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VARIATIONS IN THE QUALITY OF VIRGIN QUEENS OF HONEY BEE THAT PRODUCED COMMERCIALLY FROM DIFFERENT SOURCES IN EGYPT DURING TWO SEASONS.

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ABSTRACT

For investigating the quality of virgin queens of honey bee that produced commercially in Egypt, that purchased form four large commercial queen producers located at Assiut, Elgharbya and Menoufiya through two seasons (March and August). Forty virgin queens for each were purchased. The physical characters (fresh weigh, head length and width, thorax length and width, fore wing length and width, hind wing length and width, sum tergits length (3+4) , number of ovarioles of the right ovary , volume of the spermatheca and presence of nosema disease were measured. Queen quality significantly varied among commercial sources in physical characters and presence of Nosema disease. There were significant differences in the two months of queens rearing. It was indicated that the most suitable season for rearing queens in Egypt were late summer. The present study provides a valuable snapshot of the current status of virgin queens' quality that produce in Egypt as being comparatively low.

Keywords: Honey bee, *Apis mellifera*, virgin queen, queen quality, Nosem disease, ovariole, Season and spermatheca**.**

INTRODUCTION

Periodical requeening with young queens less than one year, results in more honey production than colonies headed by old queens (Kostarelou-Damianidou *et al.* 1995). Moreover, queen rearing is considered an essential step in the improvement of beekeeping and bee stocks.

There are many measures that can serve as proxies for queen quality such as wet or dry weight, thorax width, head width, and wing lengths (Fischer and Maul, 1991; Dedej *et al.,* 1998; Hatch *et al.,* 1999; Gilley *et al.,* 2003; Dodologlu *et al.,* 2004; Kahya *et al.,* 2008), several of which are significantly correlated with queen reproductive success or fecundity (Avetisyan, 1961; Woyke, 1971; Nelson and Gary, 1983). The diameter and volume of a queen's spermatheca is believed to be positively correlated with her body weight at emergence; hence, a larger queen is expected to have a greater ability to store sperm (Woyke, 1971; Corbella and Gonçalves, 1982; Kahya *et al.,* 2008; Delaney *et al.,* 2010). Another measure of a queen's quality is the degree to which it is parasitized. The more notable parasites of queens are the gut protozoan *Nosema apis* (Webster *et al.,* 2004, 2008).

In Egypt commercially queen rearing have been long practiced and over a long period from February to the late part of October as a result of the

diversity of bioclimatic. The prevailing environmental conditions and the growing of seasonal crops are affecting on queen's quality produced.

The produced bee queens throughout the different seasons are different qualities. Therefore, the objectives of this work were to investigate some quality characteristics of virgin queen bees that produced commercially from different sources in Egypt during two seasons.

MATERIALS AND METHOD

The experiments were carried out in Assiut, Insect Research Laboratory, Plant Protection Research Institute during the two seasons (March and August) of 2014.

In Egypt commercially queen rearing have been long practiced and over a long period from February to the late part of October as a result of the diversity of bioclimatic. Moustafa and Abdel- Rahman (2012) found to obtain a high acceptance; mating success and decreasing pre-oviposition period, it is necessary to introduce virgin queens at March or August months at Assiut governorate conditions and the following aspects.

Evaluation quality of virgin queens:

Virgin queen samples:

Virgin queens were purchased form large four commercial queen producers located in Egypt; Assiut (A) – Elgharbya (B,C) – Menoufiya (D) through two seasons (March and august). Six days after their expected date of emergence, forty virgin queens for each producer placed in standard wooding cages and attended by number of worker bees. The reproductive life of the queen begins after the ovarioles mature, which is approximately six days into adult life. All queens were of the common 'Carniolan' stock and generically coded.

Measurements of virgin queens:

All queens from a given commercial source were evaluated concurrently once and the following characteristics were studied.

Wet weight:

The queens were weighed individually by using electrical balance after anesthetized her by chilling at –20 C° until it was immobilized (approximately 4 min) .

External body characteristics:

The following characteristics measured by using a binocular microscope with the aid of micrometric lenses.

1- Head width (W) and head length (L). 2- Thorax width (W) and thorax length (L).

3- Right fore-wing width (W) and wing length (L).

4- Right hind wing width (W) and wing length (L).

5- The sum length of both the third and fourth tergites.

Internal body characteristics:

The number of ovarioles:

Right ovary were counting under a stereoscopic self-illuminated binocular with a high magnification power for the carefully dissected, isolated and especially treated ovaries according to Ibrahim (1977).

The diameter of spermatheca:

The diameter of spermatheca was measured under stereoscopic using a micrometer lens. Then, the volume of spermatheca (SV) was calculated according to the formula: SV = (4/3) (π) (r3) [r = average radius of the r for the length and the r for the width of the spermatheca and π = 3.14] (Hatch et al, 1999).

Presence of Nosema disease:

All virgin queen bees were examined for the presence of Nosema disease spores. The mid- and hind-gut of each queen were removed at the same time the ovary and spermatheca were removed. The gut material of each virgin queen were macerated 1.0 mL distilled water using a clean glass rod and mortar and pestle. A droplet of this suspension was placed onto a glass slide, and examined at X400 magnification under light microscope to determine the presence of Nosema disease spores.

Statistical analyses:

Analysis of variance (ANOVA) was carried out using MSTAT-C software program (MSTAT-C, Michigan State University Version 2.10) and least significant difference (LSD) values were calculated and presented as mean ± S.D. (standard deviation). Means were compared at o.o5 probabilities according to the method of Waller and Duncan (Waller and Duncan, 1969).To compare between spring and autumn seasons, the means of the studied characters of the two seasons were tested for differences using T – test ($p < 0.05$)

RESULTS

Table (1): Mean body weight (± SD) of virgin queens from different sources in Egypt during two seasons (March and august) of 2014.

Sources of virgin queens	Mean body weight (mg) of queens (n = 40 / source)			
	March	range	August	range
A	137.9 a ± 0.012	110 - 165	150.8 a± 0.027	109 - 244
B	128.6 b ± 0.02	100 - 167	134.6 d ± 0.011	111 - 162
C	128.7 b ± 0.015	105 - 161	143.2 c ± 0.017	108 – 183
D	115.2 c ± 0.015	100 -154	148.06 b ± 0.019	108 – 182
General mean	127.6 ± 0.0175		144.17± 0.0202	-9.0012*

Means followed by the same letter in the same column are not significantly different according to Duncan's multiple range test at 0.05 level of probability.

Differences in Queen Weights:

The average weights of the virgin queens from the four sources that reared at different locations in Egypt through two months (March and August) of the year are presented in (Table 1). There were significant differences among the sources and between two months rearing in terms of queen weight. On average, source A were significantly (P≤0.05) heavier in weight (137.9 mg) than other sources of queens in March while the lowest queen weights were source D. Also source A were significantly (P≤0.05) heavier in weight (150.8 mg) than other sources of queens in October while the lowest queen weights were source B. The general mean of highest queen weights of all sources were recorded in August (144.17 ± 0.0202 mg) than those reared in March (127.6 ± 0.0175 mg).

Measurements of morphometric traits:

Table (2): Mean values (± SD) of morphometric characters (mm) of virgin queens from different sources in Egypt during two seasons (March and August) of 2014.

Characters	Mean values (mm) of morphometric characters (n = 40 / source)									T-value
	March				General mean ± SD	August				General Mean ± SD	
	A	B	C	D		A	B	C	D		
Head L	3.878 a ±0.248	3.987 a ±0.330	3.947 a ±0.319	3.882 a ±0.254	3.92± 0.30	3.902 a ±0.195	3.838 a ±0.182	3.890 a ±0.175	3.892 a ±0.223	3.89 ± 0.20	1.7468
Head W	3.572 c ±0.191	3.705ab ±0.247	3.648 bc ±0.245	3.62bc ±0.178	3.64± 0.22	3.86a ±0.172	3.787 a ±0.192	3.815 a ±0.178	3.858 a ±0.195	3.83 ± 0.18	-8.26*
Thorax L	3.832 b ±0.544	4.148 a ±0.315	3.957 b ±0.430	3.882 b ±0.454	3.95± 0.46	3.543 b ±0.380	3.945 a ±0.339	3.852 a ±0.348	3.902 a ±0.255	3.81 ± 0.36	3.6247
Thorax W	3.570 a ±0.435	3.405 b ±0.274	3.355c ±0.318	3.320 c ±0.357	3.41± 0.36	3.630 a ±0.324	3.475 c ±0.203	3.475 c ±0.307	3.525b ±0.289	3.53 ± 0.33	-3.274*
Fore wing L	9.115 a ±0.183	8.998 b ±0.185	9.040 b ±0.115	9.015 b ±0.097	9.04± 0.11	9.173 a ±0.115	9.128c± 0.08	9.110 c ±0.130	9.1422b ±0.145	9.14 ± 0.12	-5.44*
Fore wing W	3.182 ab ±0.226	3.158 ab ±0.206	3.105 b ±0.185	3.023 c ±0.153	3.1± 0.21	3.193 a ±0.05	3.140 b ±0.05	3.137 b ±0.067	3.14 b ± 0.000	3.2 ± 0.06	3.907*
Hind wing L	6.193 a ±0.417	6.175 a ±0.331	6.095 a ±0.215	6.075 a ±0.181	6.13± 0.29	6.628 a ±0.160	6.110 d ±0.059	6.195 c ±0.196	6.313 b ± 0.210	6.31 ± 0.26	-6.705*
Hind wing W	2.110 a ±0.145	2.178 a ±0.254	2.140 a ±0.196	2.200 a ±0.254	2.16± 0.23	2.035 b ±0.048	2.005 c ±0.045	2.050 b ±0.082	2.105 a ± 0.032	2.05 ± 0.08	5.5716
Sum tergits L 3,4	4.733 a ±0.368	4.262 b ±0.516	3.995 c ±0.587	3.750 d ±0.434	4.18± 0.61	4.840 a ±0.269	4.265 c ±0.42	4.602 b ±0.423	4.665 ab ± 0.523	4.59 ± 0.47	-8.533*

Means followed by the same letter in the same row are not significantly different according to Duncan's multiple range test at 0.05 level of probability

Data in table 2 show that the significant differences among the sources with respect to all morphometrical measurements of the virgin queens, including Head L (F =1.197; df =39,156; P = 0. 3143), Head W (F =5.211; df = 39,156; P = 0.006),Thorax L (F=3.6025 ; df =39,156; P =0.008),Thorax W (F =22.177; df = 39,156; P = 0.000), Fore wing L (F =3.833; df = 39,156; P = 0.0054), Fore wing W(F =8.0246; df =39,156; P = 0.000), Hind wing L (F =1.1284; df = 39,156; P = 0.3452), Hind wing w (F =1.1705; df =39,156; P = 0.326), and sum. tergits L 3,4 (F =35.369;df= 39,156; P = 0.000) in March rearing. Similarly, in August rearing there were significant differences among the sources Head L (F=0.034; df =39,156; P =0.8309), Head W (F =1.387; df = 39,156; P = 0.2502),Thorax L (F =11.771; df = 39,156; P = 0.000),Thorax W (F =18.4143; df =39,156; P =0.000), Fore wing L (F =2.2853; df = 39,156; P =0.045), Fore wing W(F =21.506; df = 39,156; P =0.000), Hind wing L (F =71.435; df =39,156; P =0.000), Hind wing w (F =20.332; df = 39,156; P = 0.000), and Sum tergits L 3,4 (F =12.917; df = 39,156; P =0.000). Statistical

analysis indicated that there was no significant difference between Head L of the virgin queens reared in both two months rearing. While the mean Head W of virgin queens reared in August was a significantly higher values than March rearing.

There was no significant difference between Thorax L of the virgin queens reared in both two months rearing. While the mean Thorax W of virgin queens reared in March was a significantly higher values than August rearing.

The mean Fore wing L and W of virgin queens reared in August was a significantly higher values than March rearing. The mean Hind wing L of virgin queens reared in August was a significantly higher values than March rearing. While there was no significant difference between Hind wing W of the virgin queens reared in both two months rearing.

The mean sum L of tergits (3+4) of virgin queens reared in August was a significantly higher values than March rearing.

Number of ovarioles:

Table (3): Mean ovarioles number (± SD) of virgin queens from different sources in Egypt during two seasons (March and August) of 2014.

Sources of virgin queens	Ovariole numbers (right ovary) (n = 40 / source)			
	March	range	August	range
A	143.15 a ± 5.016	139-160	149.33 a ± 6.829	140 -165
B	141.13 b ± 4.877	140-155	141.55 c ± 2.498	135 -145
C	141.05 b ± 1.973	141-145	141.2 c ± 9.710	135 -165
D	118.2 c ± 3.784	115-125	146.2 b ± 5.374	140 -160
General mean	135.88 ± 11.29		144.57 ± 7.9	- 8.3181*

The mean values with different letters in the same column are significantly different (P < 0.05)

The average number of the ovarioles virgin queens from the four sources that reared at different locations through two months, March and August of the year are presented in (Table 3). The number of ovarioles in March rearing varied from 115 to 160, with an average of 135.88 ± 11.29 while the number of ovarioles August rearing varied from 135 to 165 with an average of 144.57 ± 7.9.

Table (4): Mean values (± SD) spermathecal volume (SV) of virgin queens from different sources in Egypt during two seasons (March and August) of 2014.

Sources of virgin queens	Spermathecal volum SV (mm) (n = 40 / source)			
	March	range	August	range
A	1.014 a ±0.183	0.8-1.37	1.206 a ±0.131	1.0-1.4
B	0.9275 bc ±0.184	0.7-1.3	1.053 b ±0.118	0.9-1.3
C	0.8975 cb ±0.159	0.8-1.3	1.1 b ±0.150	0.9-1.3
D	0.82 d ±0.130	0.7-1.2	1.196 a ±0.141	0.9-1.4
General mean	0.915±0.19		1.139±0.66	-4.8582*

The mean values with different letters in the same column are significantly different (P < 0.05).

Spermatheca volume (SV) from the four sources that reared at different locations through two months, March and August of the year are presented in table 4. The Spermatheca volume in March rearing varied from 0.7mm to 1.37mm, with an average of 0.915±0.19, while the Spermatheca volume in August rearing varied from 0.9mm to 1.4 mm with an average of 1.139±0.66 mm. additionally, significant difference was found in the spermathecal volume between the two rearing months.

Presence of Nosema disease:

Table (5): Percentages of Nosema infection in virgin queens from four sources during two seasons (March and August) of 2014.

Sources of virgin queens	Nosema infections %	
	March rearing	August rearing
A	12	5
B	12	7.5
C	15	7.5
D	15	7.5
General mean	13.5	6.875

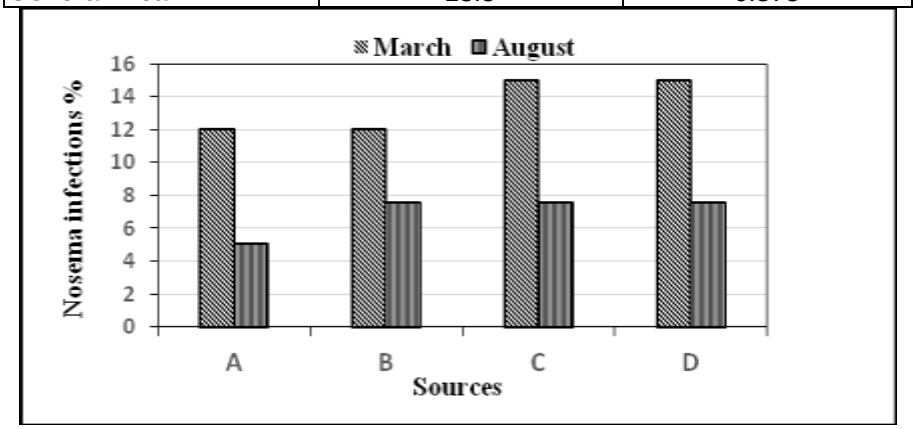

Fig. (1): Percentages of Nosema infections in virgin queens from four sources during two seasons (March and August) of 2014.

The percentages of Nosema infections in virgin queens from four sources during two seasons (March and August) are presented in table 5 and illustrated in fig.1. The highest

Percentage of Nosema infections in virgin queens was found in March rearing (13.5%) while the lowest percentage was in August rearing (6.87%).

DISCUSSION

The present study concentrated on many possible aspect of virgin queen's quality that obtained from some commercial queen producers in Egypt. The data confirm the existence of substantial variation in all characteristics virgin queens that produce in Egypt under this study.

Kaftanoglu and Peng (1980) and Nelson (1989) found that queen weight is a recommended criteria to assess queen quality as it relates to different management practices in commercial queen. There were significant differences among the sources and among two months rearing in terms of queen weight. The highest mean weights was recorded when queens were reared in late summer (August). Shawer *et al.* (1980) found that the body weight of virgin queens differed significantly depending on the rearing season. They also reported that queens produced in Egypt during May and August were heavier than those reared in other months. Delaney et al. (2010) found the mean wet weight for non-laying queens to be 0.184±0.217g; they also reported significant differences between the various sources of queen bee suppliers. 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). Mustafa *et al.* (2002) indicated that the most suitable seasons for rearing queens in Egypt were late summer, followed by summer and then spring. The reason(s) for the variations in weight between queens of different sources relates to different management practices in commercial queen producers. Woyke (1971) reported that queen bees grafted from one and two day old larvae and weighed at emergence ranged in weight between 0.156-0.201 g.

Data of the present study also showed that certain morphological characteristics of queens were not significantly affected by rearing them either in March rearing or in August rearing, except that the width of the head, thorax width, fore wing length, hind wing length and summation of length tergits 3th + 4th were highly significantly recorded when queens were reared in late summer (August). Rawash et al. (1983) found that the mean length and width of the wings obtained were from queens of 1-day-old grafted larvae.

The data confirm the existence of substantial variation in ovariole number in virgin queens. The data also revealed that, although the number of ovarioles differed slightly by source, all queens sampled had ovariole counts within the expected range. There are few historical records of ovariole number that could serve as a basis for comparison in Egypt. Mustafa et al. (2002) indicated that the greatest number of ovarioles was recorded in queens reared in late summer. Jackson J.T. *et al.* (2011) found that 7.5% of 75 commercial queens had fewer than 125 ovarioles per ovary (250 total per queen) while from our study, 20% of 160 queens had fewer than 125 ovarioles at March rearing. Van Eaton (1986), examining commercial strains of queen bees in New Zealand, reported a mean number of 148 ovarioles per ovary, with a range of 100-182 ovarioles.

The volume of the spermatheca were significantly affected by rearing queens in different two months, the maximum volume of the spermatheca was recorded in August. Hegazy (1974) stated that the mean diameter of the spermatheca of newly emerged queens were not significantly affected by rearing them in different seasons. Van Eaton (1986) reported the mean spermatheca diameter of commercial New Zealand queen bees as 1.217 mm. Woyke (1971) identified differences in spermatheca diameter between queens grafted from larvae of different ages, with one-day-old larvae producing newly emerged queens with spermatheca diameters between 1.225- 1.375 mm, and two-day-old larvae produced queens ranging between 1.150-1.300 mm.

Nosema disease, caused by the intestinal parasites, *Nosema* spp*.,* is considered to affect queen bee introduction and performance success. This effect may be direct, through infection of the queen bee from worker bees either in the colonies used for queen bee production or from the hive into which the queen is introduced; or indirect, as a result of the queen being adversely affected through the infected worker bees not being able to provide the amount of food and care required, either in the colony used for producing the queen or from the colony into which the queen was introduced. The highest percentage of Nosema infection in virgin queens was found in March rearing (13.5%) than in August rearing (6.87%). These result agreed with that obtained by Lotfi *et al*. (2009) found that the infection of the honey bee colonies was of its highest level in the spring (59.5%), however the amount was considered to be low in the fall (0%) and in the summer (3.33%).and the highest level of humidity in the spring bring about Nosema spreads. due to the lack of humidity in the summer and fall, in these seasons the incidence of Nosema was observed in very lower rates. Van Eaton (1986) found 18 % of New Zealand queens examined contained *Nosema* spp. spores, with a mean of 3.06 x 106 spores per queen. However, Delaney et al. (2010) examined queen bees for *Nosema. apis* and *Nosema. ceranae* from a range of sources within the USA, finding an absence of both species among sampled queens suggesting to them that commercial queen producers had utilized effective management practices for the prevention and spread of these parasites.

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 dependent upon the nurse bee activity and quality, especially those producing bee wax. 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.

Results of the current survey will be a potential standard for queen characteristics determinations in future queen rearing practice. Together with survey of queens' infection the entire queen quality parameters as a result of rearing practice will be considered and applied into beekeeping practice.

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الأختلافات في جودة الملكات العذارى لنحل العسل والتي تنتج تجاريا من مصادر مختلفة في مصر خلال موسمين.

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للتحقق من جودة الملكات العذارى لنحل العسل والتي تنتج تجاريا في مصر. تم شراء الملكات العذراي من أربعة منتجين كبار للملكات تقع في (أسيوط - الغربية – المنوفية) خلال موسمين مارس وأغسطس لتربية الملكات (اربعون ملكلة عذراء لكل منها). بعد ذلك تم قياس الخصائص الطبيعية التالية (الوزن الطازج، طول وعرض الجبهة ، طول وعرض الصدر ، طول وعرض الجناح الامامي ، طول وعرض الجناح الخلفي، مجموع اطوال الترجات الثالثة والرابعة، عدد انابيب البيض للمبيض الأيمن ، حجم القابلة المنوية ووجود مرض النوزيما. لوحظ اختلاف في جودة الملكات بشكل كبير بين المصادر التجارية المختلفة في الخصائص الطبيعية ووجود مرض النوزيما. وكان واضحا أن هناك اختلافات كبيرة بصورة معنوية في موسمي تربية الملكات. خلصت الدراسة الي إلى أن الموسم الأكثر مناسبة لتربية الملكات في مصر كان أواخر الصيف. وتقدم هذه الدراسة صورة واضحةعن الوضع الحالي لجودة الملكات العذراء التي تنتج في مصر بأنها منخفضة نسبيا.