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Effect of the Multiple Queens Within Colony on Some Honeybee Activities, *Apis mellifera carnica* and Sustainability of their Colonies

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ABSTRACT

Multiple honey bee queen colonies contained two to four mated queens which able to move freely were produced by removing the mandibles of queens which trigger fighting and queen elimination within the colony. This method showed the high success, Twenty five out of twenty seven queens were accepted after their introduction in Carniolan colonies, and the success ratio reaches 92.6%. Twenty seven multiple queens introduced in nine colonies were sustainable enough for long period without losing any queen from the spring to fall in the first year. Twenty seven were over wintered successfully and no queen loss was recorded until the next fall in the second year. The multiple queen colonies which contained four queens were more efficient than the other tested multiple queen colonies in the workers brood production activity, it was recorded an average 14460 $inch^2$/colony/ year for amount of worker brood, followed by colonies which contained three queens then colonies which contained two queens while the single queen colonies as control indicated the lowest mean amount of workers brood 5776 $inch^2$/colony / year. Also, multiple queen colonies contained four queens were scored highest value of mean amounts 348, 9692 and 4120 $inch^2$/colony / year for drone brood rearing, honey and pollen production, respectively. The single queen colonies indicated the lowest amount of 124, 4968 and 1864 $inch^2$/colony / year for drones brood rearing, honey and pollen production, respectively. Also, multiple queen colonies contained four queens were scored highest value of mean amounts citrus, clover and cotton flow seasons, while single queen colonies were recorded lowest amount in same flow seasons. From the obtained results it was shown that the multiple queen colonies are more efficient for helping the beekeepers to increase the bee population and thus increase the honeybee products. In addition the multiple queens technique can be used in the commercial domain.

INTRODUCTION

Honey bees have an important economic role, where it contributes to the increase in the gross national product of many countries with agricultural activity through various of the honey bees products such as honey, pollen, bee wax, royal jelly and propolis (Krell, 1996). In addition the honeybees play an important role in cross-pollination of agricultural crops, which increases the quantity of crops and improves their quality (Morse and Calderone, 2000 & Abou-Shaara ,2014). The high population of the honey bee colonies is one of the most important factors for obtaining the highest productivity of hive products (Chen, 2001). For this reason, many researches have been conducted in developing methods that increase brood rearing to produce strong colonies beyond the natural capacity of a normal single queen colony. One of the methods to achieve this is to create a colony containing more than one queen so that they were mated and able to egg laying (Zheng *et. al.*, 2009). The honey bee societies are normally monogynous but the Polygyny may occur naturally during swarming and supersedure, and this stays in a period from hours to days (Hepburn and Radloff, 1998; Gilley and Tarpy, 2005).

The aim of the present study is (1) Produce the multiple queen colonies by modulating biological factors that provoke fighting, eliminating the queens within the colony and testing the effecting of this method in obtaining stable multiple queen colonies and providing the appropriate social context in the colony composed of several freely moving queens and able to egg lay. (2) Determination the effects of multiple queens on some honeybee activities (workers and drones brood rearing, storage honey). (3) Determination the effects of multiple queens on honey production (citrus, clover and cotton).

MATERIALS AND METHODS

The experiments were carried out in an apiary located in Motobes district, kafr El-Sheikh Governorate, Egypt during the period from 2013 to 2015.

1. Effects of multiple queens on honeybee activities and sustainability of these queens.

a. The used strains and the number of queens.

Twelve Carniolan *(Apis mellifera carnica*) colonies were used and grouped according to the number of the used queens in four groups, each group consisted of three colonies, The first group was used one queen into each colony as control. In the second group two queens were introduced into each colony. Three queens were introduced in the third group into each colony and in the fourth group four queens were introduced into each colony.

b. Preparation of the colonies destined to host multiple queens.

The colonies destined to host the multiple queens were prepared as follows:

Sealed brood combs on the verge of hatching and emerging brood combs were selected then shaken, to remove the flight of the older bees from combs, while the young bees were remained on the combs. The combs were placed in the hive with the young workers bees of the age of 1- 3 day that still exist on them. The young worker bees were used to build up the colonies in order to avoid workers balling and killing the introduced queens, which is the typical behavior of the older workers bees. (Robinson, 1984; Sigg *et al*., 1997 and Masry, 2010). The host colonies were placed at a distance of 5-10 m away from their original location to ensure that all remaining older foragers bees will didn't re enter their colonies. (Lindauer, 1953 & Abou-Shaara ,2014).

Additional combs of pollen and honey were used beside brood combs to provide sufficient food, as these colonies were deprived from the foragers workers at the beginning.

c. Preparation of queens.

Two days after the colonies preparation, the queens that would be introduced into each colony were selected so that they were mated, able to egg laying, at the same age of 6-12 month old from the same strain in order to promote equality in their status of the acceptance by workers.

One third to half of both mandibles of these queens were removed with micro-scissors under a stereoscopic binocular. The ablation of the mandibles of queens reduced their tendency to fight and kill each other. In addition the mandibles of queen contain glands which produce queen substance pheromones and consequently, the workers bees recognizes the queens and recruitment for aggressive and defensive behavior (Zheng *et al*., 2009). Also, the large abdomens of the

egg laying queens contributed to reduce their ability to fight against other queens (Spiewok, 2006).

Drops of diluted honey were sprayed onto the workers and queens before queen introduction to stimulate the grooming behavior and gives some time for the odour of the queens to become acceptable to the workers colony. Drops of Thymian liquid were applied on the top of combs for change the odour of the colonies, and it was covered the hive for 3 minutes. Two to four queens were introduced to different locations in the host colonies. The colonies were examined in the next day to make sure of the success of the queens acceptance.

Multiple queen colonies were established in the spring when the nectar flow was present and availability of pollen.

The acceptances of the queens introduced were monitored, and the sustainability of the colonies successfully created was recorded.

The effect of multiple queens on some colonies activities were studied as follows:

a- Effect on brood rearing (mean amounts of workers and drones brood every 14 days intervals inch^2/colony.)

b- Effect on stored honey (mean amounts of storage honey every 14 days intervals inch^2/colony.)

c- Effect on honey production in citrus, clover and cotton flow seasons (actual amount of produced honey was weighed every flow season kg. /colony).

d- Effect on stored pollen (mean amounts of storage pollen every 14 days intervals inch^2/colony).

The effect of multiple queens was compared with the effect of single queen colonies.

RESULTS AND DISCUSSION

1. The acceptances of the queens the sustainability of the multiple queen colonies.

The multiple queen colonies were contained two to four queens cohabiting peacefully, free moving and laying eggs (Fig. 1.)The results gathered in the two years showed the high success of this method. When we compared the number of queens acceptance vs. the number of queens introduced. Twenty five out of twenty seven queens were accepted after their introduction in Carniolan colonies. The success ratio reaches 92.6% for multiple queens which introduced into host colonies (Table 1). The success ratio was 100% in case of single queen colonies (control).

Our results are in agreement with these finding by Zheng *et al.* (2009) which found that the 89.8% multiple queens colonies were accepted after their queens introduction, on other hand Morse, (1979) reported that the success rate of 95-100 % where happened when introducing queens into single queen colonies.

Twenty seven multiple queens introduced in nine colonies, these colonies were sustainable enough for long period without losing any queen from the spring to fall in the first year (Table 2). Twenty seven were overwintered successfully and no queens loss were recorded until the next fall in the second year. Only one queen was lost in the next winter. The obtained results are in agreement with those of Zheng *et al.* (2009) recorded that the 55 out of 80 multiple queen colonies were overwintered successfully and no queen was lost in the next spring while two queens were lost in 30 colonies. (Spiewok, 2006) reported that the young queens are more aggressively to their rivals. On the other hand Spoja (1953) suggested that queens need not be of the same age.

Table 1. Acceptances of queens introduced into hosting colonies.

strain	Number of queens in each colony	Total queens introduced / three colonies	Queens accepted / three colonies
Carniolan	2	6	6
	3	9	8
	4	12	11
Total Carniolan		27	25 (92.6%)

Table 2. Sustainability of multiple queens during the period from 21/3/2013 – 20/3/2015 in the Carniolan honeybee strain.

years	Season	Queens introduced	Queens loss
2013 - 2014	Spring	27	No queen loss
	Summer		No queen loss
	Fall		No queen loss
	Winter		No queen loss
Total of queens loss in the first year			0
2014 - 2015	Spring	27	No queen loss
	Summer		No queen loss
	Fall		No queen loss
	Winter		One queen
Total of queens loss in the second year			1 (3.7%)
Total of queens loss			1(3.7%)

2- The effect of multiple queens on some colonies activities:

a. Effect on workers brood rearing

Data in Table (3) summarized the clear significant differences in the mean amount of workers brood reared in the tested colonies in act of two, three and four queens and compared the effect of multiple queen colonies with colonies which containing one queen during the study period. The mean amount of workers brood reared was indicated the highest value in the spring in the act of four queens (5454 inch^2 / colony), when the mean amount of workers brood reared recorded 4788 and 3729 inch^2/colony in act of three and two queens per colony respectively. The single queen colonies as control recorded the lowest values (2615 inch^2/colony) during the same season. The mean amounts of workers brood (inch^2/colony) reared in Carniolan multiple queen colonies decreased at the summer season which recorded 4861, 4536 and 3250 inch^2/colony in act of four, three and two queens respectively. The amount was 1844 inch^2/colony in the single queen colonies. Then the value decreased gradually in the fall season till reached the minimum in the winter season it was recorded in the multiple queen colonies 1864, 1359 and 990 inch^2/colony for four , three and two queens respectively. The amount was 594.3 inch^2/colony in the single queen colonies (Table 3).

It means that the high amount was reared in spring followed by summer season and in descending order comes fall and winter.

Another comparison was achieved between the average amounts of all workers brood reared per colony / year in act of two, three and four queens in colony and compared with single queen in colony. Data of Table (3), illustrated that the mean amount of workers brood reared of multiple queen colonies in act of two queens was 9180 inch^2/colony / year, while the mean amounts scored 12344 and 14460 inch^2/colony / year for multiple queen colonies in

act of three and four queens respectively. Single queen colonies recorded an average 5776 inch^2/colony / year (Table 3). It means that the multiple queen colonies which contained four queens more efficient than the other tested multiple queen colonies in the workers brood production activity, followed by colonies which contained three queens then colonies which contained two queens while the single queen colonies as control indicated the lowest mean amounts of workers brood.

Table 3. Mean amount of workers brood inch^2/ colony /season in the act of multiple queens by Carniolan strain during the period from 2013 –2014.

Queens no./ colony Season	One queen (control)	Two queens	Three queens	Four queens	Mean
Spring	2615	3729	4788	5454	4146 a
Summer	1844	3250	4536	4861	3622 b
Fall	723.3	1210	1661	2282	1469 c
Winter	594.3	990.7	1359	1864	1202 d
Total	5776 d	9179c	12344b	14461 a	
Mean	1444 d	2295c	3086 b	3615 a	

L.S.D $_{0.05}$ between seasons= 21.9
L.S.D $_{0.05}$ between queens number = 12.1

The obtained results are in agreement with Elaidy and Abdo Tolba, (2017) which indicated that the highest amount of worker brood was reared in the spring 522 inch^2/colony for Carniolan strain followed by summer 328 inch^2/colony, then fall was 272 inch^2/colony, while the lowest mean amount of worker brood was in winter 195 inch^2/colony. On the other hand Marzouk (2009), and Salem, (2011) mentioned that the highest mean amount of worker brood observed in summer season followed by spring and fall season while the lowest mean amount were in winter.

b. Effect on drones brood rearing.

As shown in Table (4) there were clear significant differences in the mean amounts of drones brood reared in the tested multiple queen colonies and the single queen colonies. In the spring season the mean amounts of drones brood reared in act of four queens showed high mean (177 inch^2/colony / season) followed by colonies which contained three and two queens were recorded 155and 1140 inch^2 / colony/ season respectively. The single queen colonies as control recorded the minimum value (72 inch^2/colony). In the summer season data of Table (4), revealed that the mean amounts of drones brood (inch^2/colony) reared in multiple queen colonies decreased it was recorded 142, 120 and 68 inch^2/colony in act of four , three and two queens respectively. While the amount was 39 inch^2/colony in the single queen colonies (control). Then the value decreased gradually in the fall season till reached the minimum in the winter season. It was recorded in the multiple queen colonies 13, 11 and 10 inch^2/colony for four, three and two queens respectively. While the amount scored 5.6 inch^2/colony in the single queen colonies (Table 4). It means that the high amount of drone brood was reared in spring followed by summer season and in descending order comes fall and winter.

From the standpoint of the mean amounts of drones brood rearing through the different seasons it was fond that not significant different in the mean amounts of drones brood reared in the tested multiple queen colonies and the single queen colonies in the fall and winter seasons. Data of Table (4), illustrated that the mean amount of drone brood reared of multiple queen colonies in act of two queens was 232 inch^2/colony / year while the mean amounts scored 300 and 348 inch^2/colony / year for multiple queen colonies in act of three and four queens respectively. The single queen colonies recorded an average 124 inch^2/colony / year.

The obtained results are in agreement with those of; Marzouk, (2009) and Shaheen, (2012) they indicated that the highest amount of drone brood was recorded in spring followed by summer season, on the other hand, they were scored the lowest mean amount of drone in fall season.

Table 4. The mean amount of drones brood inch^2 / colony in the act of multiple queens during the period from 2013 –2014.

Queens no./ colony Season	One queen (control)	Two queens	Three queens	Four queens	Mean
Spring	72	140	155	177	136 a
Summer	39	68	120	142	92.2 b
Fall	8	13	15.7	16.7	13.3 c
Winter	5.6	10	11	13	9.9 c
Total	124 d	231 c	301 b	348 a	
Mean	31.1 d	58 c	75 b	87 a	

L.S.D $_{0.05}$ between seasons= 18.0
L.S.D $_{0.05}$ between queens number = 3.2
Means with the same latter are not significant different

c. Effect on honey storage

Data in Table (5) summarized the clear significant differences in the mean amount of stored honey in act of two, three and four queens in comparing the effect of multiple queen colonies with colonies which containing one queen. In the multiple queen colonies the mean amount of stored honey was indicated the highest value in the summer season in the act of four queens (3953 inch^2 / colony), while the mean amount of stored honey recorded 3661 and 3157 inch^2/colony in act of three and two queens per colony respectively. The single queen colonies as control recorded the lowest values (2189 inch^2/colony) during the same season. The mean amounts of stored honey (inch^2/colony) in Carniolan multiple queen colonies decreased at the spring season and recorded 3560, 3206 and 2627 inch^2/colony in act of four, three and two queens respectively. The amount was 1840 inch^2/colony in the single queen colonies (control). Then the value decreased gradually in the fall season till reached the minimum in the winter season it was recorded 750, 570, 453 and 293 inch^2/colony in act of four , three, two and one queen inside the colony respectively (Table 5). It means that the summer season was the highest followed by spring season and in descending order come fall season. The winter season indicated the lowest mean amounts of stored honey.

Table 5. Mean amount of stored honey inch^2 / colony in the act of multiple queens by Carniolan strain during the period from 2013 – 2014.

Queens no./ colony Season	One queen (control)	Two queens	Three queens	Four queens	Mean
Spring	1840	2627	3206	3560	2808b
Summer	2189	3157	3661	3953	3240a
Fall	648	951	1173	1429	1050c
Winter	293	453	570	750	516 d
Total	4970 d	7188 c	8610 b	9692 a	
Mean	1242 d	1797 c	2152 b	2423 a	

L.S.D $_{0.05}$ between seasons= 10.5
L.S.D $_{0.05}$ between queens number= 6.9

From the obtained results in Table (5) the total mean of stored honey during the experimental period showed clear differences among of queens number inside the colony, the mean amounts were recorded 4968, 7188, 8608 and 9692 inch^2/colony / year for one, two, three and four queens per colony respectively. The multiple queen colonies which contained four queens were more efficient than the other

tested multiple queen colonies in the honey storage activity while single queen colonies scored the lowest value.

d. Effect on honey production.

Another comparison was made between the activities of the tested multiple queen colonies in honey production at the end of flow seasons and single queen colonies as control. As shown in Fig. (1), there were clear significant differences in the mean of seasonal honey production. In the citrus flow season the highest amount of honey production was recorded by multiple queen colonies in the act of four queens (9.8 kg. / colony), followed by multiple queen colonies in the act of three queens (8.3 kg. / colony), then the multiple queen colonies in act of two queen (6.2 kg. / colony). The single queen colonies as control attained the lowest amount (4.7 kg. / colony). The obtained results in Fig (1) indicated that the total mean of honey production in the clover flow season was occupied the first place for the multiple queen colonies in act of four queens inside the colony (12.1kg. / colony) followed by the multiple queen colonies in act of three and two queens inside the colony there were recorded an average 10 and 8.1 kg. / colony respectively . The colonies which contained single queen came in the last position it was recorded 6.3 kg. / colony (Fig 1).

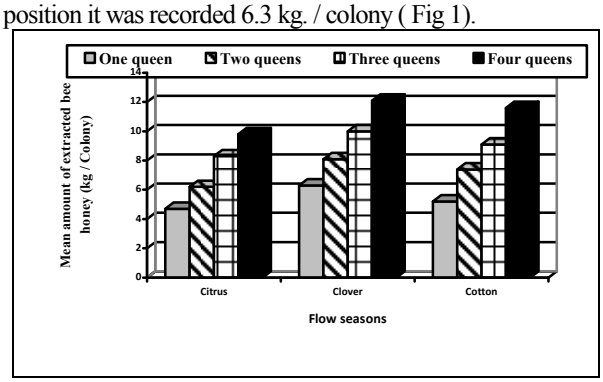

Fig. 1. Mean amount of extracted citrus, clover and cotton honey (kg / Colony) produced by the multiple queen colonies at the end of flow seasons during the period from 2013 -2014.

In the cotton flow season data in Fig (1) summarized the clear significant differences in the mean amount of honey produced in multiple queen colonies in the act of four queens indicated the higher value than the other tested colonies (11.6 kg. / colony). While the single queen colonies as control occupied the last level (5.2 kg. / colony). It means that the multiple queen colonies which contained four queens more efficient than the other tested multiple queen colonies in the honey production activity, followed by colonies which contained three queens then colonies which contained two queens while the single queen colonies as control indicated the lowest mean amounts of honey production.

The obtained results are in agreement with Salem, (2011) stated that there were two major flow seasons in Fayoum the first was clover which gathered an average amount of honey 733 inch^2 / colony and the second major flow season was cotton, the average amount of stored honey was 806.33 inch^2 / colony. Shaheen, (2012) found that the highest amount of stored honey were recorded in spring season while, the lowest amount of the stored honey was in winter.

e. Effect on pollen storage.

As shown in Table (6) the clear significant differences was found in the mean amounts of stored pollen in the tested multiple queen colonies and the single queen colonies as control for Carniolan strain. In the multiple queen colonies the mean amount of stored pollen was indicated the highest value in the summer season in the act of four queens (1679.7 inch^2 / colony), while the mean amount of stored pollen were recorded 1494.7 and 1155 inch^2/colony in act of three and two queens per colony respectively. The single queen colonies as control recorded the lowest values (877.7 inch^2/colony) during the same season. The mean amounts of stored pollen (inch^2/colony) decreased at the spring season it were recorded 1190.7, 1059.7 and 836 inch^2/colony in act of four, three and two queens respectively. The mean amount was recorded 711 inch^2/colony in the single queen colonies. The value decreased gradually in the fall season till reached the minimum in the winter season it was recorded 306, 194.3, 132.7 and 73.3 inch^2/colony in act of four , three, two and one queen respectively.

Data of Table (6), revealed that the mean amount of stored pollen of multiple queen colonies in act of two queens was 2528 inch^2/colony / year while the mean amounts scored 3440 and 4120 inch^2/colony / year for multiple queen colonies in act of three and four queens respectively. The single queen colonies was recorded an average 1864 inch^2/colony / year. It means that the multiple queen colonies which contained four queens more efficient than the other tested multiple queen colonies in the pollen storage activity, followed by colonies which contained three queens then colonies which contained two queens while the single queen colonies as control indicated the lowest mean amounts of stored pollen.

Table 6. The mean amount of stored pollen inch^2 / colony in the act of multiple queens by Carniolan strain during the period from 2013 – 20/3/2014.

Queen no./colony Season	One queen (control)	Two queens	Three queens	Four queens	Mean
Spring	711	836	1059.7	1190.7	949.3 b
Summer	877.7	1155	1494.7	1679.7	1301.7 a
Fall	200.7	405.7	690.7	943.7	560.2 c
Winter	73.3	132.7	194.3	306	176.6 d
Total	1862 a	2529 c	3439 b	4120 a	
Mean	466 d	632 c	860 b	1030 a	

L.S.D $_{0.05}$ between seasons= 27.7

L.S.D $_{0.05}$ between queens number= 6.4

The obtained results are in agreement with those of; Elaidy and Abdo Tolba, (2017) found that the Carniolan strain stored the highest amount of pollen in summer 56 g/colony followed by 53 and 37 g/colony in spring and fall respectively, lowest amount of stored pollen was recorded in winter 35 g/colony. Also, Salem, (2011) found that the highest rate of pollen production recorded in the summer , followed by spring , then fall season, while the lowest rate of pollen production was recorded in the winter. On the other hand Shaheen, (2012) recorded the highest amount of stored pollen during spring.

These results show that the multiple queen honey bee colonies technique is valid to produce sustainable colonies for long periods it may be conclude that we can be use the multiple queen honey bee colonies for commercial use. In addition the multiple queen honey bee colonies technique will help the beekeepers to increase the population colonies and increase the productions of the honeybee colonies.

Generally there are very few published studies on multiple queen colonies. Several attempts have been made to establish multiple queen colonies by using the free moving

queen and simply introducing them among bees (Kovtun, 1949, Melnik 1951& Spoja, 1953) and the physical separation between queens (Haydak and Dietz, 1967), while Lensky and Darchen (1963) which used the queens after amputating stings. Our rustles showed that mandible ablation is an efficient method to prevent queens from fighting, while normal queens fight until only one survives (Dietemann *et al*., 2008). Improving the speed of reproduction and maintaining strong colonies are prerequisites for increasing colony productivity. Increased egg laying leads to strong colonies; in addition, the development of a large field force improves productivity as well as promotes disease resistance in the colony. The increase in genetic diversity in colony improvements work efficiency (Fuchs and Schade, 1994; Jones *et al*., 2004) as well as resistance against diseases and parasites (Palmer and Oldroyd, 2003; Hughes and Boomsma, 2004, 2006).

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تأثير الملكات المتعددة داخل الطائفة على بعض انشطة نحل العسل *Apis mellifera carnica* وإستدامة طوائفهم

شيماء ناجي مصطفى ، ماجدة حسن على سالم و أسامة الأنصاري

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في هذا البحث تم انتاج طوائف نحل العسل عديدة الملكات تحتوي على 2- 4 ملكات ملقحة وحرة الحركة عن طريق ازالة الفكوك العليا لتلك الملكات والتي تثير القتال داخل الطائفة .أظهرت هذه الطريقة نجاح كبير في طوائف النحل الكرينولي حيث تم قبول 25 ملكة من اصل 27 تم ادخالهم في 9 طوائف حيث بلغت نسبة النجاح 92.6 % واستمرت هذه الطوائف لفترة طويلة بدون ان تفقد أياً من ملكاتها وذلك من الربيع حتى الخريف في السنة الأولى ، وهذه الملكات نجحت في التشتية حتى وصلت الى الخريف التالي بدون ان تفقد أياً من ملكاتها. طوائف النحل عديدة الملكات والتي تحتوي على 4 ملكات كانت أكثر كفاءة من الطوائف عديدة الملكات الاخرى في نشاط تربية حضنة الشغالات حيث سجلت 3615 بوصة مربعة طائفة ، تليها تلك الطوائف تلك الطوائف التي تحتوي على 3 ملكات ثم الطوائف التي تحتوي على ملكتين ، في حين أن الطوائف التي تحتوي على ملكة واحدة ككنترول أظهرت اقل قيمة في تربية حضنة الشغالات 144بوصة مربعة طائفة . أيضاً سجلت طوائف الاربع ملكات اعلى قيم بالنسبة لتربية حضنة الذكور ، انتاج العسل ، انتاج حبوب اللقاح حيث كانت 87، 2423، 1030 بوصة مربعة طائفة على الترتيب . الطوائف ذات الملكة الواحدة اظهرت أقل قيم بالنسبة لتربية حضنة الذكور ، انتاج العسل ، انتاج حبوب اللقاح حيث سجلت 31، 1242 ، 466 بوصة مربعة طائفة على الترتيب. ايضاً سجلت طوائف النحل عديدة الملكات التي تحتوي على اربع ملكات أعلى قيم من حيث إنتاج العسل وذلك في مواسم الفيض كلا من الموالح والبرسيم والقطن في حين سجلت طوائف الملكة الواحدة أقل قيم في نفس مواسم الفيض. من النتائج المتحصل عليها يتضح لنا إن إستخدام الطوائف العديدة الملكات أكثر كفاءة في مساعدة النحالين لزيادة أعداد النحل وبالتالي زيادة منتجات النحل ، بالاضافة الى ذلك فان تقنية الملكات المتعددة يمكن استخدامه على نطاق تجاري