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Evaluation of commercial honey bee queens quality in Egypt

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Abstract

To evaluate numbers of morphological characteristics of virgin honey bee queens, a total of 720 queens were obtained from five queen rearing stations were used. Queen samples were collected in March and August during seasons of 2015 and 2016. General characters as wet weight, thorax width and length, sum of third and fourth tergites length, abdomen length, number of ovarioles and volume of spermatheca were measured. The present result indicated that the queen weight was ranged from 134.33 to 156.34 mg in all tested samples. Insignificant difference of queen weights between the two trials in March or August was recorded. The averages of ovariole numbers were ranged from 118.93 to 130.11 in March, whereas they ranged from 125.32 and 131.26 in August. Both queen characters, weight and ovariole numbers were under the international standard for queen quality. The general mean of queen's acceptance percentage in August (83.33%) was differed significantly from the acceptance percentage in March (69.35%). The percentage of successful natural queen mating was ranged from 81.33 to 88.8 % in March and August, respectively. The present results Manifest a lack of queen quality and queen rearing practices in queen rearing stations in Egypt.

Keywords: *Apis mellifera*, virgin queen, morphological characters, successful acceptance and mating process.

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Introduction

Honey bee (*Apis mellifera* L.) is the most beneficial insect in agriculture because it produces honey and other valuable products, and fulfills an important role as a pollinator of crops. Approximately 35% of arable crops depend directly on pollinators (Klein et al., 2007). In recent years much attention has focused on the overwintering losses of the managed honey bee population in the United States and Europe (Pettis & Delaplane, 2010; Potts et al., 2010; Van Engelsdorp & Meixner, 2010) as well as in Egypt (Moustafa et al., 2014; Hassan, 2009). Because of the central role that queens play within colonies, improving the productivity and the health of honey bee colonies is often synonymous with improving the quality of queen. The physical and internal characters of a virgin honey bee queen are vital components to the performance of the colonies, which this queen is heading later on. The present investigation is aimed to evaluate the characteristics of commercial honey bee virgin queens obtained from different local rearing sources and to examine the acceptance percentage of them and the success of natural mating as a first step for a successful maintenance of honey bee colony.

Materials and methods

The experimental works were conducted in the laboratories of Agriculture College, Assiut University, Assiut, Egypt. Nuclei, equal in strength of Carniolan honey bee hybrid (*Apis mellifera carnica*) located in a private apiary at Abnoub district, were used for field studies during March and August, 2015 and 2016 seasons.

Virgin queen samples collection: A total of 720 virgin queens obtained from five stations (Table 1); forty queens from everyone at different local geographical areas. The queen rearing stations were coded by alphabetical letters. The queens were gained on six-day from emergence date as described by Tarpy et al. (2004). Every queen was placed into standard wooding cages and attended by few honey bee workers until inspection. Virgin queens from each source were weighed individually using electrical balance with 0.0001g accuracy. After queen weighting, every sample was divided into two groups: Group A: 25 queens for external and internal morphological characters measurements, Group B: 15 queens for field studies (acceptance and mating).

Table 1: The virgin queen sources and trial times of introducing.

Sources	Cod	March	August
Assiut	(A)	+	+
Elgharbya	(B)	+	+
Menoufiya	(C)	+	+
Menoufiya	(D)	+	+
New Valley	(E)	+	-

External and internal body characteristics: Abdominal length, maximal thorax width and length, and the sum length of the third and fourth tergite were measured. Every queen was carefully dissected and numbers of ovarioles of right ovary were counting under stereoscopic self-illuminated binocular according to the method of Ibrahim (1977). The diameter of spermatheca was measured under stereoscopic using a micrometer lens. Spermatheca volume (SV) was calculated according to the formula (Hatch et al., 1999):

$$SV = (4/3)\ (\pi)\ (r^3)$$

Where r = average radius of the r for the length and the r for the width of the spermatheca and π = 3.14.

Queen acceptance and natural mating: The virgin queens obtained from different source were introduced into mating nuclei, contains five frames, using a Benton mailing cage, mating nuclei should be queenless two days before introducing the virgin queens. The cage was held between the frames with the screen facing downward. Releasing of the caged virgin queens was done after 2 days from the introduction. After 24 hours from releasing queens, the mating nuclei were examined to determine the queen acceptance percentage (Rhodes et al., 2004). Accepted queens were inspected daily until they started egg laying, then, percentages of queen mating success after queen releasing were recorded (El-Sarrag & Nagi, 1989).

Statistical analysis: Data were analyzed using one-way analyses of variance by MSTAT-C (1988) software package while means were separated using the least significant differences method, when a significant "F" test was obtained. The t-test was used to evaluate the differences between the means, when only two variables were tested.

Results

Virgin queens were obtained from different sources in Egypt to assess certain of their external and internal characters in relation to the successful acceptance in new nucleus. Queen weight is often used as a good character for overall the other variables of queen quality. The average weight of the virgin queens obtained from the five queen rearing stations introduced in the experimental nuclei at two trials on March and August of both years 2015 and 2016 is presented in Table (2). The recorded weights indicated numerical and significant differences among the inspected sources on the two trials. Queens of A and E sources showed over the heaviest weight in comparing with other sources, while those of D source presented the lowest weight. The general mean of queen weight from A source on March trial was 155.14 ± 14.70 mg over other compared sources, while the lowest 134.33 ± 17.30 mg belonged to D source. On August trial, the wet weight of queen from A source gained 156.34 ± 14.11 mg over the other compared sources, while the lowest weight 145. 4 ± 16.32 mg reported for the queens of D source. The t-test supported the insignificant difference of queen's weight introduced on two trials on March and August (Table 2).

Morphometrical characteristics of virgin queens: Data represented in Table (3) show the variations in all measurements of studied morphological characteristics including the thorax length and width, sum of third and fourth tergites length and abdomen length during March season of 2015 and 2016 years. The maximum length of thorax was 4.93 ± 0.37 mm recorded for E source, while the shortest length of 4.59 ± 0.55 mm recorded for B source. The maximum width of thorax was 4.11 ± 0.38 mm recorded for B source, while the shortest length of 2.81 ± 1.42 mm recorded for E source. The length of the sum of third and fourth tergites was 3.68 ± 0.50 mm recorded for A source, while the shortest length was 3.18 ± 0.40 mm and recorded for C source. The maximum length of abdomen (8.71 ± 0.80 mm) was recorded for E source, while the shortest length (8.05 ± 0.54 mm) belongs to B source. Data show significant differences among the sources with respect to most morphometric measurements of the virgin queens at March introducing trial. It can be concluded that all the longest measurements were concerned to queens of E source, while the lowest figures described the queen of B source, during March trial. Data represented in Table (3) show also the results of August trial in which the variations in all measurements of studied characteristics including the thorax length and width, the sum of third and fourth tergits length, and the abdomen length. The maximum length of thorax was 4.98 ± 0.30 mm recorded for A source, while the shortest length was 4.64 ± 0.26 mm which recorded for B source. The maximum width of thorax was 4.15 ± 0.25 mm and recorded for A source, while the shortest length was 4.02 ± 0.26 mm and recorded for C source. The length of the sum of third and fourth tergites was 3.93 ±0.37 mm and recorded for A source, while the shortest length was 3.79 ± 0.44 mm and recorded for C source. The maximum length of abdomen was 8.95 ±0.44 mm and recorded for A source, while the shortest length (8.74 ±0.77 mm) was recorded for E source. Data show significant differences among the sources with respect to most morphometric measurements of the virgin queens in August trail.

Table 2: Body weight of virgin queens obtained from different rearing sources, introduced into two trials March and August of 2015 and 2016.

Years	Production Date	Mean of body weight (mg) ± SD					
		Virgin queen sources					Mean ±SD
		A	B	C	D	E	
2015	March	146.81 ±11.99 a	142.45 ±18.96 a	144.95 ±20.80 a	133.85 ±17.48 a	145.99 ±17.32a	142.81 ±5.27 b
	August	155.37 ±14.53 a	136.66 ±13.30 c	147.42 ±26.18 ab	140.90 ±21.72bc	-	145.09 ±7.06 ab
2016	March	163.47 ±12.33 a	140.64 ±14.74 c	135.28 ±17.37 c	134.82 ±17.35 c	155.39 ± 11.80b	145.92 ±12.86ab
	August	157.31 ±18.70 a	154.14 ±14.41 a	150.14 ±14.75 a	152.80 ±16.11 a	-	153.60 ±2.58 a
General Mean	March (2015&2016)	155.14 ±14.70 A	141.56 ±16.91 B	140.18 ±19.66 B	134.33 ±17.30C	150.62 ±15.49A	144.34 ±8.35 ns
	August (2015&2016)	156.34 ±14.11 A	145. 4 ±16.32 B	149.13 ±20.90 B	146.60 ±20.02 B	-	149.36 ±4.74 ns

Means in the same row followed by different letters are significantly different, at 5% probability, ns: not significant.

Table 3: Morphometric characteristics of virgin queens obtained from different rearing sources introduced in March and August of 2015 and 2016.

Trials	Sources	Mean values (± SD) of external characters			
		Abdomen Length	Tergits ($3^{rd}+4^{th}$) length	Thorax	
				Length	Width
March 2015& 2016	A	8.59±0.60 a	3.68±0.50 a	4.81±0.46 ab	3.91±0.40 a
	B	8.11±0.61 b	3.26±0.43 c	4.59±0.55 c	4.08±0.38 a
	C	8.05±0.54 b	3.18±0.40 c	4.72±0.29 bc	4.04±0.33 a
	D	8.29±0.60 b	3.46±0.41 b	4.79±0.30 ab	4.11±0.30 a
	E	8.71±0.80 a	3.67±0.35 a	4.93±0.37 a	2.81±1.42 b
August 2015& 2016	A	8.95±0.4 a	3.93±0.37 a	4.98±0.3 a	4.15±0.25 a
	B	8.86±1.06 a	3.84±0.44 a	4.64±0.26 c	4.03±0.24 b
	C	8.77±0.75 a	3.79±0.44 a	4.80±0.26 b	4.02±0.26 b
	D	8.74±0.77 a	3.87±0.82 a	4.83±0.33 a	4.14±0.22 a
General mean	March	8.35±0.29 B	3.45±0.22 B	4.76 ± 0.12 ns	3.79 ± 0.55 ns
General Mean	August	8.83 ± 0.08A	3.85 ± 0.05 A	4.81 ± 0.12 ns	4.08±0.06 ns

Means in the same column followed by different letters are significantly different, at 5% probability, ns: not significant.

Internal characteristics of virgin queens: The virgin queens obtained from different rearing sources were dissected to count the ovariols. The average numbers of ovarioles were presented in Table (4). During the trial of March (2015 & 2016), the highest number of ovarioles (130.11 ± 12.60) was recorded for E source, while the lowest number of (121.32±12.26) ovarioles was recorded for C source. When the trial was impacted on August (2015 & 2016) the queens of B source hold the highest numbers of ovarioles over the other compared sources with an average of 131.35 ± 13.65. The lowest average (123.41 ± 10.02) was reported for queens of C source.

Table 4: Ovarioles number of commercial virgin queens introduced in March and August 2015 and 2016 years.

Years	Production Date	Ovarioles number /ovary (Mean ± SD)					
		Virgin queen sources					General mean ±SD
		A	B	C	D	E	
2015	March	120.81 ±13.77 ab	114.00 ±14.149 b	114.90 ±11.360 b	118.47 ±9.15 ab	123.4 ±8.5a	118.32 ±3.96 c
	August	126.67 ±9.02 ab	130.10± 14.05 a	121.63 ±12.52 b	119.95 ±9.88 b	-	124.59 ±4.03 b
2016	March	130.05 ±11.85 ab	128.86 ±11.52 b	127.75 ±14.52 b	133.19 ±10.57 ab	137.1 ±12.5a	131.39 ±3.78 a
	August	131.25± 11.21 ab	132.60 ±13.41 a	125.20 ±9.12 b	131.50 ±11.76 ab	-	130.14 ±2.89 a
General mean	March (2015&2016)	125.43 ±21.55 B	121.43 ±22.06 B	121.32 ±12.26 AB	125.83 ±14.39 B	130.1 ±12.6A	124.85 ±4.35 ns
	August (2015&2016)	128.81 ±10.04 AB	131.35 ±13.65 A	123.41 ±11.96 BC	125.72 ±10.02 C	-	127.32 ±3.06 ns

Means in the row followed by different letters are significantly different at 5% probability, ns: not significant.

Volume of the spermatheca: The average volume of the spermathecal, in virgin queens from the rearing sources introduced to nucleus through the months of March and August, are presented in Table (5). The highest volume of the spermatheca volume in March trial (2015 & 2016) was (1.28±0.30 mm^3) recorded for E source while the lowest volume (1.03±0.27 mm^3) was recorded in B source. On the other hand, the August rearing supported that the highest volume of the spermatheca (2015 & 2016) was (1.37±0.28 mm^3) recorded for A source while the lowest volume (1.07±0.23 mm^3) was recorded for B source. The general mean of the spermatheca volume in March was 1.14 mm^3 less numerically but not significantly than 1.26 mm^3 of August trial. Moreover, the E source did not put in comparison during August.

Table 5: Spermatheca volumes (mm^3) of virgin queens during two trials (March and August) of 2015 and 2016.

Years	Production Date	Spermatheca volumes means (mm^3) ± SD					
		Virgin queen sources					General mean ±SD
		A	B	C	D	E	
2015	March	1.15 ±0.33 ab	0.94 ±0.17 c	1.11 ±0.18 b	1.14 ±0.29 b	1.31 ±0.35a	1.13 ±0.13 a
	August	1.43 ±0.31 a	0.98 ±0.22 b	1.29 ±0.36 a	1.32 ±0.31 a	-	1.25 ±0.16 a
2016	March	1.25 ±0.31 a	1.12 ±0.32 b	1.11 ±0.23 a	1.11 ±0.28 a	1.25 ±0.23a	1.16 ±0.07 a
	August	1.31 ±0.25 ab	1.18 ±0.21 b	1.24 ±0.21a	1.35 ±0.34 a	-	1.27 ±0.06 a
General mean	March (2015&2016)	1.20 ±0.32 AB	1.03 ±0.27 C	1.11 ±0.20 BC	1.12 ±0.28 BC	1.28 ±0.30A	1.14 ±0.09 ns
	August (2015&2016)	1.37 ±0.28 A	1.07 ±0.23 B	1.27 ±0.31 A	1.33 ±0.3 A	-	1.26 ±0.11 ns

Means in the row followed by different letters are significantly different at 5% probability, ns: not significant.

Virgin queen's acceptance: The present study was conducted to evaluate the successful acceptance of virgin queens obtained from different commercial sources, through two trials in March and August of 2015 and 2016 years. The obtained results are presented in Table (6) and elucidate that the general means of both March and August of years 2015 and 2016 reported a high respective percentages of 69.35% and 83.33% successful acceptance. A fluctuated percentage of successful acceptance was remarkable according to the source of queens and the breeding seasons within range between 46.6 to 100%. The general means of queen acceptance percentage in August (83.33%) was highly significant over the mean of March trial (69.35%).

Virgin queens mating: The successful mating percentages of virgin queens were calculated after laying eggs and insuring in the established colonies. . The obtained results (Table 7) indicated that highly successful records for most compared colonies that had successful mating percentages ranged from 57.1 to 100%. The queens obtained from A, B and E sources registered a full percentage of successful mating, while those from C and D got the lowest position. The lowest successful mating of 57.1% was registered for D queens introduced in August trial of 2015. Moreover, the t-test supported the insignificant difference between the general means of the two trials in March and August (Table 7).

Table 6: Percentages of queen's acceptance during two trials, March and August of 2015 and 2016.

Years	Production Date	Virgin queens acceptance (%)					
		Sources					General mean (%)
		A	B	C	D	E	
2015	March	86.6	66.6	93.3	53.3	60.0	72.00
	August	86.6	100	100	93.3	-	95.00
2016	March	80.0	46.6	53.3	80.0	73.3	66.70
	August	73.3	73.3	73.3	66.6	-	71.66
General mean (%)	March (2015&2016)	83.3	56.6	73.3	66.6	66.6	69.35 ns
	August (2015&2016)	80.0	86.6	86.6	80.0	-	83.33 ns

Table 7: Percentages of successful mating of virgin queens during two trials (March and August) of 2015 and 2016.

Years	Production Date	Mating success (%)					
		Virgin queen sources					General mean (%)
		A	B	C	D	E	
2015	March	76.9	90.0	71.4	87.5	77.8	79.62
	August	92.3	100.0	66.7	57.1	-	78.94
2016	March	100.0	100.0	87.5	100.0	100.0	98.00
	August	90.9	81.8	72.7	90.0	-	83.72
General mean (%)	March (2015&2016)	88.4	95.0	79.3	93.75	88.9	88.80 ns
	August (2015&2016)	91.6	90.9	69.7	73.5	-	81.33 ns

ns: not significant.

Discussion

There are many problems currently facing beekeeping in Egypt. One of these problems is the overwintering losses of the managed honey bee population. A survey of beekeeping operations in Assiut Governorate showed that beekeepers classify the “poor queens” as the second suspected cause for colony losses (Moustafa, 2013). Similar survey of certain characteristics of virgin honey bee queens had been conducted by Hamza (2015) but the current study is the most comprehensive in the quantification of physical characters of virgin queens obtained from different rearing commercial sources in relation to the successful acceptance and mating of queens. Lack of queen importance and the current queen breeding practices may reduced the genetic diversity of Egyptian honey bee population. In turn, queen breeders buy most of their queens from a relatively small number of reliable sources; import stock; and select within their own gene pool. Weight of virgin queen used by many investigators as a good proxy among other morphological parameters to evaluate their quality. In this study queen weights found to be ranged from 133.85 to 163.40 mg in

March rearing and from 136.66 to 137.31 mg in August rearing which is no comparable with the data of Woyke (1971), Nelson and Gary (1983), Van Eaton (1986) and Delaney (2011). Queen weight is recommended criteria to assess queen quality as it relates to different management practices in commercial queens (Abd Al- Fattah et al., 2011; Nelson, 1989; Kaftanoglu & Peng, 1980). The weight of queens at emergence showed a wide range of difference because of factors such as age of larvae, season, condition of rearing colonies and racial differences (Skowronek et al., 2004; Gençer et al., 2000; Weiss, 1974; Woyke, 1971; Mirza et al., 1967). Genetically different subfamilies within a group of bee larvae can also contribute in to the variability of emerged queens (Moritz et al., 2005). There were differences among the sources and between the two rearing seasons in terms of queen weight. The highest mean weight 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. (2011) 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. Data of the present study showed that certain morphological characteristics of queens were not significantly affected by rearing them either in March rearing or in August rearing. The production and quality of queens are affected by the rearing season (Abd Al-Fattah et al., 2003 ; Hassan & Mazeed, 2003; Abou El-Enain, 2000). It was found that the numbers of ovarioles in inspected queens were varied significantly according to the source, but only numerically among the introducing time. The inspected queens introduced in August trial had more ovarioles than those of March trial. Data of Moustafa et al. (2014) confirmed our results. Moreover, the volume of spermatheca was also varied in virgin queens from different rearing sources, without statistical difference, between the two introducing trials. Hegazy (1974) supported these results. The acceptance rate of queens by queenless colonies is very important for colony survival. Beekeepers buy queens, and re-queen the colonies in the spring or in the fall. If the queens are not accepted, worker bees raise new queens from existing larvae. Lots of inexperienced beekeepers end up losing their colonies in an effort to re-queen them personally. There are many reasons for rejection of queen introduction. Moretto et al. (2004) reported that there was some seasonal variation in the acceptance rates. Generally, there was greater acceptance in months with good honey flows than other months. Also, the acceptance of introduced queens was influenced by their origin. The rate of acceptance of daughter queens from 11 different mother queens varied significantly, and ranging from 33 to 75%. There appears to be a genetic influence of the mother queen on the introduced queen's acceptance rate. Gloria et al. (2007) supported these results. The presence of old or virgin queens in the colony will always cause the rejection. Good quality queens probably produce more pheromones, start laying sooner and become accepted **(**Rangel et al., 2016). Moreover, the genetics of the colonies, climatic conditions, nectar and pollen flow, and queen introduction methods are also important factors for the successful queen introduction. The obtained results indicated highly successful records of queens mating process for most compared colonies, with numerical variation among them. Tarpy et al. (2015) found that no significant differences in mean mating frequency between the feral and managed queens, suggesting that queens in the remote, low-density population of colonies in the Arnot Forest are neither mate-limited nor adapted to mate at an especially high frequency. These findings support the hypothesis that the hyper polyandry of honey bees has been shaped on an evolutionary timescale rather than on an ecological one. These studies have shown that queen reproductive capacity and mating success are highly variable across various commercial sources, because these characteristics are impacted by numerous factors that affect queen development.

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