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# SOME MORPHOLOGICAL CHARACTERS OF QUEEN HONEY BEE *Apis mellifera carnica* ACCORDING TO DIFFERENT LOCALITIES AND SEASONAL VARIATIONS

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## ABSTRACT

The criteria used throughout the present study were: weight of the queen , length of the abdomen , length and width of the right fore wing , length and width of 3rd and 4th terga , number of ovarioles of the right ovary , diameter and volume of the spermatheca .

Two different localities for queen – rearing were selected (Giza and Alexandria) ,during 1989 and 1990.

Significant differences were found between the weight of virgin queens, the length and width of the 4th tergum of queens and also between the volume of spermatheca of queens reared in both localities .

Trials also were made to find out the most suitable season for queen rearing during the different honey flows.

It was clear that there were significant differences in the weight of virgin queens ,length of the abdomen , length and width of right fore wing, length and width of 4th tergum , number of ovarioles , and volume of spermatheca during spring , summer and late summer. Length and width of the 3rd tergum were not significantly affected by rearing queens in different seasons.

It was indicated that the most suitable seasons for rearing queens in Egypt were late summer, followed by summer and then spring.

## INTRODUCTION

The queen of the honeybee *Apis mellifera* L. is the most important individual in the colony and attracted special attention. Function of laying eggs helps to maintain and increase the population of the colony. If she cannot lay enough eggs, colony will become weak and will soon die.

Quality of the queen is not only hereditarily controlled, but also depends on the conditions in which it grows as larva. The size and vigour of a colony of honeybees are a direct reflection of the genotype of the queen, and also of her individual size and vigour. For example, as her body weight increases, the number of ovarioles increases (Hoopingarner and Farrar,1959). Also, part of variations, that are frequently observed among many queens which inherit similar size and body conformations, are the result of variations in environmental factors during rearing (Roberts,1961).

The Present work was carried out to study the effects of locality, rearing period (season) upon certain morphological and physiological characteristics of the honeybee queen (weight, length of abdomen, length and width of right fore wing (according to Bediar,1990) length and width of 3rd

and 4th terga, number of ovarioles of the right ovary (according to Hassanin and El-Banby, 1956, EL-Banby, 1958 and Moukayess, 1979), diameter and volume of spermatheca.

## MATERIALS, METHODS AND TECHNIQUES

This study was carried out during the queen – rearing period in Egypt (from March until September) for two successive years 1989 and 1990. The following section describes the factors which were expected to influence the morphological and physiological characteristics of the newly emerged queens. Two Governorates (Giza and Alexandria)were selected as two different localities in order to study their probable effects .Alexandria lies off the north to Giza and about 220 KMS away . Alexandria has a Mediterranean climate which is somewhat different from that of Giza.

Queen-rearing units were placed at the queen-rearing stations of the ministry of Agriculture , both in Giza and in Alexandria. In order to study the season effects, the queen-rearing period (from March until September) was divided into three seasons , nominated as spring (March to May), summer (June and July) and late summer (August and September).

The queen-rearing technique was carried out by transplanting (grafting) one-day-old worker larvae into queen cell cups which were given to the queen-rearing colonies (Doolittle,1909).

### I. The Queen-Rearing Process:

### I.1. The Queen-Rearing Unit:

The queen-rearing unit (Fig.1) was processed during this work (after Bookkeeping Department of Plant Protection Research Institute). It was as follows:

It consisted of three parts. The middle part (3) was separated from the two lateral parts (1and 2) by 2 queen excluders. Each lateral part (1and2) contained a colony with a good selected queen.

Covers of the three parts were planned to be opened vertically. The three entrances of the unit went through the wall of the station.

This design of a queen – rearing unit allowed bees from both lateral parts to move freely into the middle part except the queen. The middle part was used for raising the grafted queen cells. Nurse bees of both lateral parts played the main role of feeding the queen larvae.

In order to encourage and attract nurse bees to the middle part , a brood comb was usually provided in the middle part for successful raising of queens .

The procedure used here resembled that followed by the Ministry of Agriculture Research Institute. This was considered to be very promising when applied by many beekeepers.

### I.2. Preparation of young larvae for grafting:

In order to obtain larvae at the proper age (24 hours) , a prolific queen of a selected colony was confined with a marked empty worker comb in a special cage provided with queen excluders on both sides . The cage was

placed in the center of the brood nest of the colony . It was found better to use a comb from which worker bees had just emerged , because the queen preferred such comb to deposit its eggs . After 24 hours , the comb with newly laid eggs was lifted out and placed in the same colony . By using this technique the queen found no place for egg laying other than the comb provided for this purpose. Therefore, the age of the larvae which were used for grafting did not exceed 24 hours.

Thirty queen cell cups were attached to two wooden stick bars. This process was repeated for the queen-rearing unit.

**I.3. Grafting technique:**

The method of Laidlaw (1975) was followed. Chosen larvae for transferring were those which were lying on abundant royal jelly in their worker cells . The queen cell cups were prepared from bee wax after Doolittle (1909). Fifteen queen cells were fixed on a wooden bar , using melted wax. The bars were fitted singly into a frame. Two wooden bars were provided to the cell building colony at each time for few hours before grafting in order refixed to wooden pars, grafting in order to let the bees clean and prepare the queen cell cups.

The tip of the grafting tool was slipped under the larva , lifting it out with small portion of its surrounding jelly , and then deposited in the cell cup . As soon as these two wooden bars , with 30 queen cell cups , were grafted , they were fitted into the frame , and provided into the middle part of the queen – rearing unit.

After 24 hours the successful queen cells , being accepted by the bees , were collected and returned to the middle part for completion of nourishment. Sugar syrup (one part sugar to one part water) was fed to the cell builders after grafting.

Ripe queen cells (after 9 days of grafting) were carefully removed from the bars, and each cell was placed in a screened cage until emergence . Cages containing ripe queen cells were incubated in a strong colony .This process was repeated four times every month for each colony; thus,120 cell cups were introduced every month for each colony.

**II. Morphometrical Techniques:**

Newly emerged queens were weighed , and then preserved in 70% alcohol for morphological and anatomical studies. Biometrical studies of the queens and diameters of the spermathecae were obtained by utilization of a microscopic micrometer slide . Volume of spermatheca was calculated as =4/3 ~ r3 (r= radius of spermatheca).

Counting the number of ovarioles of the right ovary of the queen was achieved under a stereoscopic self-illuminated binocular microscope, with a high magnification power (x40).

Dissection was carried in 50% ethyl alcohol starting from the first tergum till the last one by cutting through the connections between the terga and sterna on both sides , and then all the terga were removed. The right ovary was taken after removal of the tracheae. Each ovary was placed separately in Bouin's solution for few hours. Then the excess Bouin's solution

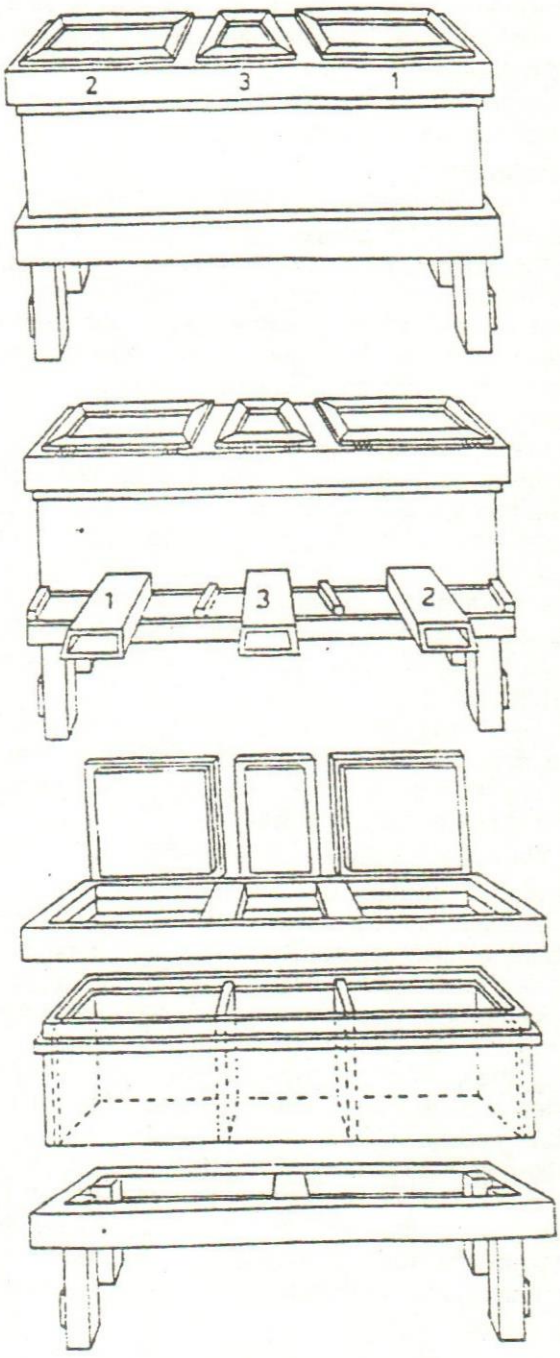


**Fig. 1 Queen rearing unit**

was removed . Following Ibrahim (1977), a drop of Puri's medium (10 ml. Distilled water , 5 ml. Glycerine,3ml.glacial acetic acid ,70 gm. Chloral hydrate and 8 gm . gum acacia) (Puri,1931) was dripped upon the fixed ovary and left for one minute. After this treatment , the specimen was washed several times with water , and then another drop of puri's medium was dripped. This process was repeated 3 times to get rid of the connective tissues. The ovary was then put on a slide with drop of distilled water , and the ovarioles were counted.

## RESULTS AND DISCUSSION

### I- Morphometrical Characteristics:

### I.1. Effect of locality:

Rearing queens was carried out from March to September for two successive years , 1989 and 1990 T-test was used for analysis of variance of data.

Two Governorates (Giza and Alexandria) were chosen to study the possible effects of different localities on the morphological and physiological characteristics of virgin queens. In comparison studies between two localities,the following characters where examined.

Table 1 summarizes the obtained results:

**Table (1): Effect of locality on the mean of certain morphological and physiological characteristics of Queens reared during 1989 and 1990.**

| | | Locality | | T - Value |
|---|---|---|---|---|
| | | Giza | Alexandria | |
| Weight of Queen (mg) | | 168.62±15.23 | 172.26±9.06 | 2.640* |
| Length of abdomen (mm) | | 13.83±0.53 | 13.88±0.51 | 1.019 |
| Right fore wing | length (mm) | 9.59±0.20 | 9.58±0.17 | 0835 |
| | Width (mm) | 3.22±0.09 | 3.20±0.06 | 1.479 |
| 3rd Tergum | length (mm) | 9.22±0.21 | 9.21±0.22 | 0.464 |
| | width (mm) | 3.33±0.11 | 3.44±0.25 | 1.803 |
| 4th Tergum | length (mm) | 10.49±0.24 | 10.41±0.24 | 3.006* |
| | width (mm) | 3.11±0.10 | 3.17±0.07 | 6.419* |
| Number of overioles | | 151.19±10.99 | 149.50±10.33 | 1.840 |
| Volume of spermatheca ($mm^3$) | | 151.19±10.99 | 149.50±10.33 | 2.190* |

n = 1311

### a) Weight of virgin queens:

Table 1 shows the mean weight of virgin queens reared in Giza and in Alexandria . There was a significant difference between weights of queens reared in both localities at 0.01 level of significance.

**b) Length of abdomen of virgin queens:**

Table 1 shows the mean length of the abdomen of virgin queens reared in Giza and Alexandria .

Statistical analysis indicated that there was no significant difference between length of the abdomen of queens reared in both localities.

**c) Length and width of right fore wing of virgin queens:**

Table 1 shows the mean length and width of the right fore wing of virgin queens reared in Giza and Alexandria . Statistical analysis indicated that there were no significant differences in both length and width of the right fore wing of virgin queens reared in both localities .

**d) Length and width of 3rd tergum of virgin queens:**

Table 1 shows the mean length and width of the 3rd tergum of virgin queen reared in Giza and Alexandria . Statistical analysis showed no significant differences in both length and width of this tergum in both localities.

**e) Length and width of 4th tergum of virgin queens:**

Table 1 shows the mean length and width of the 4th tergum of virgin queens reared in Giza and Alexandria .

Length and width of the 4th tergum of virgin queens were significantly affected by rearing queens in both localities at 0.01 level of significance.

**f) Number of Ovarioles of Virgin queens:**

Table 1 shows the mean number of ovarioles of virgin queens reared in Giza and Alexandria .

Statistical analysis indicated that the number of ovarioles was not significantly affected by rearing queens in either locality.

**g) Volume of spermatheca of virgin queens:**

Table 1 shows the mean volume of the spermatheca of virgin queens reared in Giza and Alexandria .

Statistical analysis indicated that volume of the spermatheca of virgin queens was significantly affected by rearing queens in either locality only at 0.05 level.

**I.2. Effects of Season:**

Rearing was carried out during 3 seasons: spring (March to May ), summer (June and July), late summer (August and September) for two successive years, 1989 and 1990.

F-test was used for analysis of variance. In comparison studies between different seasons, the following characters were examined .Table 2 summarizes the obtained results:

**a) Weight of virgin queens:**

The mean weight of virgin queens was 170.69±7.23mg.as recorded in late summer ,while the mean weight was 168.79±9.10mg.in summer.

Statistical analysis indicated that weight of virgin queens was significantly affected by rearing queens during different seasons only at 0.05 level.

**b) Length of abdomen of virgin queens:**

Table 2 shows the mean length of the abdomen of virgin queen during late summer. The mean length during spring.

Length of the abdomen was significantly affected by rearing queens during different seasons at 0.01 level.

**Table (2): Effects of season on the mean of certain morphological and physiological characteristics of Queens reared during 1989 and 1990.**

| | Locality | | | F - Value |
|---|---|---|---|---|
| | Spring | Summer | Late summer | |
| Weight of Queen (mg) | 170.35±9.89 | 168.78±9.10 | 170.69±7.23 | 3.404* |
| Length of abdomen (mm) | 13.57±0.34 | 13.77±0.53 | 14.06±0.51 | 27.650* |
| Right fore wing length (mm) | 9.57±0.20 | 9.65±0.19 | 9.56±0.13 | 5.308* |
| Right fore wing Width (mm) | 3.21±0.07 | 3.24±0.07 | 3.20±0.06 | 4.605* |
| 3rd Tergum length (mm) | 9.26±0.18 | 9.19±0.21 | 9.28±0.11 | 2.170 |
| 3rd Tergum width (mm) | 3.35±0.16 | 3.38±0.07 | 3.42±0.09 | 0.194 |
| 4th Tergum length (mm) | 10.49±0.20 | 10.41±0.23 | 10.52±0.15 | 29.018* |
| 4th Tergum width (mm) | 3.10±0.06 | 3.17±0.06 | 3.22±0.06 | 24.110* |
| Number of overioles | 147.53±10.59 | 148.41±10.41 | 150.93±8.95 | 6.660* |
| Volume of spermatheca ($mm^3$) | 0.606±0.02 | 0.61±0.06 | 0.60±0.05 | 3.338* |

n = 1310

**c) Length and width of right fore wing of virgin queens:**

Table 2 shows the mean length and width of right fore wing of virgin queens during summer and late summer.

So, the length and width of this wing were significantly affected by rearing queens during different seasons at 0.01 level.

**d) Length and width of 3rd tergum of virgin queens:**

Table 2 shows that the mean length and width of the 3rd tergum of virgin queens during late summer and summer.

Statistical analysis indicated that length and width of this tergum were not significantly affected by rearing queens in different seasons.

**e) Length and width of 4th tergum of virgin queens:**

Table 2 shows that the mean length and width of the 4th tergum of virgin queens during late summer. The mean length and width of this tergum during summer,the length and width of this tergum were significantly affected by rearing queens in different seasons at 0.01 level .

**f) Number of ovarioles of virgin queens:**

Table 2 shows the mean number of ovarioles of virgin queens during late summer and mean number during spring.

Statistical analysis indicated that number of ovarioles of the queens was significantly affected by rearing queens in different seasons at 0.01 level.

**g) Volume of spermatheca of virgin queens:**

Table 2 shows that the mean volume of the spermatheca of virgin queens reared in different seasons.

Statistical analysis of data indicated that volume of the spermatheca was significantly affected by rearing queens in different seasons only at 0.05 level.

**A. The Queen Honeybee**

In this work with honeybees, Volsevich (1954) and Savvin (1956), found that quality of the queen could be determined by the number of ovarioles , length of the ovary, diameter of the spermatheca length of the third and fourth terga , and length and breadth of the fore wing . They also stated that the abdomen proved to be a good guide to her potential fertility. Moreover, Zhi chog-yuan and huang wan-cheng (1987) found that weight of the queen at emergence could be a useful index for selecting rish-ovipositing queens.

In the present work these criteria were taken into consideration so as to find out the most suitable way and the best conditions for rearing queens in Egypt The effects of locality and season were mainly considered.

**1.Effects of locality:**

Results of the present study demonstrated that the mean weight of queens was significantly affected by rearing them in different localities. The mean weight of virgin queens reared in Alexandria was 172.26±9.06 mg. This was higher than that recorded in Giza (168.62±15.23mg). This differed from the results of Abdellatif (1967) who stated that the average weight of virgin queens reached only 155mg in Alexandria. On the other hand, Ibrahim (1977) reported the mean weight of virgin queens reared in the same governorate to be 174.9mg.Diab (1986), using the same race and the same technique in Giza , found that the mean weight of virgin queens was 162 mg, while Bediar (1990), in the same locality, found that the mean weight was 181.7mg.

Becker (1925) found that the weight of queens aged 24-36 hours was 160-162mg. Komarov (1934) stated that the weight of queens reared from worker larvae not more than 3 days old was 178mg. Orosi-Pal (1958) mentioned that the weight of queens ranged between 200-210 mg when developed from grafted larvae. Rawash *et al* .(1983) found that the heaviest queen was 175mg when using 1-day old larvae.

Data of the present study also showed that certain morphological characteristics of queens were not significantly affected by rearing them either in Giza or Alexandria, except that the length and width of the 4th tergum were significantly affected by rearing queens in these different localities. El-Berry (1963) found that the abdomens of queens reared from one-day-old larvae were significantly longer than those of queens reared from two days old larvae. Rawash *et al.* (1983) found that the mean length and width of the wings obtained were from queens of 1-day-old grafted larvae . Bediar (1990) found that length and width of the right fore wing of the queen were 9.85 and 3.23mm., respectively .

Data of the present study also indicated that the number of ovarioles

was not significantly affected by rearing queens in Giza and Alexandria, while the volume of spermatheca was significantly affected by rearing them in both localities.

The mean volume of spermatheca (0.61±0.06mm3) was recorded when queens were reared in Alexandria.

Becker (1925) found that the diameter of spermatheca in queens aged 24 hours was 0.77mm. Wafa (1959) found that grafted worker larvae 12-24hours old gave better queens, with an average diameter of spermatheca of 1.0mm. Diab (1986) and Bediar (1990) found the volume of spermatheca of virgin queens to be 0.505 and 0.572 $mm^3$, respectively.

Resultes obtained during this study show that the localities used played an obvious role in the production of queens. Alexandria locality proved better for queen rearing. There, queens with higher weight, greatest width of the $4^{th}$ tergum, and larger spermathecal volume were obtained.

Eckert and Shaw (1960) stated that the egg-laying capacity of queen bees was closely correlated with certain environmental factors , especially temperature of the hive. Abdellatif (1967), rearing queens in Alexandria , found that the most favorable time for queen rearing was in April and May, due to the moderate weather conditions and, the availability of nectar and pollen resources. On the other hand, lower temperature during March delayed the queen rearing.

**2. Effects of season:**

The present study demonstrated that the mean weight of queens was significantly affected by rearing them in different seasons .The highest mean weight was recorded when queens were reared in late summer (August and September). Hegazy (1974) also mentioned that the mean weight of queens was significantly affected by rearing them in different seasons. He recorded the maximum weight in summer (July). Zhdanova (1967) found that the heaviest queens were reared in early spring during the main nectar flow, while the lighter queens were reared during the swarming period. Avetisyan *et al.*(1967) obtained the heaviest queens in April and the lightest queens in March, May and June. Nagi (1984) stated that seasonal variations were found in the weight of queens at emergence.

The present work also showed that certain external characteristics of the queens were significantly affected by rearing them in different seasons.

The maximum external measurements were recorded when queens were reared in late summer (August and September), expect the length and width of the right fore wing which were recorded in summer (June and July).

Concerning the influences of external conditions upon honeybees , Alpatov (1929) found that the cuticle of workers underwent distinct changes during summer . also there was a pronounced increase in the wing length towards the end of summer . McGregor (1938) studied the environmental factors which caused size variations in honeybee appendages . He noted that measurements of tongue length and wing length indicated that these appendages seemed to retain the same general size throughout the season, and that the honey flow did not affect their size . He added that minor variations which occurred simultaneously in these two organs indicated that

some unknown external factors did influence them.

Our work showed also that the mean number of ovarioles and the volume of the spermatheca were significantly affected by rearing queens in different seasons. The greatest number of ovarioles was recorded in queens reared in late summer; the maximum volume of the spermatheca was recorded in summer. Hegazy (1974) stated that the mean number of overioles as well as diameter of the spermatheca of newly emerged queens were not significantly affected by rearing them in different seasons ; the greatest number of ovarioles was recorded in queens reared during July.

From our study , it could be concluded that summer was better for raising queens with good morphometerical characteristics , including the number of ovarioles. These results also showed that the maximum volume of spermatheca was clearly obtained during summer.

This might be due to the high activity of the bee colony in this season, and consequently of the queen. This activity was correlated with certain ecological factors, among which were temperature of the hive, quantity of food fed to the queen, availability of fresh pollen and nectar sources in the area.

Hoopingarner and Farrar (1959) reported that slight environmental differences, and unknown factors, might alter the weight of queens. Orosi-Pal (1960) indicated that weight of the queens after emergence varied according to environmental influences. Abdellatif (1965) found insignificant differences between queens reared in early spring and late spring . Our results agreed with the previous results reported by Avetisyan *et al.* (1967) and Zhdanova (1967).These workers indicated that time of the year when queens were reared influenced their external and internal characteristics . On the other hand , Abdellatif (1967) mentioned that both queen weight and size were mainly controlled by its hereditary constitution . He also found that the environmental factors were not so effective unless they were too bad . This was because the bees could control the reared number of queens to be suitable for the colony conditions and to have well developed ,normal sized queens. Diab (1986) reported that the most suitable time for queen rearing was summer (May-June),which could be recommended for beekeepers in Egypt. Thus, to obtain queens of excellent quality, the beekeeper should use a queens-rearing unit. This agrees with Rosser (1934) who stated that a colony used for rearing queens must be very strong and must have an unlimited supply of pollen.

Taber (1981) showed that 500 young nurse bees were sufficient to raise a good queen.

Mangum (1999) outlined a couple of procedures that illustrate the time and care required for rearing well-developed queens.

Evans *et al* (2000) concluded that queen production is best viewed as a dance between environmental causes , such as diet, and genetic programs that respond to these causes by adding their own effects down stream.

Significant differences between queens reared by using different techniques and in different localities were probably due to weather conditions which might play an important role in the production of queens . This depended upon locality conditions and geographical situation. In most

regions, however, rearing good queens would be during the period of late summer (August and September).This time was believed to be favorable for pollen production. In most studies some external characteristics , such as size and weight of the queen, were related to the number of ovarioles or brood production, and these two characters were correlated with each other . Phenotype differences between queens could be related to environmental or hereditary factors .Thus, the important factor in the production of good queens with good qualities might be a rich supply of royal jelly provided by nurse bees. For the best results, a comb containing pollen should be provided in the cell building colony.

Another important factor for the production of good queens was temperature of the hive, which could be regulated by the great number of bees (especially in the queen-rearing unit). The acceptance of queen cells was mainly dependant upon the nurse bee activity and quality , especially those producing beewax. In order to help building queen cells, broad combs from other colonies were regularly provided to the queen-rearing colonies to ensure the quantity of nurse bees needed . Artificial diet (sugar syrup or dilute honey)was continuously supplied.

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## بعض الصفات المورفولوجية لملكات نحل العسل تبعا لاختلاف المكان و التغيرات الموسميه.

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١ - اجريت تلك الدراسة على تربية الملكات خلال موسمي التربية للاعوام ١٩٨٩، ١٩٩٠ حيث تم أختيار منطقتين مختلفتين أحداهما تقع بمدينة الجيزة و الخرى بمدينة الاسكندرية . و قد تم تحليل النتائج إحصائيا بأستخدام أختبارات T,F.

٢- أجريت القياسات المورفولوجية (وزن الملكة العذراء ، طول البطن ،طول و عرض الجناح الامامي الأيمن , طول و عرض الحلقة الظهريه "الترجه" الثالثة و الحلقه الظهريه "الترجه" الرابعة للبطن ، عدد فريعات المبيض الأيمن ، قطر الحويصلة المنوية و منها حساب حجمها ).

وقد أظهرت الدراسة النتائج التالية :

ا - وجود فروق معنوية بين متوسطات أوزان الملكات العذارى التي ربيت في مدينتي الجيزة و الأسكندرية .ب - هناك أيضا فروق معنوية في متوسطي طول و عرض الترجة الرابعة للملكات التي ربيت في المدينتين .

ج- أظهرت الدراسه وجود فروق معنوية في حجم الحويصلة المنويه للملكات التي ربيت في المدينتين .د- من خلال الجزء الثاني للدراسة التي أجريت للحصول على أنسب المواسم لتربية الملكات خلال الانسيابات المختلفه للعسل , أوضحت النتائج وجود فروق معنويه لقيم متوسطات القياسات المورفولوجيه لأوزان الملكات العذارى , طول البطن ، طول و عرض الجناح الأمامى الأيمن ، طول و عرض الترجة الرابعه ، عدد فريعات المبيض ، حجم الحويصلة المنوية خلال الربيع ، الصيف و أواخر الصيف .

ذ- كما أوضحت الدراسة عدم وجود أية فروق معنوية لقيم متوسطات طول و عرض الترجة الثالثة لتربية الملكات في المواسم المختلفة .

مما سبق يتضح أن أفضل المواسم لتربية ملكات نحل العسل في مصر هو أواخر الصيف(أغسطس و سبتمبر) يتبعة الصيف(يونيو و يوليو) ثم الربيع(مارس - مايو) .