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Research Article

The Effects of Season and Honeybee (*Apis mellifera* L.) Genotype on Acceptance Rates and Royal Jelly Production

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Abstract: This study was carried out in order to determine the effects of honeybee genotype and season on the acceptance rates of the larvae and royal jelly production. The cell builder colonies were prepared from the Caucasian (*A. m. caucasica*), Carniolan (*A. m. carnica*) and Mugla (*A. m. anatoliaca*) bees. One-day-old larvae were grafted into queen cell cups and they were placed in queenless and queenright cell builders. They were removed from the cell builders 72 hours after grafting and the acceptance rate and the royal jelly production were recorded.

The average acceptance rate and royal jelly production per cell throughout the season were 88.2% and 0.263 g in queenless cell builders and 72.1% and 0.214 g in queenright cell builders, respectively.

The average acceptance rate and the royal jelly production per cell were 90.0 ± 0.5% (89.09%-91.03%) and 0.372 ± 0.082 g in Carniolan bees, 83.4% ± 0.72% (82.00%-84.86%) and 0.325 ± 0.067 g in Mugla bees and 77.1% ± 0.6% (75.92%-78.32%) and 0.200 ± 0.011 g in Caucasian bees, respectively ($P < 0.01$). Royal jelly production in Carniolan bees was 14.46% and 86.0% higher than that in Mugla and Caucasian bees, respectively. Carniolan and Mugla bees were found to be more suitable for royal jelly production than the Caucasian bees under the experimental conditions.

Key Words: Royal jelly production, *Apis mellifera*, genotype, season, queen cell cup

Arı Sütü Üretimi ve Aşılama Randımanı Üzerine Bal Arısı (*Apis mellifera* L.) Genotipi ve Sezonun Etkisi

Özet: Bu çalışma, arı sütü üretimi ve larvaların tutma oranları üzerine sezonun ve bal arısı genotipinin etkilerini araştırmak amacıyla yapılmıştır. Üretim kolonileri Kafkas (*A. m. caucasica*), Karniyol (*A. m. carnica*) ve Muğla (*A. m. anatoliaca*) arı kolonilerinden hazırlanmıştır. Ana arı yüksüklerine 1 günlük larvalar transfer edilerek, ana arılı ve ana arısız üretim kolonilerine verilmiştir. Yüksükler transferden 72 saat sonra alınıp tutma oranları ve arı sütü verimleri kaydedilmiştir.

Üretim kolonilerinde tutma oranı ve arı sütü verimi, erken ilkbaharda yaza göre daha fazla olduğu, sezon boyunca ortalama tutma oranının ve yüksük başına arı sütü veriminin ana arısız kolonilerde ana arılı kolonilere göre daha yüksek olduğu ana arısız kolonilerde ortalama tutma oranının % 88,2 ve arı sütü veriminin 0,263 g olduğu, ana arılı kolonilerde ise sırasıyla % 72,1 ve 0,214 g olduğu belirlenmiştir.

Karniyol arı kolonilerinde ortalama tutma oranı % 90,0 ± % 0.5 (% 89,09-% 91,03) arı sütü verimi 0,372 ± 0,082 g, Muğla arılarında, % 83,4 ± % 0,72 (% 82,00-% 84,86) ve 0,325 ± 0,067 g, Kafkas arılarında, % 77,1 ± % 0,6 (% 75,92-% 78,32) ve 0,200 ± 0,011 g olduğu belirlenmiştir ($P < 0,01$). Karniyol ırkı kolonilerde arılarında arı sütü üretimi Muğla kolonilerine göre ise % 14,46 oranında , Kafkas kolonilerine göre % 86 oranında daha fazla olduğu saptanmıştır. Araştırma koşulları altında arı sütü üretimi için Karniyol ve Muğla arılarının, Kafkas arılarından daha uygun olduğu bulunmuştur.

Anahtar Sözcükler: Arı sütü üretimi, *Apis mellifera*, genotip, mevsim, ana arı yüksüğü

Introduction

Royal jelly is a white creamy substance secreted by the young (6-12 days old) worker bees for the nutrition of the queen bee and young larvae in the colony. It is secreted from the mandibular and the hypophryngeal glands of the nurse bees. It consists of an emulsion of proteins, sugars and lipids (1).

Royal jelly is a fundamental food for the bees; however, humans have used it for a long time. It used to be a very expensive and exotic food for the rich but due to an increase in production in China and worldwide, the price is affordable for everybody. It is believed that it stimulates the immune system and strengthens the body. It is also good for many diseases and maladies such as leukemia, cancer, high blood pressure, high cholesterol, and infertility in males and females (2,3).

The use of royal jelly and other bee products has increased as the number of research and publications increased on the production, properties and the use of bee products as a supplementary food or medical remedy. Consequently, royal jelly has become a well-known and popular bee product in many countries.

There are several factors affecting royal jelly production. The most important of them are the age of transferred larvae (4), feeding (5), number of transferred queen cell cups (6-8) harvesting interval (9), whether the colony is queenless or queenright (6) and bee race (10).

We studied the effects of honeybee genotype and season on the acceptance rates of the larvae and royal jelly production in order to disseminate knowledge, and increase domestic production and the income of poor beekeepers.

Materials and Methods

This study was conducted in 2 successive years. The effects of seasons were studied in the first year and the effects of genotypes were studied in the second year.

Experiment I.

Total of 10 cell builders were used during the 1st year's experiment. Five queenless colonies and 5 queenright colonies were used over 6 months (from April to September). One-day-old larvae were transferred to each colony and they were removed from the cell builders after three days. This process was repeated 5 times with 3-day intervals. Therefore, 1500 larvae were transferred totally, 750 of them in queenless and 750 of them in queenright colonies.

Queenless starter colonies were prepared by dequeening the colonies and rearranging the frames in the brood chamber as honey, sealed brood, open brood, one space for the transferred larvae, open brood, sealed brood, honey and feeder (11). Queenless cell builders were inspected weekly and natural queen cells were removed. Queenright starter colonies were prepared by placing a queen excluder above the chamber confining the queen and rearranging the frames as in the queenless cell builders.

Bees wax queen cell cups were made by dipping queen cell cup molds in melted bees wax (11). Grafting was done in a tent at the apiary. One drop of diluted royal jelly was placed at the bottom of the new queen cell cups (4).

Experiment II.

Total of 30 cell builders were used during the 2nd year's experiment. Ten queenless colonies from each genotype, Caucasian (*Apis m. caucasica*) provided from Camili village of Artvin province, Mugla (*A. m. anatoliaca*) provided from Marmaris town of Mugla province and Carniolan (*A. m. carnica*) provided from Austria, were used. All colonies were fed with sugar syrup and pollen substitute (4 units soybean flour, 1 unit milk powder, 2 units sucrose syrup). Thirty 1-day-old larvae were transferred to each colony and they were removed from the cell builders after three days. This process was repeated 10 times with 3-day intervals. Therefore, 9000 larvae were transferred totally, 3000 of them from each genotype.

Accepted cells were counted and the acceptance rates were determined. Then larvae in the queen cells were removed with a pair of fine forceps. Royal jelly was harvested using a special plastic royal jelly collecting spoon and placed in a separate vial and weighed on an electronic balance to determine royal jelly yield.

Statistical analysis

The data obtained from the first year experiment were analyzed by using the following model for testing the different season and queen factor:

$$Y_{ijk} = m + a_i + b_j + ab_{ij} + e_{ijk}$$

where m is overall mean, a_i is the effect of the season, b_j is the effect of queen factor, ab_{ij} is interaction effect of season and queen factor, and e_{ijk} is error term. Using the model tested the effect of genotype

$$Y_{ij} = m + a_i + e_{ij}$$

where m is overall mean, a_i is the effect of the genotype and e_{ijk} is error term.

SPSS 9.05 for Windows analyzed all data and Duncan's multiple comparison test was used to compare the means (12).

Results

Experiment I (The effects of season on the acceptance rate and royal jelly production)

The effects of season on the acceptance rate were not significant, but on royal jelly production were significant ($P < 0.01$). The acceptance rates in queenless colonies from April to September are summarized in Table 1. The average acceptance rates in queenless colonies in April, May, June, July, August and September were determined to be an average of 95.6%, 93.5%, 89.9%, 88.6%, 85.6% and 75.7%, respectively.

The average royal jelly production in April, May, June, July, August and September in queenless colonies was determined to be an average of 0.345 ± 0.006 g, 0.316 ± 0.010 g, 0.293 ± 0.014 g, 0.243 ± 0.013 g, 0.209 ± 0.004 g and 0.170 ± 0.003 g, respectively (Table 1; Figures 1, 2).

The average acceptance rates in queenright colonies in April, May, June, July, August and September were determined to be an average of 79%, 77.8%, 75.1%, 70.9%, 68.3% and 61.1%, respectively (Table 2). The average royal jelly yield in queenright colonies in April, May, June, July, August and September were determined to be an average of 0.292 ± 0.008 g, 0.248 ± 0.008 g, 0.230 ± 0.005 g, 0.194 ± 0.007 g, 0.163 ± 0.004 g, and 0.158± 0.002 g respectively (Table 2, Figures 1, 2).

Table 1. The acceptance rates (%) and royal jelly per cell production (g) in different months in queenless colonies.

Months	Number of Repetitions	Number of Grafts	Number of Accepted Larvae	Acceptance Rate (Confidence Interval) (%)	Max.-Min. (%)	Royal Jelly Yield (g) Mean ± SE	Max.-Min. (g)
April	25	750	713	95.6 (93.96, 97.17)	100-87	0.345 ± 0.006 a	0.389-0.285
May	25	750	698	93.5 (91.24 , 95.8)	100-80	0.316 ± 0.010 b	0.369-0.212
June	25	750	668	89.9 (88.04 , 91.8)	100-80	0.293 ± 0.014 c	0.389-0.259
July	25	750	660	88.6 (86.08,91.19)	100-73	0.243 ± 0.013 d	0.447-0.163
August	25	750	638	85.6 (82.52 , 88.6)	100-73	0.209 ± 0.004 e	0.228-0.165
September	25	750	563	75.7 (72.34, 79.71)	93-67	0.170 ± 0.003 f	0.198-0.143
Total/Average	25	4500	3940	88.2 (86.73, 89.58)	100-67	0.263 ± 0.006	0.447-0.143

* Different letter indicates significant mean differences ($P < 0.01$).

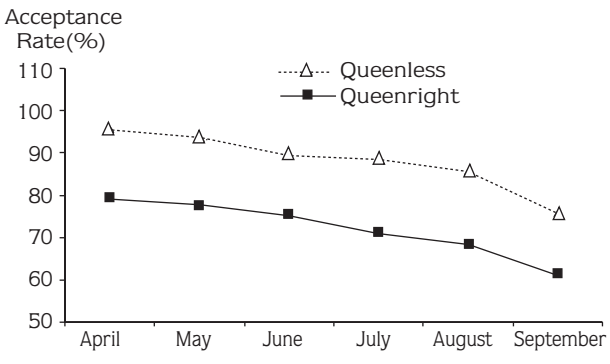

Figure 1. Royal jelly production per cell (g) in different months in queenless and queenright colonies.

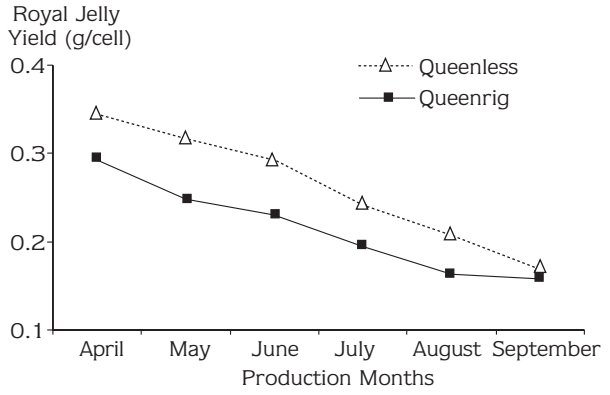

Figure 2. The acceptance rates (%) in different months in queenless and queenright colonies.

Table 2. The acceptance rates (%) and royal jelly production per cell (g) in different months in queenright colonies.

Months	Number of Repetition	Number of Grafts	Number of Accepted Larvae	Acceptance Rate (Confidence Interval) (%)	Max.-Min. (%)	Royal Jelly Yield (g) Mean ± SE	Max.-Min. (g)
April	25	750	592	79.2 (76.61, 81.7)	87-83	0.292 ± 0.008 a	0.355-0.232
May	25	750	577	77.8 (74.92, 80.76)	87-67	0.248 ± 0.008 b	0.384-0.198
June	25	750	562	75.1 (72.64, 77.6)	87-73	0.230 ± 0.005 c	0.309-0.197
July	25	750	525	70.9 (68.61, 73.22)	80-60	0.194 ± 0.007 d	0.265-0.150
August	25	750	510	68.3 (66.44, 70.28)	73-60	0.163 ± 0.004 e	0.200-0.129
September	25	750	457	61.1 (58.96, 63.28)	67-53	0.158 ± 0.002 f	0.179-0.132
Total/Average	25	4500	3223	72.1 (70.72, 73.45)	87-53	0.214 ± 0.005	0.384-0.132

Royal jelly production was highest in April and lowest in September in both queenright and queenless cell builders. However, the average production was about 22.9% higher in queenless cell builders than in queenright cell builders.

Experiment II (The effects of honeybee genotypes on the acceptance rates of the larvae and the royal jelly production)

The effects of bee genotypes on acceptance rates and royal jelly production were significantly different ($P < 0.01$). The acceptance rates and the royal jelly production in Mugla (*Apis m. anatoliaca*), Caucasian (*Apis m. caucasica*) and Carniolan (*Apis m. carnica*) genotypes are summarized in Table 3.

The average acceptance rates in Mugla, Caucasian and Carniolan bee genotypes were 83.4%, 77.1% and 90.0%, respectively. The average royal jelly production in Mugla, Caucasian, Carniolan bee genotypes was 0.325 ± 0.067 g, 0.200 ± 0.011 g, and 0.372 ± 0.082 g, respectively.

Discussion

The average acceptance rates throughout the season were 88.2% and 72.1% in queenless and queenright cell builders, respectively. Şahinler and Kaftanoğlu (4), Kaftanoğlu and Kumova (13) and Öztürk (14) reported that the average acceptance rates for queenless cell builders were 87.1%, 75.9% and 77.59%, respectively.

Royal jelly production in April was 9.2% greater than that in May, 17.7% greater than that in June, 41.9% greater than that in July, 65.1% greater than that in August and 103% greater than that in September. These results showed that royal jelly production decreased significantly over time (Tables 1, 2, Figure 2). This was due to the shortage of fresh pollen during summer and weakness of the queenless cell builders due to ageing.

The average royal jelly production was found to be an average of 0.263 and 0.214 g in queenless and queenright cell builders, respectively. Şahinler and Kaftanoğlu (4), and Öztürk (14) reported that the

Table 3. The acceptance rates (%) and royal jelly yield per cell (g) in different bee genotypes.

Months	Number of Repetition	Number of Grafts	Number of Accepted Larvae	Acceptance Rate (Confidence Interval) (%)	Max.-Min. (%)	Royal Jelly Yield (g) Mean ± SE	Max.-Min. (g)
Muğla	100	3000	2490	83.4(82.00,84.86) a	100-70	0.325 ± 0.067 b	0.475-0.170
Caucasian	100	3000	2310	77.1(75.92,78.32) b	90-70	0.200 ± 0.011 c	0.250-0.170
Carniolan	100	3000	2700	90.0(89.09,91.03) c	100- 70	0.372 ± 0.082 a	0.493-0.197
Total/Average	300	9000	7500	83.5(82.61,84.45)	100- 70	0.299 ± 0.006	0.493-0.170

*Different letter indicates significant mean differences ($P < 0.01$).

average royal jelly yield for queenless cell builders was 0.180 g and 0.303 g, respectively.

The acceptance rates in the Carniolan bee genotype were 7.9% and 16.7% more than in the Muğla and Caucasian genotypes, respectively. Similarly, royal jelly yield in the Carniolan bee genotype was 14.46% and 86% more than that in the Mugla and Caucasian genotypes, respectively. These results show clear differences among the bee races using in this study in terms of the acceptance rates and royal jelly production (Table 3).

In conclusion, the acceptance rate and royal jelly production were highest in April and lowest in September. The royal jelly production was highest in the Carniolan genotype, and lowest in the Caucasian genotype. Royal jelly producers should use faster developing colonies in order to increase the acceptance rates and royal jelly production. Even though Caucasian bees are excellent honey producers and they are the gentlest bees in Turkey, and the acceptance rate and the royal jelly production are lower in the slow developing Caucasian bees than in the faster developing Carniolan and Mugla bees in the Mediterranean climate.

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