفع كذلك وزن الملكات العذارى الناتجة و قطر بطنها عند استخدام يرقات عمر ١٢ ساعة ( ١٧٧,٥ ملجم , ٠,٥٦ سم) عن عمر ٢٤ ساعة ( ١٥١,٥ ملجم, ٠,٤٨ سم ) وأخيرا عمر ٣٦ ساعة ( ١٤٢,٥ ملجم , ٠,٤٧ ).

٢- تأثير تكنيك التطعيم المستخدم

- حقق استخدام تكنيك التطعيم المزدوج أفضل النسب المئوية للقبول و غلق البيوت الملكية و معدل فقس الملكات – ثم التطعيم المبتل و أخيرا التطعيم الجاف.
- ظهر تميز نظام التطعيم المزدوج أيضا على المواصفات البيومترية للبيوت الملكية حيث كانت أعمق و أكثر اتساعا و أكبر حجما عن استخدام التطعيم المبتل و أقل النتائج مع التطعيم الجاف .
- انعكست هذه النتائج على مواصفات الملكات الناتجة حيث كانت أقل وزنا وأكبر حجما عند استخدام التطعيم المزدوج ثم التطعيم المبتل ثم التطعيم الجاف