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MODIFIED HIVES FOR MASS PRODUCTION OF HONEYBEE QUEENS

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ABSTRACT: *This study was carried out at a private apiary in Meat-Fares Village, Bani Ebeed Region, El-Dakahlia Governorate, Egypt, during the period from the first of February, 2013 to the end of September, 2013 to compare the efficacy of modified queen rearing hives in queenright colonies with that of queen less colonies. Ten Langstrouth hives with 12 frame size were prepared by dividing it from the inside with a vertical queen excluder to two sections, each had its separate entrance. The first section presents the queen section which contains three combs with the queen, while the section presented the orphan contains five combs, one or two queen cup frames and a frame feeder. Modified queen right colonies fed on lemon juice as a natural queen pheromone inhibitor.*

The obtained results indicated that there was significant difference in the average numbers of accepted larvae. The highest larval accepted percentage was recorded at the treatment of modified hives fed on lemon juice (81.07 %) followed by modified hives (72.27 %), while the queenless hives gave only 67.07 %. In addition, There was significant difference in the average weights . The highest average weights of emerged queens was recorded with the treatment of modified hives fed on lemon juice (182.16 ± 3.04 mg) followed by modified hives (180.08 ± 3.41a), while the queenless hives gave only (168.56 ± 4.09 mg) .

Finally, it could be concluded that the use of the modified hives at queenright colonies fed on lemon juice significantly improved the process of mass production of queen rearing.

Key words: *Honeybee ,modified hives , Apis mellifera , queen rearing*

INTRODUCTION

The development of queen rearing techniques started in the 19 th century. Doolittle, 1889 in the USA developed a comprehensive system for rearing queen bees which serves as the basis of current production by using wax cups and transferred worker bee larvae to start the production of queen cells, as well as the method of queen rearing in queenright colonies with the old queen isolated by a queen excluder is still applied. In addition Doolittle,1915 emphasized the importance of simulating a swarming or supersedure situation in the cell building colonies and a constant, rich food supply for the production of high quality queens.

Many researches study the process of honeybee queen rearing all over the world, where several improvements have been made in Doolittle's grafting method. (Laidlaw ,1979) found that grafting (Doolittle's) method involves the transferring of small larvae (24 hours old) into artificial queen cell cups and is generally employed by commercial queen breeders. (Delaplane and Harbo ,1987) found that the survival of queenlessness worker was prolonged, while colony weight gain and defense behavior (number of stings) decreased, as well as queenlessness did not induce drifting . (Thakur *et al.* 1998) found that the acceptance rate of grafted larvae increased by using old wax queen cells cups. (Stace and White, 1994) reported that adding iso-leucine as a supplement feed to sugar syrup for honey bees, *Apis mellifera* L. increased acceptance of queen cells after grafting and cell production per colony, while it decreased the consumption of supplementary feeding (protein diet).

The mandibular gland pheromone of a queen honeybee, *A. mellifera* , contributes to the suppression of ovarian activation in workers (Hoover *et al.*, 2003). Absence of

the queen in the colonies leads to the developing of a pheromonal set in some laying workers, similar to that of queens (Crewe and Velthuis, 1980), which stimulates physiological responses in other workers similar to those evoked by queens (Velthuis *et al.*, 1990). In the Cape honeybee, *A. mellifera capensis*, the high level of the queenlike mandibular pheromone substance, 9-oxo-2-(E)-decenoic acid (9-ODA), in workers is related with more ovarian activation (Hepburn, 1992). High levels of 9-ODA increase aggressive attacks (Pettis *et al.*, 1995).

In Poland, (Woyciechowski and Kuszewska 2012) found that the period of queenless conditions affects female larval development, where colony continues by raising a new queen and daughter workers that are kin, but may be less than kind. (Taber, 1983) reported that rearing of queens in queenless colonies are particularly difficult in queenless, *A. mellifera capensis* colonies due to the development of laying workers which produce worker brood (thelytokous reproduction) and the workers fight each other, causing much colony disruption.

Recently, (Wilkinson and Brown 2002) described in details Doolittle's method of queen rearing method in queenright colonies, where it consisted of raising frames of brood, above a queen excluder in a strong colony, and grafting 12-18 hr old larvae into queen cell cups next to the brood in the upper chamber. A brood frame rotation schedule maintains the colony as a queen reared for further batches of queen cells. The overall acceptance rate of 6666 grafts was 81%. Furthermore, (Ahmad and Dar, 2013) compared rearing honey bee queens between queenright and queenless colonies, and found that grafted larvae acceptance percentage was higher in queenright colonies than that in queenless colonies, which reached 83 and 79% respectively.

Artificial rearing of queen allows the researcher and breeders to select stock economically and to explore honeybee behavior and genetics. It will enable us to select the specific queens with desired characters such as high honey production, high brood viability, early spring build up, good temperament, clearing behavior, incidence of disease, swarming and color (Johnstone, 2008).

From the previous studies this work aims to compare the efficacy of three queen rearing methods (queen less colonies, a modified queen right colonies and a modified queen right colonies fed on a natural queen pheromone inhibitor).

MATERIALS AND METHODS

This study was carried out at a private apiary in Meat-Fares Village, Bani Ebeed Region, El-Dakahlia Governorate, Egypt, 25 km. from Mansoura city during the period from first of February, 2013 to the end of September, 2013. The modified hives for rearing queens mentioned in this research (Fig. 1) was used in the Chinese commercial apiaries for royal jelly production (Fert, 2000) and was modified by (Wilkinson and Brown, 2002) under the European circumstances to rear queens in two floor hives with horizontal queen excluder.

1- The experimental colonies:

1-1 Five queenless colonies (*Apism. carnica*)

1-2 Five modified queenright colonies (*Apis m. carnica*)

1-3 Five modified queenright colonies (*Apis m. carnica*) fed on a natural queen pheromone inhibitor (lemon juice).

2- Queen rearing by the traditional way in queenless colonies:

Day 1: Two honey and pollen- combs and a feeder were placed in empty Langstrouth hive, 10 brood combs covered with local Carniolan young bees without the queen were shaken in the hive. The previous procedure was repeated for five hives. The hives were closed, moved to another apiary and left in dark cool place for one night. Five grafting frames each contain 30 plastic queen cup in three wooden bars were sprayed with sugar syrup and were put into strong colonies to clean it over the night (Fert ,1997).

Day 2 : Hives were set in the apiary with open entrance. Old black comb with larvae less than 36 hr. old was chosen from a good breeder colony. The graft frames prepared the day before were bring, add a drop of diluted (1:1) fresh royal jelly to each cup. Larvae at 24 h old were grafted to the wax queen cups in a room maintained at 35°C and 60%RH (Laiddlaw and Page, 1997). Frames with grafted larvae were placed in the hives in the position shown in Fig. (2) Between the two honey and pollen combs. Colonies were fed with half a liter of (1:1) sugar syrup for every hive and add pollen substitutes patties if needed.

Day 3: Colonies were inspected for the acceptance percentages of grafted larvae and two open brood combs were add for every hive in both sides of the grafting frame as shown in Fig. (3).

Days 4 – 8: Brood combs were inspected and any natural queen cells were destroyed. Colonies were fed with half liter of 50 % sugar syrup for every hive and add pollen substitutes patties if needed.

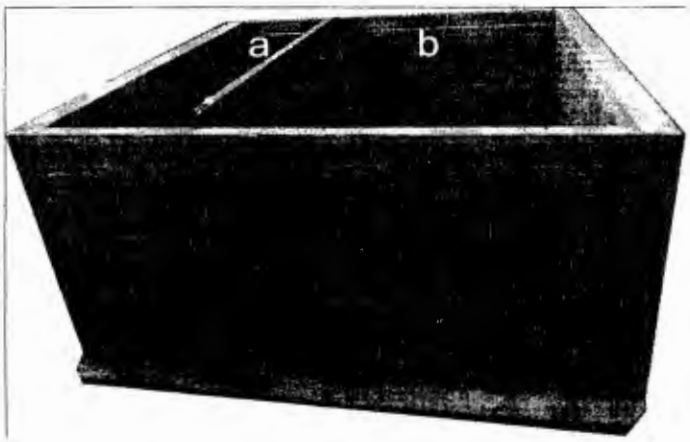

Fig. 1. The modified hive provided with a queen excluder divided it to two sections.

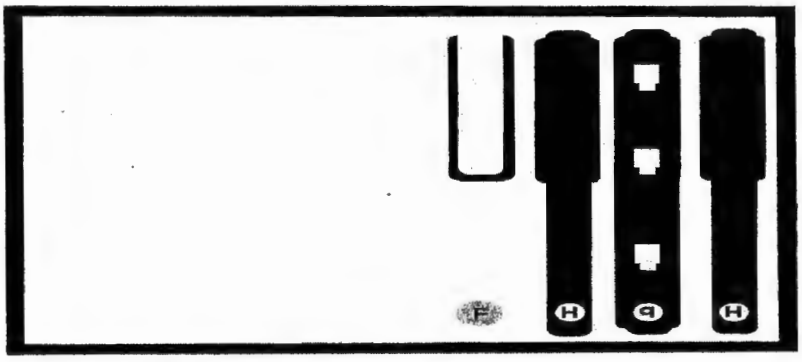

Fig. 2. The position of combs in the traditional method for queen rearing in queen less hives.
H=honey and pollen comb. q=grafted queen cups frame. F=side feeder.

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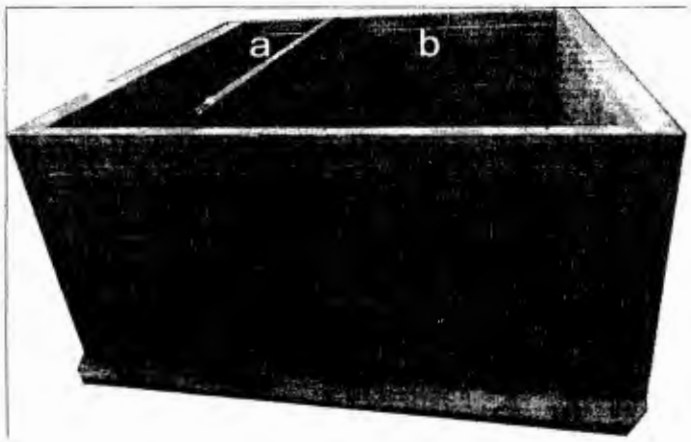

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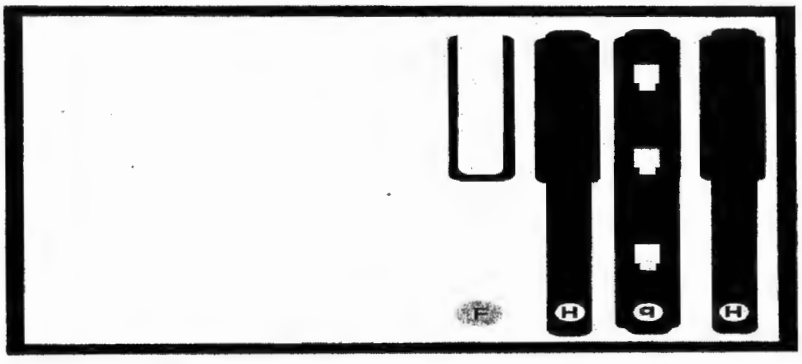

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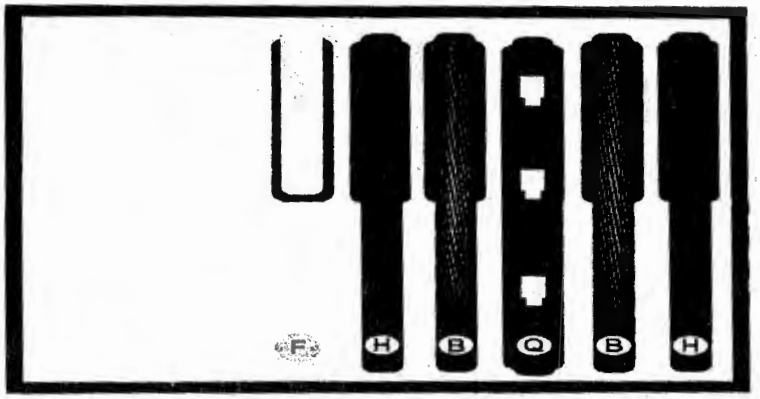

Fig. 3. The position of combs in the traditional method for queen rearing in queen less hives after add brood combs.
H = Honey and pollen comb. B = Open brood comb.
Q = Accepted queen cells frame. F = Side feeder.

Day 10: Frame with ripe queen cells was removed from the hive; queen cells were separated from the bars carefully and incubated in electric incubator in 34.5°C and 70%RH, individually in special queen cages until emergence. A frame with new grafted queen cells placed immediately in the same place of the removed queen cells frame within 15 minute.

Day 12: Incubator was checked every four hours for emerged virgin queens in cages. Immediately after emergence queens were counted, weighted in mg so queens had no chance to fed with and data were recorded (Hatch *et al.*, 1999). Hatched brood combs were replaced with new brood combs contain young larvae covered with young bees.

3-Queen rearing by the modified method in queen right colonies:

Five special Langstrouth hives with 12 frame size were prepared. All hives were divided from the inside with a vertical queen excluder to two sections and each had its separate entrance. The first section presents the queen section which contains three combs with the queen, while the Section presented the orphan which contains five combs, one or two queen cup frames and a frame feeder Fig. (4).

Study procedures:

Day 1: Five young local Carniolan mated queens with a good colonies (about 8-10 combs) were chosen in the apiary. Eight combs with the queen were moved to the modified hive and extra combs were shaken on them. The combs were arranged as shown in Fig. (4). Colonies were to fed with half liter of 50% sugar syrup and pollen substitutes patties (1 part dead yeast : 2 parts corn flour : 6 parts dust sugar).

Day 3 – 6: Combs in the modified hives were inspected; any natural queen cells in brood combs in the orphan section were destroyed. Colonies were fed with half liter of 50 % sugar syrup for every hive and add pollen substitutes patties if needed.

Day 8: Five grafted frames were used each contain 30 plastic queen cup in three wooden bars were sprayed with sugar syrup and were put into strong colonies to clean it over the night.

Day 9: All the 5 modified hives were inspected, any natural queen cells in brood combs in the orphan section were destroyed and brood combs were rearranged Fig. (4). Old black comb with larvae less than 36 hr. was chosen from a good breeder colony. The grafted frames prepared the day before were bring, add a drop of diluted (1:1) fresh

royal jelly to each cup. Young larvae bout 24 hr. old were grafted to the wax queen cups in a room maintained at 35°C and 60%RH (Laidlaw and Page, 1997). Frames with grafted larvae were placed in the modified hives in the position shown in Fig. (5), between brood comb No (3) and brood comb No (4). Colonies were fed with half liter of 50% sugar syrup for every hive and add pollen substitutes patties if needed.

Day 12: Colonies were inspected for the acceptance percentages of grafted larvae; any natural queen cells in brood combs in the orphan section were destroyed. Brood combs were rearranged Fig. (5). Colonies were fed with half liter of 50% sugar syrup for every hive and add pollen substitutes patties if needed.

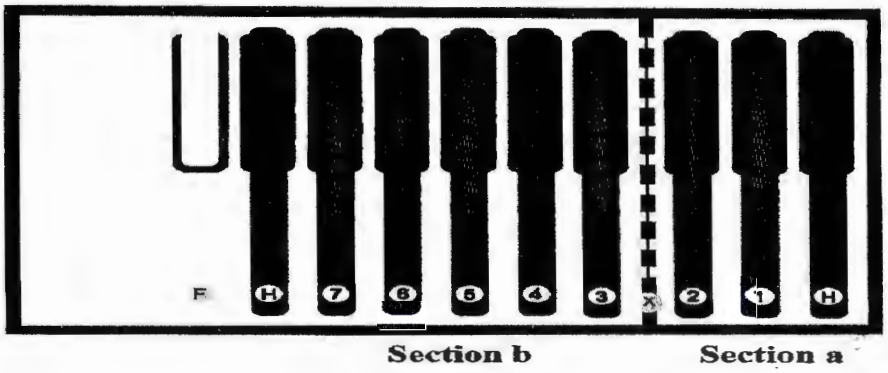

Fig. 4. The position of combs inside the modified hive

Queen Section (a): H = Honey and Pollen comb 1 = Empty comb for queen to lay in
2 = Young larvae comb x = Queen Excluder
Orphan Section (b): 3 = Old larvae comb 4 = Just capped brood comb
5 = Capped brood comb 6 = Capped brood comb
7 = about to hatch and hatching brood comb F = Frame feeder

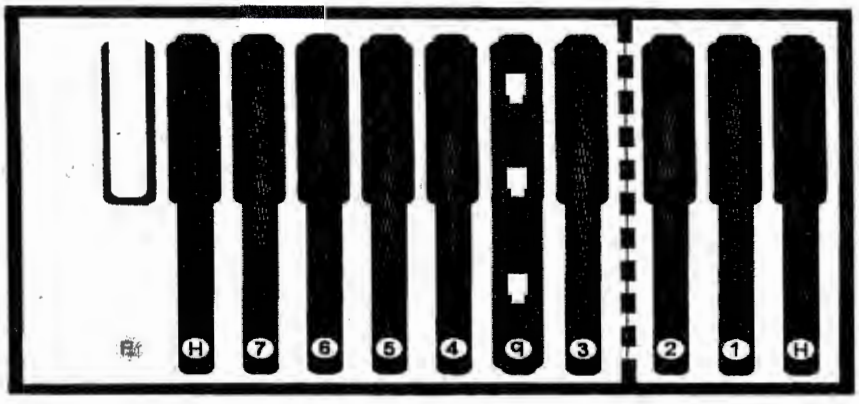

Fig. 5. The position of combs with grafted queen cups frame (q).

Day 15: Queen cells in brood combs in the orphan section were destroyed. Brood combs were rearranged Fig. (5), frame with sealed queen cups was moved to the position between comb No (5) and comb No (6) and a new frame with another 30 new grafted larvae was placed in its place. Colonies were fed with half liter of 50% sugar syrup for every hive and add pollen substitutes patties if needed.

Day 18: The first frame with ripe queen cells was removed from the hive, queen cells were separated from the bars carefully and incubated in electric incubator in 34.5°C and 70%RH Fig. (6), individually in special queen cages until emergence Fig. (7), second frame with sealed queen cells was moved to the position of the removed frame and a third frame with another 30 new grafted larvae was placed in its place. Natural queen cells in brood combs in the orphan section were destroyed. Brood combs were rearranged Fig. (5).

Colonies were fed with half liter of 50% sugar syrup for every hive and add pollen substitutes patties if needed.

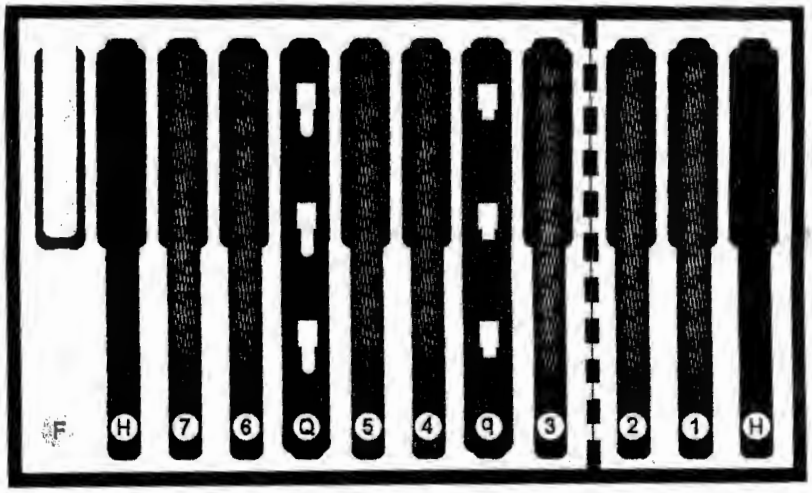

Fig. 6. The position of combs with grafted queen cups frame (q) and sealed queen cells frame (Q).

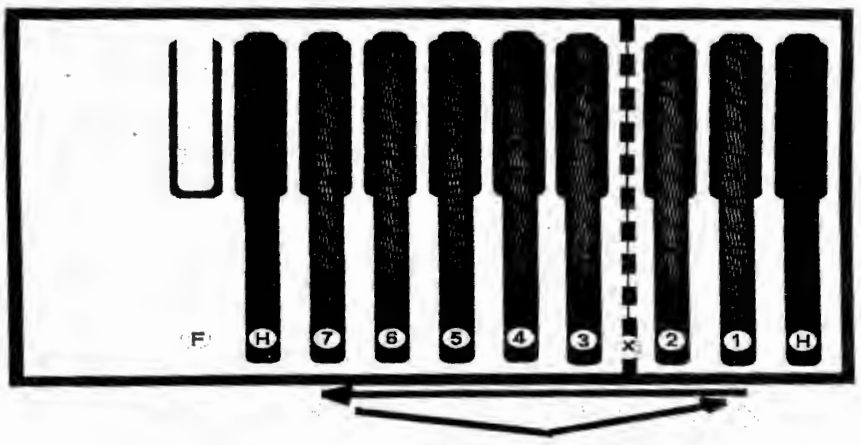

Fig. 7. Brood combs rotation during the study.

Day 21: Natural queen cells in brood combs in the orphan section were destroyed. Brood combs were rearranged Fig. (5). Colonies were fed with half liter of 50% sugar syrup for every hive and add pollen substitutes patties if needed.

Incubator was checked every four hours for emerged virgin queens in cages. Immediately after emergence queens were counted, weighted in mg so queens had no chance to feed with and data were recorded (Hatch *et al.*, 1999).

Brood combs rotation:

Brood combs were moved to the lift one step every three days so comb No (1) placed in the position of comb No (2) and comb No (2) placed in comb No (3) place and so until hatching brood comb No (7) goes to its first place of brood comb No (1) Fig. (7).

4- Rearing queen by the modified method in queen right colonies fed with natural queen pheromone inhibitor (lemon juice):

The same procedure used in the previous method was used plus adding a solution of sugar syrup contains lemon juice at the rate of 5 ml of concentrated natural lemon juice (*Citrus aurantifolia*) per one liter 50 % sugar syrup.

5- Statistical analysis:

The obtained data was statistically analyzed using analysis of variance (ANOVA) at 5 % probability. The measurements were separated using Duncan's Multiple Range Test (DMRT) through CoStat software program (Version 6.400). CoStat version 6.400 Copyright © 1998-2008 . Cohort Software. 798 Lighthouse Ave. PMB 320 , Monterey, CA, 93940, USA.

RESULTS AND DISCUSSION

Data presented in Table (1) show the effect of queen rearing methods on the larval acceptance reared under three methods. From total of 2250 grafted larvae a 1653 were accepted in the three methods with a overall acceptance of (73.47%) with an average of 22.04±2.86 per patch (30 cup).

The highest percentages in accepted cups was recorded with the modified queen rearing hives fed on lemon juice (81.07), followed by the treatment of queenless colony (72.27%) , while the modified queen rearing hives had the lowest acceptance percentage (67.07%).

The statistical analysis of the obtained data (Table 1) indicated that there were significant differences in the numbers of accepted larvae between both of modified hives and queenless colonies.

The highest average numbers of accepted larvae were accepted at the treatment of queenright colonies with lemon juice (24.32±1.31 per patch) (608 of 750 cups) , followed by the treatment of queenless colonies (21.68±2.58 per patch) (542 of 750 cups) , while the treatment of queenright colonies accepted only (24.32±1.31 per patch) (608 of 750 cups).

Table 1. Effect of queen rearing methods on the acceptance of queen grafted larvae

Methods	Queenless Colonies	Queenright Colonies	Queenright Colonies with lemon juice	LSD 5%
Ave. no. T.G.L/ colony	750	750	750	-
Ave. no. T.A.L.	542 b	503 c	608 a	12.8
Acceptance %	72.27	67.07	81.07	-
Ave. no T.A.L. / patch	21.68±2.58 b	20.12±2.73 b	24.32±1.31 a	1.4

Means with the same letter for each row are not significantly different at 0.05 level .

Data also indicate that adding natural lemon juice as a food supplement to the sugar syrup increased the grafted queen larvae acceptance when queen cups were introduced to queenright colonies, such results goes in line with (Gao *et al.*, 2010) who stated that increased phenolic compounds in nectar and bee food enhanced worker bee resistance to queen signals in honeybee colony and to build many queen cells in their colony. (Beatriz *et al.*, 2012) investigated the Polyphenol profile of lemon juice and found that fifty eight phenolic compounds of five different classes were identified in Citrus juices. In addition (Ebadi and Gary 1980), (Whiffler and Hepburn 1991), (Abo Lila and Khattaby 1997), (Dietemann *et al.*, 2006) and (Peso *et al.*, 2013) reported that queen pheromones inhibits queen rearing behaviors.

Data indicate too that grafted queen larvae acceptance was higher in Queenless colony than in Queenright colony with lemon juice or Queenright colony and such results in agreement with (Shawer 1980),(Dodologlu *et al.* 2004), (Şahinler and Kaftanoğlu 2005) and (Cengiz *et al.*, 2009) who demonstrated that queen larvae acceptance percentage was higher when larvae were introduced to queenless colonies than when larvae introduced to queenright colonies. While that data disagree with (Ahmad and Ahmad 2013) who stated that queen larvae acceptance percentage was higher when larvae were introduced to queenright colonies than when larvae introduced to queenless colonies.

Data also reveal that the average of accepted queen larvae increased across the study in the Queenright colony with lemon juice and Queenright colony groups while it was decreased in the Queenless colony group. The increase on acceptance in the Queenright colony with lemon juice and Queenright colony groups could be explained that new young bees (needed to rear queens) emerge every day and that the young nurse workers were getting used to the position of the larvae cup moreover the presence of the queen inhabit the development of worker ovary, while in Queenless colony group the number of young bees decreased a day after a day and laying worker could be seen at any time.

This explanation coincides with (Al-Shaarawi *et al.*, 2002) who stated that in case of rearing queens in queenless colonies for a long period (until the appearance of laying workers), it need continuously provide the queenless colonies with sealed brood. The presence of laying workers or unsealed brood combs in the colony drop the acceptance percentage significantly. (Wilkinson and Brown 2002) stated too that the queenright method could be re-used for successive patches of queen cells without causing laying worker problems. (Cengiz *et al.*, 2009) added that rearing queen bees in queenright colonies is more advantageous than in queenless colonies. Also (Peso *et al.*, 2013) observed that the presence of mated honey bee queen reduces worker ovary activation.

Data also observe that there were no significant differences among the Queenless colony hives and could be returned that the bees used was collected from different colonies before it were re-split in the rearing hives. While there were significant differences among Queenright colony hives returned to genetic differences there were no significant differences among the Queenright colony with lemon juice hives but this could be back that using of lemon juice inhabit queen pheromone and other pheromones.

The statistical analysis of the obtained data (Table 2) indicated that there were significant differences in the weights of emerged queens between both of modified hives and queenless colonies.

Table 2. Effect of queen rearing methods on the weight (mg) of virgin queens.

Methods	Queenless colonies	Queenright colonies	Queenright colonies with lemon juice	LSD 5%
Ave. weight / queen mg	168.56±4.09 b	180.08±3.41a	182.16±3.04 a	11.2

Means with the same letter are not significantly different at 0.05 level

Data in Table (2) indicated that the heavier virgin queens weight was recorded in Queenright colony with lemon juice group (182.16±3.04) mg followed by Queenright colonies group (180.08±3.41) mg, whereas the lighter virgin queens weight was produced by the Queenless colonies group (168.56±4.09) mg.

Data indicate that adding natural lemon juice as a food supplement to the sugar syrup increased the virgin queen weight such results are similar to previous results obtained by (Tharwat, 2002) who mentioned that queens reared in colonies treated with formic acid were heavier than others. (Sahinler *et al.*, 2005) mentioned that the supplementary feeding with vitamin E increased royal jelly production. (Gao *et al.*, 2010) stated that increased phenolic compounds in nectar enhanced worker bee resistance to queen signals in honeybee colonies.

Also that data are in agreement with (Woyke ,1999) who found that in queenright colonies, larvae of all ages received more nourishment than in queenless colonies. (Skowronek *et al.*, 2004) noticed that body weight of queens is significantly affected by rearing conditions. Ahmad and Ahmad 2013, who indicated that the virgin queen emergence weight was heavier in queenright colonies than in queenless colonies.

On the other hand (Shawer,1980) verified that the weight of newly emerged queens increased with increasing degree of orphans for queen rearing colonies. Wilkinson and Brown 2002 indicated that there was no significant difference in the weights of the queen pupae reared in the queenright or the queenless colonies. (Cengiz *et al.*, 2009) verified that there was no significant difference in queen weight at emergence and that rearing queen bees in queenright colonies is more advantageous than in queenless colonies.

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خلايا معدلة للإنتاج الموسع لملكات نحل العسل

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الملخص العربى

أجرى هذا البحث بمنحل خاص بقرية ميت فارس مركز بنى عبيد محافظة الدقهلية خلال الفترة من فبراير 2013 و حتى نهاية سبتمبر 2013 و ذلك بغرض دراسة مقارنة كفاءة تربية الملكات فى خلايا يتيمة (بدون ملكة) مع تربية الملكات فى خلايا ذات ملكة من طابق واحد ومعدلة بغرض إنتاج الملكات وذلك بتغيير الطريقة

الصينية لإنتاج غذاء الملكات لتناسب إنتاج و تربية الملكات العذارى ونفس الخلايا المعدلة مع إستخدام عصير الليمون كمادة طبيعية مثبطة لفرمونات الملكة . تم تصنيع خلايا خشبية تتسع ل (12) قرص شمعى مقاس لانجستروث ووضع بها حاجز ملكات رأسى يقسم الخلية إلى قسمين لكل قسم مدخل منفصل ، القسم الأول به الملكة و ثلاثة أقراص وباقى الأقراص وكئوس التربية بالقسم الثانى وتم مقارنتها بإنتاج الملكات فى خلايا بدون ملكة (يتيمة) و كانت اهم النتائج المتحصل عليها كما يلى:

كان متوسط عدد الكؤوس المقبولة لكل سدابه هو 24.32 ± 1.31 ، 21.68 ± 2.58 ، 20.12 ± 2.73 فى معاملات تربية الملكات فى خلايا ذات ملكة (المعدله) مع إستخدام عصير الليمون ، تربية الملكات فى خلايا يتيمة ، تربية الملكات فى خلايا ذات ملكة (المعدله) ، على التوالى.

بالنسبة للنسبة المئوية للكؤوس المقبولة ، كان متوسط نسبة قبول الكئوس (81 ٪ ، 67 ٪ ، 72 ٪) فى معاملات تربية الملكات فى خلايا ذات ملكة (المعدله) مع إستخدام عصير الليمون ، تربية الملكات فى خلايا ذات ملكة (المعدله) ، ثم تربية الملكات فى خلايا يتيمة على التوالى.

بالنسبة لمتوسط وزن الملكات المرباة ، كان متوسط الوزن للملكة الواحدة هو (168.56 ±4.09 ملليجم) ، (180.08 ±3.41 ملليجم) ، (182.16±3.04 ملليجم) فى معاملات تربية الملكات فى خلايا يتيمة ، تربية الملكات في خلايا ذات ملكة (المعدله) ، تربية الملكات فى خلايا ذات ملكة (المعدله) مع إستخدام عصير الليمون على التوالى.

يوصى البحث بتربية الملكات على نطاق تجارى باستخدام الخلايا المعدلة فى طوائف ذات ملكات مع استخدام عصير الليمون الطازج فى عمليات التغذية كمادة مثبطة للفرمون الملكى .