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Assessment of the impact of several factors on the acceptance rate of larvae and the royal jelly production of the *Apis Mellifera Carnica* breed

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Abstract

The aim of this study was to evaluate the effects of season, feeding, and the location of the apiary on the acceptance rate of larvae and milk production in bee colonies. The study was conducted in two seasons (late spring and mid-summer) in two different regions of Albania, using ten colonies (five as suppliers and the other five as nurse colonies) of the Apis Mellifera Carnica breed. In each season, two consecutive plantings were monitored. The study was conducted at the end of spring in the Elbasan region and in mid-summer in the region of Pogradec. In each nurse colony, a grafting frame with 60 cups was placed, and indicators such as larval acceptance and royal jelly production were recorded following standard procedures. The results showed that in the second planting, due to the utilization of foraging areas in Elbasan, there was a significant increase in the amount of royal jelly harvested per cup compared to the first planting (33.3%, $p<0.05$). From the first season to the second, a significant decline in harvested royal jelly per frame and per cup was recorded (by 15.84% and 15%, respectively). The best results for royal jelly production per frame and per cup were achieved at the end of spring in Elbasan.

Keywords: apiary, larvae grafting, larval acceptance rate, royal jelly production, season.

INTRODUCTION

Royal jelly is a nutrient-rich and therapeutic substance produced by 6-12 day-old worker bees of Apis mellifera L. (Deseyn and Billen 2005). Royal jelly is an important element for larval development (Fujita et al. 2013, Wright et al. 2018) which also contributes to the health of the colony. It is produced as a result of larval feeding (Zeng 2013, Chuan Ma et al. 2022) and is influenced by diet and many other factors. The quantity and quality of the produced and harvested royal jelly are affected by a series of

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biotic and abiotic factors (Sahinler N. et al. 2005). From many studies it has been evidenced that the production of royal jelly, is influenced by several factors such as genetics (Cao L F, et al. 2016, Li J. et al. 2000, Saleh 1999), larval release age (Sahinler N et al. 1997), feeding methods, harvesting intervals (EL-Din HAS 2010), and environmental circumstances (Li. J. K. et al. 2010, EL-Din 2010, Hussain ARE et al. 2020).

Previous data suggest that nutrition (El-Shemy 1997) and colony management can significantly increase milk production and larval acceptance. Studies have indicated that the quantity and quality of feed, as well as environmental circumstances, play an important role in the success rate of larval development and consequently, in royal jelly production. According to several recent studies, temperature and humidity also play a critical role in bee productivity, affecting their behavior and capacity for royal jelly production. Additionally, bee migration has shown its impact on larval acceptance and the quantity of the harvested royal jelly (Gonzalez et al. 2021).

This study aims to assess the effect of season, feeding methods, and the location of the apiary on the rate of larval acceptance and royal jelly production. By conducting this study in two regions and during two different seasons, we aim to provide a significant contribution to better understanding some of the factors that influence royal jelly production and ways to optimize beekeeping and management practices.

MATERIAL AND METHODS

To assess the effect of various factors on the rate of larval acceptance and the quantity of the harvested royal jelly, an experiment was conducted in 2023 during two seasons (at the end of spring and in mid-summer) and in two different regions of Albania. For this purpose, in each season, a group of 10 bee colonies of the Apis Mellifera Carnica breed was created (5 bee colonies were used as suppliers and the other 5 as nurse colonies). In both planting sessions conducted for each season, one grafting frame with two drawers containing 60 cups was placed in each of the five nurse colonies. All colonies were selected to have the same strength, with equal feed supplies, similar cell patterns with sealed generations, and two-years-old queens.

In the first season, conducted in the park on the outskirts of Elbasan, 500 meters above sea level, two consecutive plantings were carried out on May 26 and 30. In the second phase, monitoring was conducted in the apiary near Pogradec, 1250 meters above sea level. The same work procedure was followed, and two consecutive plantings were carried out on July 1 and 4.

Queen bee excluder was used to prevent the queen from entering the area containing the queen bee cells. Worker larvae (less than 24 hours old) were transferred to the grafting frame (with 60 previously polished cups). The latter was placed in the nurse colony between two frames of bees. At the bottom of each cup, a droplet of prime food for the larvae was placed (a mixture of royal jelly: honey: distilled water in equal ratios of 1:1:1). Three days later, the frame was checked for the rate of larval acceptance and for royal jelly production according to standard procedures. The royal jelly was harvested out of the cells with a micro spatula into a plastic container and weighed using an electronic scale. This procedure was repeated twice at three-days intervals for each season.

In the first season, during the first planting, as rainfall began and the bees could not utilize the floral sources, they were fed sugar syrup (1 liter/72 hours). In the second planting, under improved weather conditions, the bees only utilized the natural floral sources.

To be highlighted is the fact, that during the second season (in both plantings), there were rainfalls and temperatures of 25°C in the first planting and 22°C in the second planting were registered. However, since the colonies were strong and had sufficient food supplies, no supplemental feeding was applied.

During the period of royal jelly harvesting, the following indicators were recorded:

- The number of cups with planted larvae into the grafting frames;
- The number of accepted larvae by the bees (fed) in each grafting frame per each bee colony;
- The quantity of produced royal jelly by each colony per each planting.

The obtained and recorded results from this monitoring were subjected to statistical processing through ANOVA method and descriptive analysis, and the t-Test was applied for comparisons.

RESULTS AND DISCUSSION

First season

The results for both plantings in the apiary of Elbasan are presented in the table below:

Table 1. Acceptance rate of larvae and quantity of harvested royal jelly in the apiary of Elbasan

No. of planting	Colonies' feeding during the harvesting time	Number of planted larvae	Number of the accepted larvae	% of the accepted larvae	Quantity of the harvested royal jelly (g/frame)	Royal jelly production per cell (g)
1	Sugar Syrop	60	55±5.43	91.66±9.07	19.8±1.48	0.36±0.02
2	Natural floral sources	60	49.6±6.54	82.68±10.92	23.8±2.77	0.48±0.02
Average				**87.17±6.35**	**21.8±2.83**	**0.42±0.08**

Although in the second planting the acceptance rate of larvae was 8.98% lower than in the first, the differences were not statistically significant. The use of floral sources in the second planting allowed the larvae to be intensively fed with royal jelly, which resulted in an increasing trend in the harvested royal jelly per frame (4 ml, or 20.2% more) and a noticeable increase in the harvested royal jelly per cell (0.12 g), or 33.3% (with significant differences for $p<0.05$).

Weiss K. 1983, reported that stimulative feeding does not affect the queen acceptance rate and royal jelly (RJ) production. In contrast, the availability of floral sources, particularly pollen and nectar, fluctuates throughout the year, affecting the stimulation of bee glands (Xun L. et al. 2020, Dimitrios Kanelis et al. 2024, Reda Omar, 2006).

Second season:

Table 2 presents the results from two consecutive harvests in the apiary of Pogradec.

Table 2. Acceptance rate of larvae and quantity of harvested royal jelly in the apiary of Pogradec

No. of planting	Colonies' feeding during the harvesting time	Number of planted larvae	Number of the accepted larvae	% of the accepted larvae	Quantity of the harvested royal jelly (g/frame)	Royal jelly production per cell (g)
1	Natural floral sources	60	51.4±3.36	85.64±5.60	20.2±1.48	0.40±0.05
2	Natural floral sources	60	50.6±8.20	84.34±13.67	17.0±3.08	0.34±0.01
Average				**84.99±0.92**	**18.6±2.26**	**0.37±0.04**

As it can also be seen in the table, there is a small decrease of 1.3% in the percentage of accepted larvae from one planting to the next. However, this can be considered merely a trend, as the differences are not statistically validated. This cannot be said for the other two indicators. A significant decline in the production of royal jelly per frame and per cell is observed in the second planting, with statistically validated differences for $p<0.05$. In the second planting, there was a decrease in the value of the harvested royal jelly per frame of 3.2 ml, or 15.84%, while for the royal jelly harvested per cell, there was a decrease of 0.06 ml, or 15%. Although temperatures were optimal (22-25°C) during this period and food supplies in the hive were sufficient, rainfall affected the adequate use of floral sources.

This is also confirmed by other studies: Manino (1982) studied the effect of temperature and rainfall on the growth of queens and concluded that the grafting and development of queen cells were slightly influenced by climatic conditions.

Table 3. Comparison of the acceptance rate of larvae and the produced royal jelly depending on the season and location of the apiary

	% Acceptance of larvae	Harvested royal jelly per frame (g)	Produced royal jelly per cell (g)
Season 1	87.17±6.35	21.8±2.83	0.42±0.08
Season 2	84.99±0.92	18.6±2.26	0.37±0.04

From Table 3, although a decrease for all three parameters during the summer season is observed, the differences between the two seasons were not statistically validated for the acceptance rate of larvae (for $p<0.05$). However, the changes are significant for the amount of the harvested royal jelly per frame and the amount of harvested royal jelly per cell. Additionally, there is a strong correlational relationship between the harvested royal jelly per frame and the harvested royal jelly per cell in both seasons (0.64 and 0.70, respectively).

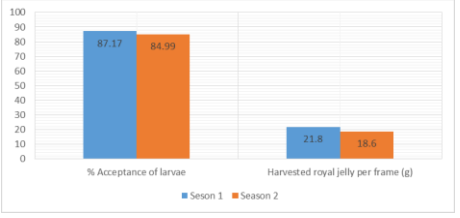

Figure 1. Acceptance rate of larvae and harvested royal jelly per frame depending on the season and location of the apiary

The results of this study regarding the impact of food sources and season on the acceptance rate of larvae and the amount of harvested royal jelly are confirmed by various literature sources (Fratini F et al. 2016, Xun L et al. 2020, Shakeel M et al. 2020, Helaly K 2018, Şahinler N et al. 2005, Hussain A.R.E. 2020).

CONCLUSION

At the beginning of summer, the intensive use of the natural floral resources from the second planting led to the larvae being fed intensively with royal jelly, resulting in an upward trend in the harvested royal jelly per frame (20.2% more) and a noticeable increase in the harvested royal jelly per cell (33.3%), with significant differences for $p<0.05$. During the second phase, from one planting to another, a slight decline in the percentage of accepted larvae was observed (by 1.3%), while the differences in royal jelly production per frame and per cell were significant (for $p<0.05$). The largest amounts of harvested royal jelly per frame and per cell were obtained at the beginning of summer (for $p>0.05$).

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