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Original article

# Evaluation of the standards compliance of the queen bees reared in the Mediterranean region in Turkey

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A B S T R A C T

The influence of different commercial queen producers on the quality of *Apis mellifera* queens was assessed. It was aimed to determine the quality characteristics of queens reared by commercial queen producers located in the province of Antalya, which is an important region in queens production due to its climatic characteristics. For this purpose, the quality characteristics of a total of 105 queen bees obtained from 21 enterprises were determined. Differences between the enterprises in terms of the number of spermatozoa ($P < 0.01$) were determined. In terms of the diameter of spermatheca, spermatheca volume and live weight, statistical differences between the enterprises were also observed ($P < 0.05$). When the relationships between the measured characteristics were examined, significant values were obtained statistically between live weight and diameter of spermathecae (0.268) and spermatheca volume (0.258). It was also determined that there is a significant correlation between spermatheca diameter and spermatheca volume (0.995). The spermatheca diameter of a good quality queen bee should not be <1.2 mm, spermatheca volume 0.90 $mm^3$ and live weight not <200 mg. Only live weight was found to be within the normal quality standard values when the average results of the quality criteria are taken into consideration. Other characters such as spermathecae diameter, spermathecae volume and number of spermatozoa in spermathecae seem to be below quality standard values.


## 1. Introduction

Although the queens are of great importance for beekeeping activities (Dolasevic et al., 2020), there is still little importance attached to them in Turkey compared to developed countries. As the developed countries have been carrying out controlled queen rearing for approximately a hundred years, they have established a national bee breeding studies and registration system. The controlled queen bee breeding in Turkey started 25–30 years ago and the desired level of queen rearing, unfortunately, has not yet been reached. The queen rearing in spring season is also done in the Aegean and Mediterranean regions of Turkey (Adanacioğlu et al. 2019). Breeding enterprises in queen bee rearing are extremely important (Adgaba et al., 2019). As for the breeding activities, breeding enterprises have to supply queens with high-quality breeding value and meet the breeding needs of the production enterprises. Turkey has 125 queen bee production enterprises authorized by the Ministry of Agriculture (Anonymous 2021). The queens bred by these enterprises are Caucasian breed bees which grow as pure breeds in their regions. Turkey has rich bee breeds with its six local bee species. On the other hand, the breeding value of these queens produced in Turkey is not known and there are almost no studies carried out to determine the quality of the reared queens. Quality and standard queen production is carried out under controlled conditions and using modern methods. The Doolittle method is the standard method used in queen bee rearing around the world (Büchler et al., 2013). This method has several advantages over others. These advantages are, respectively, the planning of a standard, producing an average standard of queen due to the use of breeding larvae at the same age, having an easy and practical application, and all stages being under

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control. This method is used by all commercial queen breeders in Turkey (Arslan et al., 2015). In this method, the quality difference arises from the practices of the beekeepers, the season and the environmental conditions.

The quality of queens affects many genetic and environmental factors (Hatjina et al., 2014; Amiri et al., 2017; Köseoğlu et al., 2017; Okuyan & Akyol, 2018; Lee et al., 2019). Genetic properties are transferred to the colonies through a breeder queen and drone production colonies (Güler, 2008; Czekonska et al., 2015; Metz & Tarpy, 2019). The selection program on the appropriate genetic race and ecotype is genetic factors that influence the quality of queens (Büchler et al., 2013; Güler, 2017; Genç & Cengiz, 2019). Environmental factors have a great effect on queens reproductive physiology and behaviors. These environmental factors include seasons, flora, feeding, strength of the colony, queen cell cup properties, number and age of larvae grafted. Additionally, the queen rearing season and technique, age and number of the transferred larvae, characteristics of queen rearing colonies, structure of mating colonies, amount and quality of the drone are the environmental rearing factors that affect queen quality (Büchler et al., 2013; Arslan et al., 2015; Njeru et al., 2017; Güler, 2017; Prešern & Škerl, 2019). The properties of queens such as emergence weight, ovulation weight, the diameter and volume of spermathecae, the number of spermatozoa and number of egg tubes are important criteria that determine these factors (Woyke, 1971; Gençer et al., 2000; Koç & Karacaoğlu, 2011; Mahbobi et al., 2012; Payne & Rangel, 2018).

The weight of a queen bee changes throughout its lifespan. The average weight of a queen is 214.4 mg 18 days after taken from the mating nucs, but its weight reduces to 207.9 mg 8 days after queen was accepted by production colonies. During an intense nectar flow, the average weight of a queen reaches its highest level (292.9 mg) (Nelson & Gary, 1983). Many researchers have reported that there is a high correlation among queen emergence weight, diameter of spermatheca, spermatheca volume, and the amount of spermatozoa stored in the spermatheca (Gilley et al., 2003; Dodoloğlu et al., 2004; Arslan et al., 2015). Woyke (1971) argued that emergence weight can be used as a selection criterion. The queens are divided into quality classes according to their emergence weights, and according to their live weight, and three groups are formed: light (<190 mg), medium (190–200 mg) and heavy (>210 mg). The queens with a weight of 200 mg and above are considered of good quality (Kahya et al., 2008; Akyol et al., 2008).

It has been reported by many investigators that the use of young larvae in queen bee rearing affects the diameter of the spermathecae, and as the larval age decreases, the diameter of the spermathecae increases. The ability of a queen to store sperm depending on the size of the spermathecae was associated with the queen's productivity and longevity (Woyke, 1971; Rhodes & Somerville, 2003). It was reported that more spermatozoa could be stored in spermathecae with larger diameter and the queens who store more spermatozoa could lay fertilized eggs and thus live longer (De Souza et al., 2013; Brutscher et al., 2019).

## 2. Material and methods

### 2.1. Supply of queen bee material

The queen bees used in this study were obtained in May 2017 from 21 different queen bee production enterprises in Antalya, all having different production capacities in spring. Five queens from each enterprise were randomly selected from the mating nucs. A total of 105 queens were obtained.

### 2.2. Methods

The queens obtained from the producers were brought to the Department of Biotechnology at the Faculty of Agriculture of Akdeniz University. In a laboratory, the live weights of the queen bees (mg/queen), the diameters of their spermathecae (mm) and number of spermatozoa in spermathecae (million/queen) were determined (Woyke, 1979; Güler et al., 1999; Njeru et al., 2017).

The egg-laying queens were weighed using an analytical balance with 0.001 mg precision (Akyol et al. 2008). The spermatheca is isolated with the tracheal net around it and the spermathecal diameters were measured and the number of spermatozoa stored in their spermathecae was determined (Dodoloğlu et al. 2004) (Fig. 1). The spermathecae were then discharged with a fine insect needle and fine forceps in 1 mL of saline solution (0.9%). The final volume was completed by 9 mL tap water adding to 10 mL. The sample taken from this mixture was dropped between the lamella and the lamella slide, and the image from the microscope was transferred to the closed circuit television monitor. Then, the number of the spermatozoa in the square part of the Thoma slide were counted (Fig. 2), and the total amount of spermatozoa (million pieces/queen) found in the 10 mL mixture and also in the sperm sac of the queens was calculated. The calculation was done as

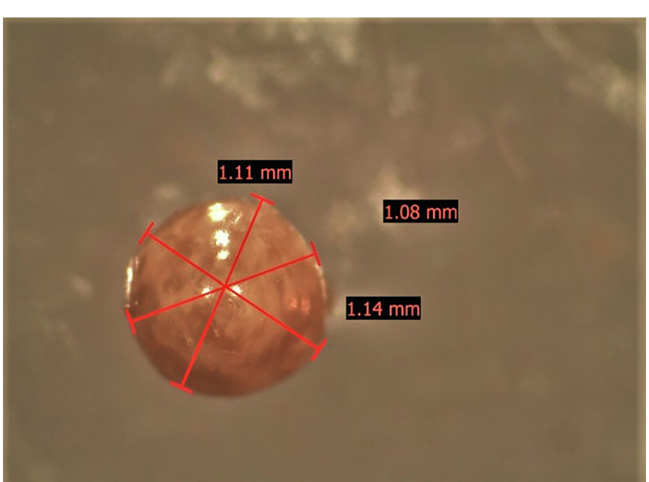

**Fig. 1.** Measuring of spermatecae under a 4.5 × 10 magnification microscop.

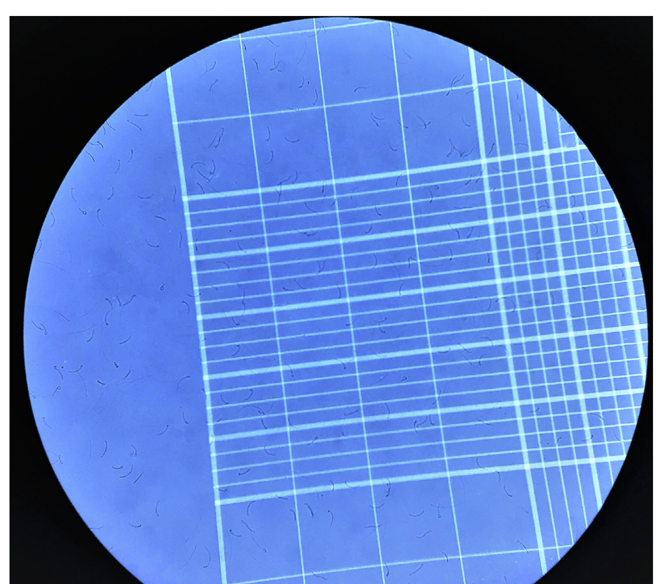
**Fig. 2.** Spermatozoa count with a Thoma slide.

following; The volume of the squared part of the thoma slide = 1 mm × 1 mm × 0.1 mm = 0.1 mm$^3$ calculated as.

$$\frac{\text{The observed number of spermatozoa}}{\text{The observed number of square}} \times 10.000$$

When this value is multiplied by 10, the number of spermatozoon in 10 mL is found, which gives the number of spermatozoon in spermateca (Woyke, 1979; Güler et al., 1999; Koç & Karacaoğlu, 2005; Arslan et al., 2015; Cengiz et al., 2019).

### 2.3. Statistical analysis

In the statistical analysis of the data, first of all, Kolmogorov-Smirnov test was used to test the suitable of data to normal distribution. After the data were determined to be suitable for normal distribution, one-way analysis of variance (ANOVA) was used to compare the means of live weight, spermateca diameter, spermatheca volume and number of spermatozoa in the enterprises, and Duncan multiple comparison test was used to compare the averages of the important characteristics.

## 3. Results and discussion

### 3.1. Live weight

The live weights of the queens reared in different enterprises were found to be between 171.20 ± 3.800 and 223.40 ± 1.657 mg. The difference between the mean weight of the queen bees was statistically significant (P < 0.01). According to the Duncan test performed to determine the difference between the averages, four different groups were formed in terms of live weight. The lowest average live weight was obtained from the 20th enterprise (171.20 mg), while the highest average live weight was found in the 2nd enterprise (223.40 mg). Other enterprises differed between these two groups. The average live weight of the queens reared in 21 enterprises was determined as 191.04 ± 2.094 mg. It was observed that there were very high quality (>220 mg) and medium quality (<190 mg) groups in terms of live weight among the measured enterprises. Although the average of the 21 queen producers was above the standards (191.04 ± 2.094 mg), it is seen that the values of 11 queen producers were close to the standard limit.

High live weight for a queens is generally desired and is considered as a quality criterion. In fact, it has been determined that the queens, who have higher live weight, store more spermatozoa, lay more eggs and generally control the colony better (Skowronek et al., 2004). The average live weight obtained in the present study was found to be higher than the average (167.8 mg) values reported in the study conducted by Güler et al. (1999) in the Mediterranean region conditions. However, these findings are still lower than the average live weight (206.23 ± 20.150 mg) obtained by Arslan et al. (2015). This result indicates that the beekeepers prepared good starter colony, made good feeding and thus learned queen rearing. As previously reported by many researchers, queen emergence weight is influenced by many factors such as season, population of starter colony, quantity and quality of nectar and pollen coming to the starter colony, number of larvae given to the starter colony, weather conditions and genetic structure. However, it can be said that the starter colony is much more important (Güler et al., 1999; Medina & Gonçalves, 2001). The average live weight determined in the present study shows that, in the Mediterranean region, quality queens can be reared in April and May. Paying more attention to the preparation and feeding of starter colonies and reducing the number of grafted larvae can positively affect the queen's emergence weight. In a study conducted in this region, it was reported that it is possible to rear a quality queen in April and May, but not in June, July and August (Güler et al., 1999).

### 3.2. Diameter of spermatecae

The mean diameter of the spermathecae of the queens reared in 21 enterprises was found to be significantly different from one another (P < 0.01). The diameter of the spermathecae ranged from 1.162 ± 0.086 to 0.944 ± 0.376 mm. The spermatheca with the largest diameter was obtained from the 6th and 4th enterprises, while the smallest diameter was determined in the 14th enterprise. The diameters of the other queen producers differed among these values.

The average spermatheca diameter was determined as 1.044 ± 0.071 mm in the studied enterprises. This value was higher than the value (0.98 ± 0.01 mm) reported by Dodoloğlu et al. (2004) for queens produced with the Doolittle method, while it was lower than the value (1.258 ± 60.2 mm) reported by Akyol et al. (2008) for the heavy group. The result of the present study was found to be similar to the value (1.061 ± 60.3 mm) reported by Akyol et al. (2008) for the medium group. According to these results, it can be said that the spermathecae diameters of the queens reared in the early period of spring in the Mediterranean Region are in accordance with the standards.

### 3.3. Spermathecae volume

The difference between the mean spermathecae volume of the queens was statistically significant (P < 0.01). When the difference between the averages is examined, the largest volume of spermathecae was determined in the 6th, 4th and 2nd enterprises, while the smallest spermathecae volume was determined in the 14th enterprise. Other enterprises differed among these enterprises. The average spermathecae volume ranged from 0.448 ± 0. 053 to 0.824 ± 0.017 mm$^3$. As it can be seen, the difference is very high and almost double.

In this study, the average spermathecae volume was 0.605 ± 0. 012 mm$^3$. While this finding was found to be lower than the values determined by Woyke (1960), Güler et al. (1999) and Güler & Alpay (2005) (1.093, 0.768 and 0.793 mm$^3$, respectively), it was similar to the value (0.667 ± 0.096 mm$^3$) reported by Arslan et al. (2015) for the Mediterranean region. It is noteworthy that the queen bees with higher live weight were found to be larger in spermathecae volumes. Therefore, it can be said that the great difference between the enterprises in terms of the spermathecae volume is due to the weight of the queens.

### 3.4. Number of spermatozoa in spermathecae

The average number of the stored spermatozoa was determined as 4.454 ± 0.177 million and the exchange rate between the enterprises was 7.455 ± 0.737 and 2.11 ± 0.463 million. It is seen that the difference between the enterprises is three-fold (Table 1). The maximum number of spermatozoa was determined in the 21st enterprise and the minimum was in the 18th enterprise. It was observed that the queens reared in different enterprises stored a different number of spermatozoa at a significant level (P < 0.01). When the standards and quality concept are taken into consideration, it can be said that the number of spermatozoa stored must be 5 million or more. However, as seen in the present study, there were only three enterprises above the standards in terms of the number of spermatozoa stored by the queens. The other 18 enterprises are below the standards and quality.

The average number of spermatozoa determined in this study (4.454 ± 0.177) was found to be lower than the average number of spermatozoa previously determined by Dodoloğlu et al. (2004)

**Table 1**
Average values for live weight (mg), the diameter of spermathecae (mm), spermathecae volume ($mm^3$) and number of spermatozoa ($\times 10^6$) of queens reared in different enterprises.

| Queen rearing enterprises | N | Live Weight (mg) | Diameter of spermathecae (mm) | Spermathecae volume ($mm^3$) | Number of spermatozoa ($\times 10^6$) |
|---|---|---|---|---|---|
| 1 | 5 | 188,60 ± 5,946 $^{bdc}$ | 1.024 ± 0.124 $^{edf}$ | 0.560 ± 0.021 $^{efd}$ | 4.280 ± 0.350 $^{edc}$ |
| 2 | 5 | 223,40 ± 1.657 $^{a}$ | 1.142 ± 0.182 $^{ba}$ | 0.784 ± 0.036 $^{a}$ | 4.833 ± 0.505 $^{bdc}$ |
| 3 | 5 | 186,00 ± 2,469 $^{bdc}$ | 1.120 ± 0.288 $^{bac}$ | 0.744 ± 0.053$^{ba}$ | 3.336 ± 0.389 $^{edc}$ |
| 4 | 5 | 189,00 ± 6,496 $^{bdc}$ | 1.158 ± 0.066 $^{a}$ | 0.816 ± 0.013$^{a}$ | 4.237 ± 0.614 $^{edc}$ |
| 5 | 5 | 175,60 ± 9,851 $^{dc}$ | 1.084 ± 0.132$^{bdc}$ | 0.670 ± 0.025$^{bc}$ | 2.599 ± 0.539 $^{ed}$ |
| 6 | 5 | 203.00 ± 5,856 $^{bac}$ | 1.162 ± 0.086$^{a}$ | 0.824 ± 0.017$^{a}$ | 2.895 ± 0.102 $^{ed}$ |
| 7 | 5 | 183.80 ± 5,678 $^{dc}$ | 1.040 ± 0.141$^{ed}$ | 0.589 ± 0.024$^{ecd}$ | 4.704 ± 0.616 $^{bdc}$ |
| 8 | 5 | 192.80 ± 10,209$^{bdc}$ | 1.072 ± 0.174 $^{edc}$ | 0.648 ± 0.032 $^{bcd}$ | 5.593 ± 0.774 $^{bac}$ |
| 9 | 5 | 179.20 ± 5,141$^{dc}$ | 1.048 ± 0.048 $^{ed}$ | 0.602 ± 0.007 $^{ecd}$ | 3.885 ± 0.609 $^{edc}$ |
| 10 | 5 | 198.60 ± 2,767$^{bdac}$ | 1.048 ± 0.174 $^{ed}$ | 0.604 ± 0.030 $^{ecd}$ | 4.097 ± 0.582 $^{edc}$ |
| 11 | 5 | 190.40 ± 4,545 $^{bdc}$ | 1.072 ± 0.174 $^{edc}$ | 0.648 ± 0.032 $^{bcd}$ | 4.677 ± 0.833 $^{edc}$ |
| 12 | 5 | 213.20 ± 15,100 $^{ba}$ | 1.008 ± 0.135 $^{ef}$ | 0.538 ± 0.022 $^{efg}$ | 4.640 ± 0.970 $^{bdc}$ |
| 13 | 5 | 198.80 ± 15,856 $^{bdac}$ | 0.964 ± 0.365 $^{gf}$ | 0.476 ± 0.054$^{fg}$ | 3.705 ± 0.574 $^{edc}$ |
| 14 | 5 | 176.60 ± 8,364 $^{dc}$ | 0.944 ± 0.376$^{g}$ | 0.448 ± 0.053$^{g}$ | 4.188 ± 0.594$^{edc}$ |
| 15 | 5 | 178.60 ± 7,187 $^{dc}$ | 0.968 ± 0.135 $^{gf}$ | 0.474 ± 0.018$^{fg}$ | 4.348 ± 0.953 $^{edc}$ |
| 16 | 5 | 181.20 ± 8,668 $^{dc}$ | 0.968 ± 0.287 $^{gf}$ | 0.479 ± 0.039$^{fg}$ | 4.883 ± 0.872 $^{bdc}$ |
| 17 | 5 | 200.20 ± 3,624 $^{bac}$ | 1.048 ± 0.135 $^{ed}$ | 0.603 ± 0.023 $^{ecd}$ | 3.895 ± 0.256 $^{edc}$ |
| 18 | 5 | 203.60 ± 7,833 $^{bac}$ | 1.016 ± 0.040 $^{ef}$ | 0.549 ± 0.006 $^{efgd}$ | 2.111 ± 0.463 e |
| 19 | 5 | 202.20 ± 3,597 $^{bac}$ | 1.016 ± 0.172 $^{ef}$ | 0.550 ± 0.028 $^{efgd}$ | 4.566 ± 0.675 $^{bdc}$ |
| 20 | 5 | 171.20 ± 3,800 $^{d}$ | 1.024 ± 0.160 $^{edf}$ | 0.563 ± 0.026 $^{efcd}$ | 6.802 ± 0.302 $^{ba}$ |
| 21 | 5 | 176.00 ± 2,529 $^{dc}$ | 1.012 ± 0.280 $^{ef}$ | 0.548 ± 0.043 $^{efgd}$ | 7.455 ± 0.737 $^{a}$ |
| **Average** | | **191.04 ± 2,094** | **1.044 ± 0.071** | **0.605 ± 0.012** | **4.454 ± 0.177** |

*a,b,c,d,e,f,g: The difference between the averages carrying different letters is important (Duncan, P < 0.01).

and Güler & Alpay (2005) (4.65 ± 0.08 and 5.61 ± 0.10 million, respectively). However, Arslan et al. (2015) conducted a study in the same region on 11 enterprises and found the number of spermatozoa to be higher than the average (2.2481 ± 0.816 million). A significant increase in the number of spermatozoa compared to three years ago can be related to the beekeepers' having made progress in the rearing of the drones in the apiary. The reason why the number of spermatozoa was below the standards in the present study is that the breeders did not seem to fully acknowledge the importance of drone rearing. For a good quality queen bee breeding, factors such as good starter colony preparation, suitable age larvae grafted, good nectar and pollen flow and very good weather conditions (temperature, wind) alone are not sufficient. The reared queens are assessed for quality standards by considering their live weights. However, it is not possible to say this in terms of the number of spermatozoa. Therefore, the main element in the quality of queen bee rearing is the drone. According to the results of this study, it is not possible to rear high quality and efficient queens without quality and healthy drone rearing. Various researchers have reported that one queen bee uses 3 million spermatozoa to lay fertilized eggs from March to October (Woyke 1962; Yu & Omholt, 1999; Güler, 2017). It was observed in the present study that the queens reared in seven of the 21 enterprises did not store enough spermatozoon to last for even one season. Indeed, one of the most important problems in Turkey is that the worker bees refuse the queen bee purchased by beekeepers. As the number of spermatozoa is inadequate, the queen will start laying eggs without fertilization after a while and the colony will have to change the queen bee. Various researchers have reported that queen rearing cannot reach the desired levels without high-quality drone rearing (Koeniger & Koeniger, 2007; Delaney et al., 2011).

### *3.5. Relationships between the evaluated reproductive criteria of queens*

In the present study, significant relationships were found between the examined properties of queens. A positive correlation (r = 0.268) was found between the live weight of the queen bee and the diameter of spermathecae. A positive correlation (r = 0.258) was also observed between the live weight of the queen bee and

**Table 2**
Relationships between the evaluated reproductive criteria of queens.

| | | Live Weight (mg) | Diameter of spermathecae (mm) | Spermathecae volume ($mm^3$) | Number of spermatozoa ($\times$106) |
|---|---|---|---|---|---|
| Live Weight (mg) | Correlation | 1 | 0,268** | 0,258** | -,014 |
| | Sig. (2-tailed) | | 0,006 | 0,008 | ,893 |
| | N | 105 | 105 | 105 | 98 |
| Diameter of spermathecae (mm) | Correlation | 0,268** | 1 | 0,995** | -,068 |
| | Sig. (2-tailed) | 0,006 | | ,000 | ,507 |
| | N | 105 | 105 | 105 | 98 |
| Spermathecae volume ($mm^3$) | Correlation | 0,258** | 0,995** | 1 | -,092 |
| | Sig. (2-tailed) | 0,008 | 0,000 | | ,370 |
| | N | 105 | 105 | 105 | 98 |
| Number of spermatozoa ($\times 10^6$) | Correlation | −0,014 | −0,068 | −0,092 | 1 |
| | Sig. (2-tailed) | 0,893 | ,507 | ,370 | |
| | N | 98 | 98 | 98 | 98 |

**. Correlation is significant at the 0.01 level (2-tailed).

the spermathecae volume. Furthermore, a positive and significant relationship (r = 0.995) was determined between the diameter of spermathecae and the spermathecae volume (table 2).

A high and positive relationship (0.995) observed between the diameter of spermathecae and the spermathecae volume was an expected result. The similarity between the live weight and the spermathecae volume may be due to the differences in the starter colony, feeding conditions, queen rearing method and especially the genetic structure.

## 4. Conclusions

The system regarding queen production has started to be effectively applied in Turkey although it is still not at the desired level. Significant progress has been made for queen bee production to become a new profession in the sector. When the existence of 7.5 million colony is considered, the conditions in Turkey should be seen as a sector where there is a need of>3.5 million queen bee per year. In Turkey, queen bee producers are far behind the production and quality to meet this demand. However, as it was also determined in this study, the sector is not able to produce material at the desired quality and standards. The most important shortcoming is the lack of importance given to drone rearing and its biology. As the queen bee production is encouraged and supported, this shortcoming can be minimized by supervision and occasional trainings.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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