Grana

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/sgra20

A comparative melissopalynological study of royal jelly from Turkey

Gulsah Yil, Semiramis Karlidag, Abuzer Akyol, Abdurrahman Koseman, Ayşe Burçin Uyumlu, Murat Yilmaztekin, Selim Erdogan & Ibrahim Seker

To cite this article: Gulsah Yil, Semiramis Karlidag, Abuzer Akyol, Abdurrahman Koseman, Ayşe Burçin Uyumlu, Murat Yilmaztekin, Selim Erdogan & Ibrahim Seker (2022): A comparative melissopalynological study of royal jelly from Turkey, Grana, DOI: 10.1080/00173134.2022.2049862

To link to this article: https://doi.org/10.1080/00173134.2022.2049862

Published online: 29 Apr 2022.

Grana, 2022
https://doi.org/10.1080/00173134.2022.2049862

A comparative melissopalynological study of royal jelly from Turkey

GULSAH YIL[1], SEMIRAMIS KARLIDAG[2], ABUZER AKYOL[2], ABDURRAHMAN KOSEMAN[2], AYŞE BURÇIN UYUMLU[3], MURAT YILMAZTEKIN[4], SELIM ERDOGAN[3] & IBRAHIM SEKER[5]

[1]*Battalgazi Vocational School, Plants and Animal Production Department, Malatya Turgut Ozal University, Malatya, Turkey,* [2]*Akcadag Vocational School, Plants and Animal Production Department, Malatya Turgut Ozal University, Malatya, Turkey,* [3]*Faculty of Pharmacy, Basic Pharmaceutical Sciences Department, Inonu University, Malatya, Turkey,* [4]*Faculty of Engineering, Food Engineering Department, Inonu University, Malatya, Turkey,* [5]*Faculty of Veterinary Medicine, Zootechny Department, Firat University, Elazig, Turkey*

Abstract
Three treatments (Glucose, Sucrose, Bee Feed Syrup) were used to examine the influence of supplementary feeding on the pollen spectrum of the royal jelly produced by *Apis mellifera* L. colonies. *Apis mellifera* colonies were located in the Battalgazi and Dogansehir districts of Malatya province in Turkey. In total 255 royal jelly samples were investigated and the analysis recorded over 30 taxa. In both sites the control group heavily utilised *Quercus*, *Verbascum*, *Astragalus*, and Lamiaceae. The feeding treatments saw *Quercus*, *Verbascum*, and *Astragalus* still being used but in different proportions. The utilisation of Lamiaceae was much reduced and *Echium* became an important component in colonies that had supplementary feeding.

Keywords: *Malatya, pollen, royal jelly, Turkey*

Honey bees require proteins, carbohydrates, lipids, vitamins, minerals in their diet for optimum nutrition and adult bees can use glucose, fructose and sucrose as carbohydrates as energy source (Standifer 1980; Brodschneider & Crailsheim 2010). Pollen and nectar are the main food source of adult workers and queens (Haydak 1970). However, pollen and nectar are not present enough in the first days of spring so these nutrients cannot be supplied, therefore supplementary foods are required for the survival and fostering of a colony (Standifer et al. 1977). When a colony has poor nutrition they are more susceptible to pathogens, viruses and parasites (Naug 2009). Consequently, beekeepers provide the hives with support nutrients and food to maintain the health of the colony.

Honey, wax, propolis, venom, pollen pellets and royal jelly (RJ) are qualified as bee products. *Apis mellifera* is unique in being able to turn pollen to RJ and has the highest concentration of sugar in RJ within bee taxa (Wright et al. 2018). RJ is a product that is a result of the honey and pollen digestion process and secretion from hypopharyngeal and mandibular glands of nurse bees to nourish the hive individuals, including all larvae and adult queens (Haydak 1970; Witherell 1978; Deseyn & Billen 2005). However, the honey bee larvae, either queen or worker, are fed with this mandibular and glandular secretion; the queen larvae fosters RJ throughout the larval feeding period while the worker larvae within a certain time of larval feeding period (Hartfelder et al. 2015). Furthermore, the worker larvae receive these secretions in different proportions

Correspondence: Gulsah Yil, Battalgazi Vocational School, Plants and Animal Production Department, Malatya Turgut Ozal University, Malatya, Turkey. E-mail: *gulsah.saatcioglu@ozal.edu.tr*

(Received 9 June 2021; accepted 7 November 2021)

to the queen larvae (Jung-Hoffmann 1966; Haydak 1970). RJ contains 60–70% water, 30% crude proteins, 20–30% reducible sugars; worker jelly consists of 75% water and 12% sugars only (Shuel & Dixon 1968; Dietz & Haydak 1971; Asencot & Lensky 1976, 1988; Takenaka & Takahashi 1980). The studies show that the higher sugar content of RJ works like a phagostimulant for queen larvae (Asencot & Lensky 1984, 1988). Besides the queen larvae are fed these secretions up to 1500 times while the worker larvae approximately 150 times (Lindauer 1952). This diet determines critically the caste system of RJ among female bees in the hive (Hartfelder et al. 2015).

Because of the high nutritional quality RJ is classed as a superfood (Bilikova et al. 2015; Wang et al. 2016; Strant et al. 2019; Kafantaris et al. 2021). RJ mainly consists of water, protein, lipids and carbohydrates (Barth 2005). RJ contains 7.5–15% sugars and fructose and glucose reach 90% ratio of total sugar content; RJ sugar composition is diverse among samples depending on seasons, bee species and races, production method and geographical and botanical origin (Wytrychowski et al. 2013; Xue et al. 2017; Kunugi & Ali 2019).

Pollen grains with larval exuviae and wax fragments constitute approximately about 5% of the weight of RJ (Krell 1996). The pollen in RJ comes from pollen collected by bees or indirectly from honey stomach (Simpson 1955; Renner et al. 2003). Pollen is vital for growth and survival, immunity and reproduction of brood and the colony, as it contains a wealthy source of proteins, amino acids, carbohydrates, vitamins, lipids and minerals (Dietz 1978; Brunner et al. 2014; Sajwani et al. 2014; Vaudo et al. 2015; Conroy et al. 2016). Pollen grains determination in bee products is an effective method to identify geographical origin (Luz et al. 2018). Melissopalynological studies are useful for determining the RJ origin and the preferences of the bee colony (Barth 2005) but also for determining the botanical origin of RJ is important for marketing. The high nutrient value of RJ makes this product popular in alimentation of community and the popularity provides commercial profit to beekeepers.

In this study, we aim to determine whether honey bees would use different plant sources for making RJ when fed with different sugar groups.

Material and methods

Study area

Battalgazi and Dogansehir are districts of Malatya city in East Anatolian region of Turkey. Battalgazi is part of eastern (38° 42' 47" N, 38° 36' 59" E) and Dogansehir is part of southern (38° 09' 48" N, 37° 87' 91" E) Malatya (Figure 1). The city is located southeast of Anatolian Diagonal and is floristically rich with 21.1% of the flora endemic (Karakuş 2016). Karakuş (2016) observed the vegetation of the city covers three phytogeographic regional elements; Irano-Turanian (42.81%), Mediterranean (7.82%), Euro-Sibirian (3.84%) with the Asteraceae, Fabaceae, Brassicaceae, Lamiaceae, Poaceae being the most abundant taxa in native flora.

The southern branch of south-eastern Toros Mountains forms the Malatya Mountains while the Dogansehir Plain is the largest lowland area of the city (Yakar et al. 2014). Battalgazi is bordered by Karakaya Dam and the altitude decreases from approximately 900 m to less than 700 m in this district (Arslan & Hayli 2007).

Feeding methods

Different feeding methods were used for determining the effect on pollen composition in RJ. The Caucasian race, *Apis mellifera caucasica* L. colonies were supplemented with different sugars. The hives were set up initiatively and queenless. Moulds made of

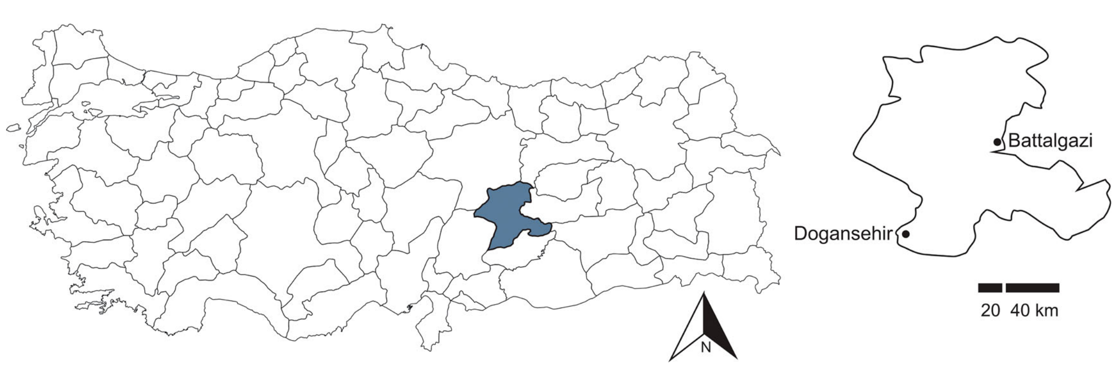

Figure 1. Map of Malatya city (marked are the two study areas).

hard wood with the length of 10 cm and a diameter of 9 mm were used to make the cells. Of these moulds, 15 were mounted on a carrier lath at 2.5 cm intervals, and the 10 mm edges of the frames connected to the mandril prepared were dipped into melted bee wax 3–4 times. The cells obtained were fixed to the carrier laths (15 cells/bar). Each treatment colony was given a cell grafted with 45 larvae (three bars).

Colonies were fed with a plastic bee hive feeder (25 cm × 48 cm × 3 cm) placed in hives. The supplementary sugar types were regularly topped up as the bees depleted them. Three feeding types were tested: Sucrose, Glucose and Bee Feed. For the Sucrose group (S) the syrup was prepared with commercial 'crystallized granulated sugar', by the ratio 1:1 water. For the Glucose group (G) commercial 'glucose syrup' (Brix 82,DE 37, Dextrose 14, Maltose 12) was diluted with water by the ratio 1:1 and for the Bee Feed group (BF) 'Pasteurized Bee Feed Syrup' (sucrose 30–36%, glucose 27–30%, fructose 37–40%, dry matter 72 ± 2%) was given to the colonies without any processing. The Control group (C) were not given any supplementary feeding and were left for the colony to forage in the local vegetation. Every feeding group, including the control group, had five beehives arranged side by side at the same location. The control groups access to feeding hives can be considered negligible since sugar additions were made frequently as sugar was depleted and in this short time the bees never had the opportunity to access feeding groups.

Palynological method

Battalgazi and Dogansehir districts were visited each seven times between 22 June 2018 and 6 July 2018 to collect the RJ samples produced by the different feeding methods. The study period had to be interrupted due to a lack of RJ production because of dry weather flow. The samples (0.5 g) were prepared following the Ricciardelli d'Albore and Battaglini Bernardini (1978) method for palynological analysis. Every feeding and control group were represented by five hives in both locations and the pollen samples were prepared as two investigation material. The samples of each feeding and control group of the five hives were investigated separately within each group and averaged on calculating. Seventy samples for each feeding treatment, G, S, BF and C, were analysed. At the end of the study 255 samples (some days RJ samples could not be collected) were examined palynologically. A Nikon Eclipse E100 microscope, 40× lens was used and pollen grains were counted from the whole area over 22 × 22 cover glass.

The pollen grains were compared with descriptions from Erdtman (1952, 1969), Wodehouse (1965), Aytuğ (1967), Charpin et al. (1974), Faegri and Iversen (1975). In addition, during the study the two areas were visited for collecting the native flora and then from these specimens we made reference pollen samples following the method in Wodehouse (1965).

Results

The RJ samples, collected from Battalgazi and Dogansehir, were examined palynologically, across the different feeding treatments. Results were evaluated as a percentage (Table I).

The results determined the taxa and over 5% were accepted as dominant taxa (Saavedra-Carhuatocto et al. 2014). For Battalgazi district; *Quercus* (32.93%), *Verbascum* (12.39%), *Astragalus* (7.40%), Lamiaceae (7.40%) are the dominant pollen types in the control group (C) (Figure 2); *Echium* (25.57%), *Quercus* (21.40%), *Verbascum* (14.49%), *Astragalus* (12.50%), Asteraceae (5.16%) are the dominant pollen types in the glucose group (G) (Figure 2); *Verbascum* (45.75%), *Astragalus* (14.91%), *Echium* (14.53%), *Quercus* (5.87%) are the dominant pollen types in the sucrose group (S) (Figure 2); *Astragalus* (28.33%), *Quercus* (23.11%), *Echium* (10.43%), *Verbascum* (9.62%), Poaceae (6.29%) are the dominant pollen types in the bee feed group (BF) (Figure 2).

For Dogansehir district; *Quercus* (20.36%), *Astragalus* (19.46%), *Verbascum* (8.29%), *Lamiaceae* (6.85%), *Salix* (6.13%), *Dianthus* (5.23%), *Poaceae* (5.23%) are the dominant pollen types in the control group (C) (Figure 3); *Echium* (23.31%), *Quercus* (19.51%), *Astragalus* (17.62%), *Verbascum* (8.20%) are the dominant pollen types in the glucose group (G) (Figure 3); *Verbascum* (43.37%), *Astragalus* (29.31%) are the dominant pollen types in the sucrose group (S) (Figure 3); *Astragalus* (30.80%), *Quercus* (18.63%), *Verbascum* (15.86%), *Echium* (13.37%) are the dominant pollen types in the bee feed group (BF) (Figure 3).

In total, 33 taxa belonging to 23 families were identified in Dogansehir and 31 taxa belonging to 22 families were identified in Battalgazi RJ samples. Anacardiacae and *Salix* were not observed in Battalgazi RJ samples.

A pollen frequency classification following Louveaux et al. (1978) was used in determining the botanical origin of the RJ (Table II). If the percentage of

Table I. The pollen spectra of royal jelly comparison according to the feeding methods for the two localities (%).

	Control		Glucose		Sucrose		Bee feed	
	Battalgazi	Dogansehir	Battalgazi	Dogansehir	Battalgazi	Dogansehir	Battalgazi	Dogansehir
Anacardiaceae	0.00	1.26	0.00	1.67	0.00	0.71	0.00	0.28
Anchusa	1.81	0.54	0.52	0.15	1.08	0.29	0.63	0.21
Apiaceae	0.91	0.18	1.75	1.37	0.18	0.86	0.18	0.36
Asteraceae	3.32	3.24	5.16	4.25	0.00	1.52	0.54	1.07
Astragalus	7.40	19.46	12.50	17.62	14.91	29.31	28.33	30.80
Boraginceae	1.36	1.80	0.33	1.29	0.09	0.14	0.00	0.36
Brassicaceae	1.51	1.98	0.52	0.15	1.98	0.76	0.99	0.78
Dianthus	1.81	5.23	0.66	1.82	0.20	0.48	0.45	0.50
Carduus	0.91	0.36	0.66	0.08	0.18	0.19	0.72	0.14
Cichorideae	0.76	0.54	0.62	1.06	0.50	0.76	1.08	0.36
Cistaceae	1.21	1.98	0.43	0.30	0.61	0.19	1.53	0.21
Convolvulus	0.15	1.08	0.95	0.53	0.09	0.19	0.36	0.28
Cup/Tax	2.42	1.62	0.85	0.61	0.79	0.29	1.26	0.57
Cyperaceae	0.15	0.72	0.14	0.15	0.00	0.10	0.09	0.14
Echium	0.91	0.90	25.57	23.31	14.53	4.23	10.43	13.37
Fabaceae	1.06	4.14	1.33	1.52	1.40	2.47	1.62	1.14
Lamiaceae	7.40	6.85	2.89	3.04	2.19	1.62	1.35	0.78
Liliaceae	0.60	0.00	0.52	0.23	0.44	0.19	0.36	0.28
Malva	0.00	0.54	0.00	0.08	0.18	0.10	0.18	0.07
Medicago	4.68	0.36	1.70	0.61	0.53	0.43	1.08	1.07
Onobrychis	1.96	0.36	0.00	2.05	0.00	0.81	0.00	1.85
Papaver	2.11	0.18	0.24	0.00	0.23	0.62	3.51	1.56
Pinus	0.45	0.36	0.09	0.23	0.06	0.10	0.18	0.21
Plantago	2.42	0.18	0.62	0.00	1.49	0.43	1.26	0.57
Poaceae	3.17	5.23	4.36	1.52	2.80	2.52	6.29	2.35
Populus	1.06	1.08	0.14	0.38	0.35	0.24	1.17	0.28
Quercus	32.93	20.36	21.40	19.51	5.87	1.95	23.11	18.63
Rosaceae	1.21	0.54	0.33	0.15	0.79	0.14	0.18	0.21
Rumex	1.21	0.90	0.14	0.08	0.12	0.14	0.27	0.14
Salix	0.00	6.13	0.00	3.72	0.00	2.61	0.00	4.05
Urtica	1.06	1.98	0.05	3.42	1.08	1.24	1.98	1.07
Verbascum	12.39	8.29	14.49	8.20	45.75	43.37	9.62	15.86
Vicia	0.76	0.90	0.80	0.61	1.46	0.81	0.72	0.07
Undefined	0.91	0.72	0.24	0.30	0.15	0.24	0.54	0.36

pollen > 45%, the frequency was regarded as 'very frequent'; the percentage of pollen between 16% and 45%, was recorded as 'frequent'; 3–15% was referred to as 'rare'; if the percentage of pollen taxa was below 3% it was regarded as 'sporadic' (Louveaux et al. 1978).

Statistical analysis

Descriptive statistics of the data obtained in the study were calculated. Afterwards, the normal distribution of data for all properties examining their suitability was evaluated. It was determined that the data did not show normal distribution and did not provide the parametric test assumptions. Therefore, locations Mann–Whitney U test was used to compare the differences between Battalgazi and Dogansehir, Kruskal–Wallis variance analysis was used to compare the differences between feeding groups (C, G, S, and BF) (Özdamar 2003; Akgül 2005). All analyses and calculations were performed with SPSS 11.5 version program (SPSS 2005). The p values of comparison are shown in Table III and Figure 4.

Discussion

Our study is the first palynological research on RJ samples in Turkey. There are a few researches on palynology of RJ around the world. In Minas Gerais, Brazil *Eucalyptus* and *Senecio* are the most abundant taxa in RJ samples (Barth 2005). In Barth's study (2005) 38 plant taxa were identified. Piana et al. (2006) examined Italian (northern and central) RJ compared with imported (Chinese and Vietnamese) RJ samples. In Greece there are two studies on the palynology of RJ. For the first, the

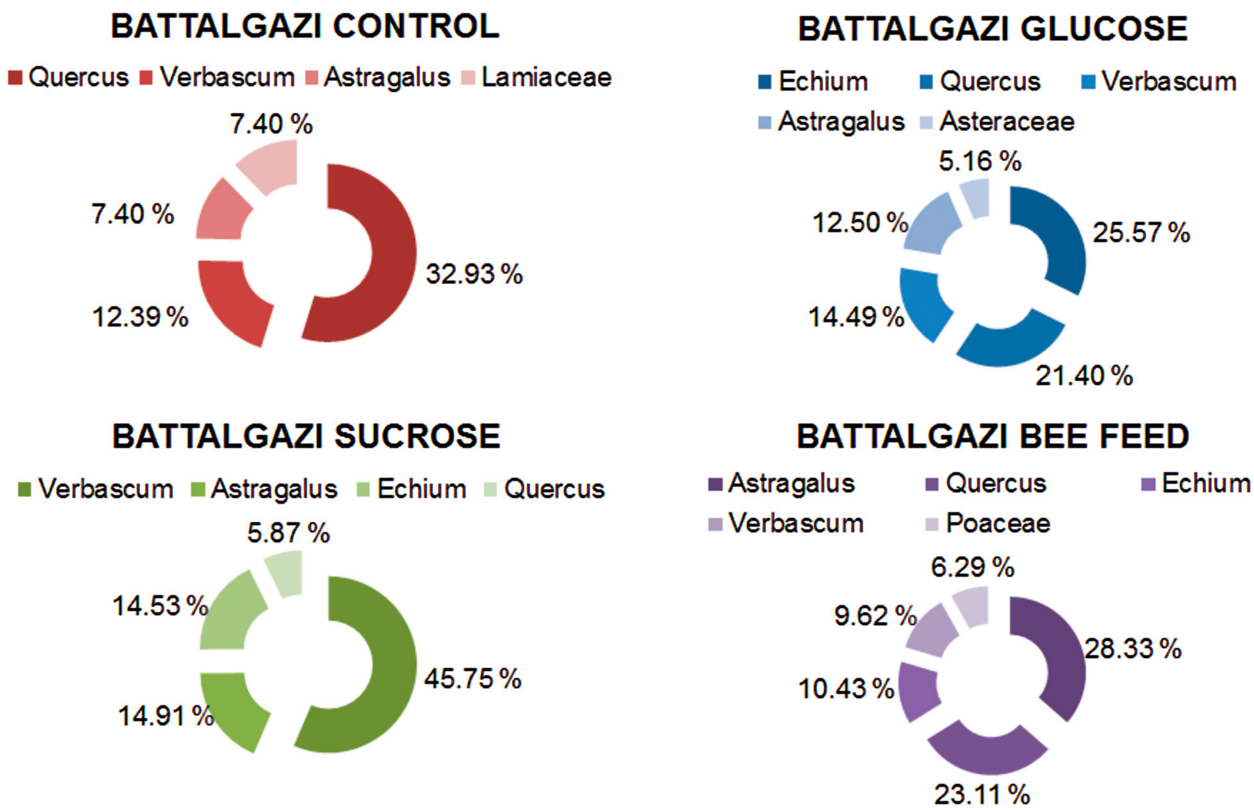

Figure 2. The dominant pollen types in Battalgazi province royal jelly samples according to feeding groups during the study period.

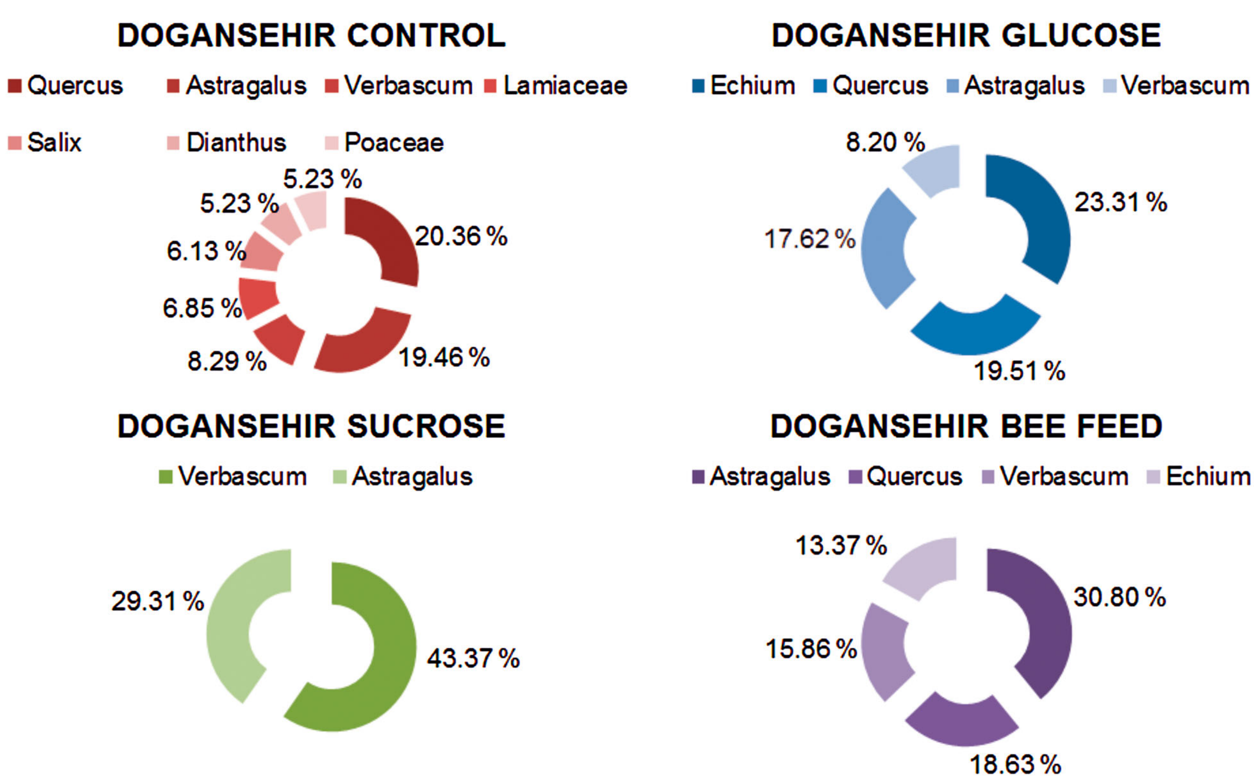

Figure 3. The dominant pollen types in Dogansehir province royal jelly samples according to feeding groups during the study period.

Table II. Pollen frequency in royal jelly (RJ) samples: ■ = very frequent, ■ = frequent, ■ = rare, □ = sporadic.

BATTALGAZI	C	G	S	BF
Anchusa	sporadic	sporadic	sporadic	sporadic
Apiaceae	sporadic	sporadic	sporadic	sporadic
Asteraceae	rare	rare	sporadic	sporadic
Astragalus	rare	rare	rare	frequent
Boraginceae	sporadic	sporadic	sporadic	sporadic
Brassicaceae	sporadic	sporadic	sporadic	sporadic
Dianthus	sporadic	sporadic	sporadic	sporadic
Carduus	sporadic	sporadic	sporadic	sporadic
Cichorideae	sporadic	sporadic	sporadic	sporadic
Cistaceae	sporadic	sporadic	sporadic	sporadic
Convolvulus	sporadic	sporadic	sporadic	sporadic
Cup/Tax	sporadic	sporadic	sporadic	sporadic
Cyperaceae	sporadic	sporadic	sporadic	sporadic
Echium	sporadic	frequent	rare	rare
Fabaceae	sporadic	sporadic	sporadic	sporadic
Lamiaceae	rare	sporadic	sporadic	sporadic
Liliaceae	sporadic	sporadic	sporadic	sporadic
Malva	sporadic	sporadic	sporadic	sporadic
Medicago	rare	sporadic	sporadic	sporadic
Onobrychis	sporadic	sporadic	sporadic	sporadic
Papaver	sporadic	sporadic	sporadic	rare
Pinus	sporadic	sporadic	sporadic	sporadic
Plantago	sporadic	sporadic	sporadic	sporadic
Poaceae	rare	rare	sporadic	rare
Populus	sporadic	sporadic	sporadic	sporadic
Quercus	frequent	frequent	rare	frequent
Rosaceae	sporadic	sporadic	sporadic	sporadic
Rumex	sporadic	sporadic	sporadic	sporadic
Urtica	sporadic	sporadic	sporadic	sporadic
Verbascum	rare	rare	very frequent	rare
Vicia	sporadic	sporadic	sporadic	sporadic

DOĞANŞEHİR	C	G	S	BF
Anacardiaceae	sporadic	sporadic	sporadic	sporadic
Anchusa	sporadic	sporadic	sporadic	sporadic
Apiaceae	sporadic	sporadic	sporadic	sporadic
Asteraceae	rare	rare	sporadic	sporadic
Astragalus	frequent	frequent	frequent	frequent
Boraginceae	sporadic	sporadic	sporadic	sporadic
Brassicaceae	sporadic	sporadic	sporadic	sporadic
Dianthus	rare	sporadic	sporadic	sporadic
Carduus	sporadic	sporadic	sporadic	sporadic
Cichorideae	sporadic	sporadic	sporadic	sporadic
Cistaceae	sporadic	sporadic	sporadic	sporadic
Convolvulus	sporadic	sporadic	sporadic	sporadic
Cup/Tax	sporadic	sporadic	sporadic	sporadic
Cyperaceae	sporadic	sporadic	sporadic	sporadic
Echium	sporadic	frequent	rare	rare
Fabaceae	rare	sporadic	sporadic	sporadic
Lamiaceae	rare	rare	sporadic	sporadic
Liliaceae	sporadic	sporadic	sporadic	sporadic
Malva	sporadic	sporadic	sporadic	sporadic
Medicago	sporadic	sporadic	sporadic	sporadic
Onobrychis	sporadic	sporadic	sporadic	sporadic
Papaver	sporadic	sporadic	sporadic	sporadic
Pinus	sporadic	sporadic	sporadic	sporadic
Plantago	sporadic	sporadic	sporadic	sporadic
Poaceae	rare	sporadic	sporadic	sporadic
Populus	sporadic	sporadic	sporadic	sporadic
Quercus	frequent	frequent	sporadic	frequent
Rosaceae	sporadic	sporadic	sporadic	sporadic
Rumex	sporadic	sporadic	sporadic	sporadic
Salix	rare	rare	sporadic	rare
Urtica	sporadic	rare	sporadic	sporadic
Verbascum	rare	rare	frequent	frequent
Vicia	sporadic	sporadic	sporadic	sporadic

Note: S, Sucrose group; G, Glucose group; BF, Bee Feed group; C, control group.

most frequent pollen types are *Lilium* type, *Portulaca oleraceae* L., *Polygonum aviculare* L., *Sonchus* sp. and *Zea mays* L. (Dimou et al. 2007). In the study 17 taxa were identified (Dimou et al. 2007). The second study (Dimou et al. 2013) the most abundant pollen grains belonging to Brassicaceae, *Olea europaea* L., *Eucalyptus*, *Trifolium*, *Rubus*, *Carduus*-type, *Hypericum*, *Daucus*- type and *Cistus* (Dimou et al. 2013). In this study 60 taxa were identified within the RJ (Dimou et al. 2013). An experimental study in São Paolo, Brazil recorded Mimosaceae (*Acacia* sp., *Anadenanthera* sp., *Mimosa caesalpiniaefolia* Benth, *Mimosa scabrella* Benth, *Mimosa verrucosa* Benth) are the most frequent taxa within six RJ samples examined (Morgado & Barth 2011).

In our study, 33 taxa belonging to 23 families were determined in Dogansehir district and 31 taxa belonging to 22 families were determined in Battalgazi district RJ samples. Anacardiacae and *Salix* were not observed in Battalgazi RJ samples. In Dogansehir there are aquatic areas therefore *Salix* is more abundant around the study area. *Rhus coriaria* L. 'from Anacardiaceae' is a widely grown taxon because of the ethnobotanical usage.

Honey bees prefer diverse forage for their nutritional requirements (Donkersley et al. 2017). Pollen is known as a protein source for honeybees but pollen sugars are valuable supplements as carbohydrate food of honeybees and especially as glucose and fructose are present in high ratio within pollen extracts (McLellan 1977; Palmer-Young &

Table III. Correlation between pollen taxa percentages of royal jelly according to location and feeding methods.

Taxa	Location Asymptotic significance (two-tailed)	Feeding method Asymptotic significance
Anacardiaceae	**0.014***	0.925
Anchusa	**0.043***	0.418
Apiaceae	1	0.224
Asteraceae	0.773	0.112
Astragalus	0.83	0.3
Boraginceae	0.248	0.16
Brassicaceae	0.468	0.177
Dianthus	0.248	0.129
Carduus	0.083	0.572
Cichorideae	0.663	0.908
Cistaceae	0.248	0.367
Convolvulus	0.386	0.321
Cup/Tax	0.149	0.212
Cyperaceae	0.243	0.133
Echium	0.564	0.112
Fabaceae	0.248	0.919
Lamiaceae	0.773	0.083
Liliaceae	**0.021***	0.983
Malva	0.559	0.654
Medicago	0.083	0.572
Onobrychis	0.139	0.831
Papaver	0.248	0.321
Pinus	0.386	0.16
Plantago	**0.021***	0.801
Poaceae	0.149	0.682
Populus	0.773	0.367
Quercus	0.149	0.212
Rosaceae	0.149	0.418
Rumex	0.554	0.143
Salix	**0.014***	0.925
Urtica	0.191	0.998
Verbascum	0.564	0.212
Vicia	0.564	0.184
Undefined	0.885	0.092

Note: $p < 0.05$ values are marked with an asterisk (*) and are shown in bold typeface.

Thursfield 2017). Sugars are one of the main components of bee pollen and form 40% of dry matter (Szczęsna 2007). According to researchers the sugar content of pollen grains collected by bees are 34% upward than the pollen grains in flowers because of the additional secretions of bees while preparing the pollen pellet (Todd & Bretherick 1942; Conti et al. 2016). Liolios et al. (2018) noted that botanical origin of bee pollen, include nectar and affects the sugar profile. The shift in pollen preferences but also nectar preferences could be the reason for the change in frequency of the pollen grain percentages between the different feeding methods.

Apis mellifera L. colonies consist of approximately 60 000 worker bees and this number of individuals need a large amount of food for nutrition, survival, development and health of the colony (Southwick & Heldmaier 1987; Abou-Shaara 2017). Nectar is insufficient for such great colonies for this reason beekeepers feed the colonies with different sugars (Abou-Shaara 2017). Nectar is provided by phloem liquid and contains saccharose, fructose and glucose (De la Barrera & Nobel 2004). Bees break down the saccharose into glucose and fructose for nourishment however they lose energy with the ratio 23.0% during this inversion (Ceksteryte & Racys 2006). For Zaboenko (2000) inverted sugar (glucose and fructose) feeding may be more suitable for bees not to consume their biological sources as they lose energy in this process.

Echium pollen and nectar are collected by honey bees and this taxon is an accepted bee plant because of providing a good source of nectar and pollen for food (Chwil & Weryszko-Chmielewska 2011). *Echium* flowering season has been recorded

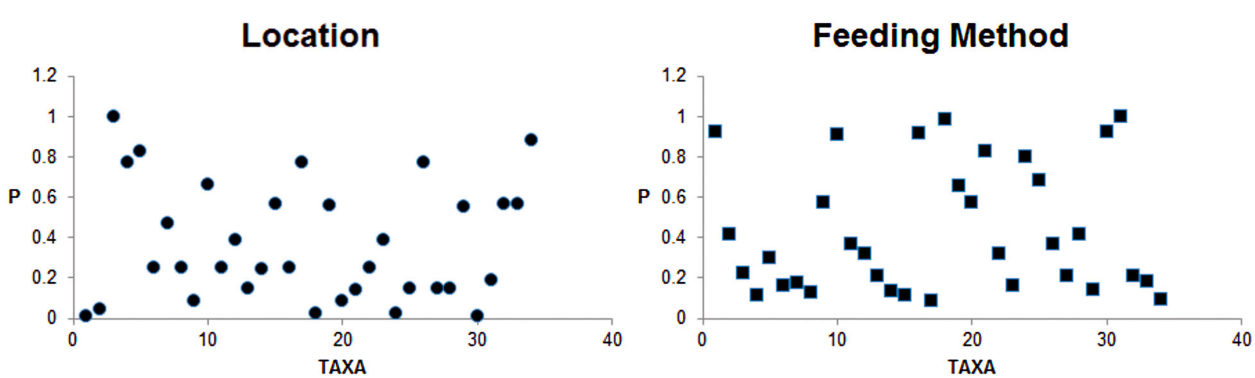

Figure 4. Graphical presentation of p values for the effects of location and feeding method on pollen taxa ratios in royal jelly.

as occurring between March and August in Turkey (Karaköse et al. 2018). The colour of *Echium* flowers and pollen grains (violet-blue) make it an attractive plant for insects (Maurizio & Grafl 1969; Prabucki 1998; Chwil & Weryszko-Chmielewska 2011). For the glucose group (G) *Echium* pollen was the dominant palynomorph for each study area. According to previous studies for *Echium* nectar sugar content, sucrose is the predominant component (Maurizio & Grafl 1969). It has been reported that the high sugar content of *Echium* monofloral honey is related to the presence of a high amount *Echium* pollen and these honeys have abundance of fructose and glucose (sucrose) (De La Fuente et al. 2011). Therefore, the lack of sucrose in glucose feeding could be resolved with *Echium* pollen grains. When sucrose is hydrolysed to monomers it forms glucose and fructose molecules. The absence of fructose may be balanced with *Echium* pollen grains.

Verbascum is also attractive for pollinators with its taller stem and strong apical dominance, this situation is explained by the 'effective pollination' hypothesis (Gross & Werner 1978; Aarssen 1995; Lortie & Aarssen 1999). For hives fed on sucrose *Verbascum* is the dominant taxon in both Battalgazi and Dogansehir. McCaskill and Turgeon (2007) identified that the glucose content of wild type of *Verbascum* is higher than the sucrose content. Wykes (1952) noted that honey bees prefer sucrose, glucose and fructose sugars equally for alimentation. However, Furgala et al. (1958) postulated that some plants are more important for honey bees because they enable the balancing of sucrose–glucose–fructose over sucrose-dominant plants. A general opinion is that honey bees prefer sucrose primarily but if they have a chance to forage variable sugar types they would harvest hexose sugars rather than waste energy unnecessarily when foraging (Hagler 1990; Zaboenko 2000).

For bee feed feeding *Astragalus* is the dominant taxon for Battalgazi and Dogansehir. *Astragalus* is one of the primal nectar sources of honeybees also this taxon has papilionaceous flowers (Green & Bohart 1975; Decker & Anderson 2004; Uzun et al. 2019). Bee feed syrup is composed of a mixture of three sugars with the percentage of sucrose 30–36%, glucose 27–30%, and fructose 37–40%. The ratio of the sugars was approximately equal. However, glucose is lower than the others. According to some studies of *Astragalus* sugar content, the glucose ratio is higher than the other monosaccharides in most species (Ebrahimzadeh et al. 2000; Niknam & Lisar 2004).

For the control group *Quercus* is the most widely represented genus. This arboreal taxon is identified as an important pollen sources for honeybees (Ghosh & Jung 2017). When the control group is examined, it could be seen that different pollen grains (Lamiaceae, *Salix*, *Dianthus* and Poaceae) were dominant in both locations unlike the feeding groups. Here, we suggest that the bees may be trying to complete the lack of feeding sugars with these different pollen types.

With this study we determined the geographical origin of RJ samples in two different districts of Malatya. Additionally, the study was conducted with different feeding methods, based on this, pollen content variations were obtained in RJ samples. Pollen grains are known as a good food source for bee nutrition and contain carbohydrates, lipids, and various vitamins and minerals (Roulston & Cane 2000; Kieliszek et al. 2018). The differences of pollen spectrum according to feeding methods and positions of hives could change the nutritional value positively or negatively of bee products.

Conclusions

In this melissopalynological study on RJ, the types and percentages of pollen grains were found differently by spatial and nutritional values simultaneously in 2018.

The study was carried out in two different districts of Malatya city; Battalgazi and Dogansehir. According to results, the types of pollen grains and their percentages differ by location. *Verbascum*, *Astragalus*, *Echium*, *Quercus* pollen grains were used as plant resources dominantly by honeybees and Anacardiaceae and *Salix* pollen grains were not observed in Battalgazi RJ samples.

In both locations, hives with the same feeding groups (glucose, sucrose, bee feed and control group) were established. Eventually it was seen that the pollen types and their percentages differ depending on feeding groups. Plant target of bees changes according to feeding groups.

Consequently, when the bee colonies are manipulated by different feeding methods, the bees will shift resource utilisation in the plants that they harvest.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This study was supported by Inonu University Scientific Research Project Unit [grant number: FCD-2018-1118].

References

Aarssen LW. 1995. Hypotheses for the evolution of apical dominance in plants: Implications for the interpretation of overcompensation. Oikos 74: 149–156. doi:10.2307/3545684

Abou-Shaara H. 2017. Effects of various sugar feeding choices on survival and tolerance of honey bee workers to low temperatures. Journal of Entomological and Acarological Research 49: 6–12. doi:10.4081/jear.2017.6200

Akgül A. 2005. Tıbbi Araştırmalarda İstatistiksel Analiz Teknikleri. Ankara: Emek.

Arslan Ö, Hayli S. 2007. Population geography of Battalgazi country. F.Ü. Sosyal Bilimler Dergisi 17(2): 1–30.

Asencot M, Lensky Y. 1976. The effect of sugars and Juvenile hormone on the differentiation of the female honeybee larvae (L.) to queens. Life Sciences 18(7): 693–699. doi:10.1016/0024-3205(76)90180-6

Asencot M, Lensky Y. 1984. Juvenile hormone induction of ‘queenliness’ on female honeybee (*Apis mellifera* L.) larvae reared on worker jelly and on stored royal jelly. Comparative Biochemistry and Physiology Part B: Comparative Biochemistry 78(1): 109–117. doi:10.1016/0305-0491(84)90153-6

Asencot M, Lensky Y. 1988. The effect of soluble sugars in stored royal jelly on the differentiation of female honeybee (*Apis mellifera* L.) larvae to queens. Insect Biochemistry 18(2): 127–133. doi:10.1016/0020-1790(88)90016-9

Aytuğ B. 1967. Polen Morfolojisi ve Türkiye’nin Önemli Gymnospermleri Üzerinde Palinolojik Araştırmalar. İstanbul Üniversitesi Orman Fakültesi Yayınları. İstanbul: Kutulmuş.

Barth OM. 2005. Botanical resources used by *Apis mellifera* determined by pollen analysis of royal jelly in Minas Gerais, Brazil. Journal of Apicultural Research 44: 78–81. doi:10.1080/00218839.2005.11101153

Bilikova K, Krakova TK, Yamaguchi K, Yamaguchi Y. 2015. Major royal jelly proteins as markers of authenticity and quality of honey. Archives of Industrial Hygiene and Toxicology 66: 259–267. doi:10.1515/aiht-2015-66-2653

Brodschneider R, Crailsheim K. 2010. Nutrition and health in honey bees. Apidologie 41(3): 278–294. doi:10.1051/apido/2010012

Brunner FS, Schmid-Hempel P, Barribeau SM. 2014. Protein-poor diet reduces host specific immune gene expression in *Bombus terrestris*. Proceedings of the Royal Society of London. Series B: Biological Sciences 281: 1–10.

Ceksteryte V, Racys J. 2006. The quality of syrups used for bee feeding before winter and their suitability for bee wintering. Journal of Apicultural Science 50(1): 5–14.

Charpin J, Surinyach R, Frankland AW. 1974. Atlas of European allergenic pollens. Paris: Sandoz Editions.

Chwil M, Weryszko-Chmielewska E. 2011. Nectar production and pollen yield of *Echium vulgare* L. in the climatic conditions of Lublin. Acta Scientiarum Polonorum. Hortorum Cultus = Ogrodnictwo 10(3): 187–196.

Conroy TJ, Palmer-Young EC, Irwin RE, Adler LS. 2016. Food limitation affects parasite load and survival of Bombus impatiens (Hymenoptera: Apidae) infected with Crithidia (Trypanosomatida: Trypanosomatidae). Environmental Entomology 45(5): 1212–1219. doi:10.1093/ee/nvw099

Conti I, Medrzycki P, Argenti C, Meloni M, Vecchione V, Boi M, Mariotti G. 2016. Sugar and protein content in different monofloral pollens – building a database. Bulletin of Insectology 69(2): 318–320.

Decker K, Anderson D. 2004. *Astragalus anisus* M.E.Jones (Gunnison milkvetch): A technical conservation assessment (Online). USDA Forest Service, Rocky Mountain Region. Corpus ID: 135458396.

De la Barrera E, Nobel PS. 2004. Nectar: Properties, floral aspects, and speculations on origin. Trends in Plant Science 9(2): 65–69. doi:10.1016/j.tplants.2003.12.003

De La Fuente E, Ruiz-Matute AI, Valencia-Barrera RM, Sanz J, Castro IM. 2011. Carbohydrate composition of Spanish unifloral honeys. Food Chemistry 129(4): 1483–1489. doi:10.1016/j.foodchem.2011.05.121

Deseyn J, Billen J. 2005. Age-dependent morphology and ultrastructure of the hypopharyngeal gland of Apis mellifera workers (Hymenoptera, Apidae). Apidologie 36(1): 49–57. doi:10.1051/apido:2004068

Dietz A. 1978. Nutrition of the adult honey bee. In: Graham M, ed. The hive and the honey bee, 125–156. Carthage: Dadant & Sons.

Dietz A, Haydak MH. 1971. Caste determination in honeybees I. The significance of moisture in larval food. Journal of Experimental Zoology 177(3): 353–357. doi:10.1002/jez.1401770309

Dimou M, Goras G, Thrasyvaoulou A. 2007. Pollen analysis as a means to determine the geographical origin of royal jelly. Grana 46: 118–122. doi:10.1080/00173130701393874

Dimou M, Tananaki C, Goras G, Karazafiris E, Thrasyvoulou A. 2013. Melissopalynological analysis of royal jelly from Greece. Grana 52(2): 106–112. doi:10.1080/00173134.2012.719538

Donkersley P, Rhodes G, Pickup RW, Jones KC, Power EF, Wright GA, Wilson K. 2017. Nutritional composition of honey bee food stores vary with floral composition. Oecologia 185: 749–761. doi:10.1007/s00442-017-3968-3

Ebrahimzadeh H, Niknam V, Maassoumi AA. 2000. Mucilage content and its sugar composition in Astragalus species from Iran. Pakistan Journal of Botany 32: 131–140.

Erdtman G. 1952. Pollen morphology and plant taxonomy, angiosperms. Stockholm: Almqvistand Wiksell.

Erdtman G. 1969. Hand book of palynology. New York: Hafner.

Faegri K, Iversen J. 1975. Textbook of pollen analysis. 3rd ed. Copenhagen: Munksgaard.

Furgala B, Gochnauer TA, Holdaway FG. 1958. Constituent sugars of some northern legume nectars. Bee World 39: 203–205. doi:10.1080/0005772X.1958.11095065

Ghosh S, Jung C. 2017. Nutritional value of bee-collected pollens of hardy kiwi, *Actinidia arguta* (Actinidiaceae) and oak, *Quercus* sp. (Fagaceae). Journal of Asia-Pacific Entomology 20: 245–251. doi:10.1016/j.aspen.2017.01.009

Green TW, Bohart GE. 1975. The pollination ecology of *Astragalus cibarus* and *Astragalus utahensis* (Leguminosae). American Journal of Botany 62(4): 379–386. doi:10.1002/j.1537-2197.1975.tb14060.x

Gross KL, Werner PA. 1978. The biology of Canadian weeds. 28. *Verbascum thapsus* L. and *V. blattaria* L. Canadian Journal of Plant Science 58(1): 401–413. doi:10.4141/cjps78-062

Hagler JR. 1990. Honey bee (*Apis mellifera* L.) response to simulated onion nectars containing variable sugar and potassium concentrations. Apidologie 21: 115–121. doi:10.1051/apido:19900204

Hartfelder K, Guidugli-Lazzarini KR, Cervoni MS, Santos DE, Humann FC. 2015. Old threads make new tapestry-rewiring of signalling pathways underlies caste phenotypic plasticity in the honey bee, *Apis mellifera* L. Advances in Insect Physiology Genomics, Physiology and Behaviour of Social Insects 48: 1–36. doi:10.1016/bs.aiip.2014.12.001

Haydak MH. 1970. Honey bee nutrition. Annual Review of Entomology 15: 143–156. doi:10.1146/annurev.en.15.010170.001043

Jung-Hoffmann I. 1966. Die determiantion von königin und arbeiterin der honigbiene. Z. Bienenforsch 8: 296–322.

Kafantaris I, Amoutzias GD, Mossialos D. 2021. Foodomics in bee product research: A systematic literature review. European Food Research and Technology 247: 309–331. doi:10.1007/s00217-020-03634-5

Karaköse M, Polat R, Rahman MO, Çakılcıoğlu U. 2018. Traditional honey production and bee flora of Espiye, Turkey. Bangladesh Journal of Plant Taxonomy 25(1): 79–91. doi:10.3329/bjpt.v25i1.37184

Karakuş Ş. 2016. Malatya ili Florası. PhD Thesis, Inonu University, Malatya, Turkey.

Kieliszek M, Piwowarek K, Kot AM, Błażejak S, Chlebowska-Śmigiel A, Wolska I. 2018. Pollen and bee bread as new health-oriented products: A review. Trends in Food Science & Technology 71: 170–180. doi:10.1016/j.tifs.2017.10.021

Krell R. 1996. Value added products from beekeeping. FAO agricultural services bulletin. Rome: FAO.

Kunugi H, Ali AM. 2019. Royal jelly and components promote healthy aging and longevity: From animal models to humans. International Journal of Molecular Sciences 20(19): 4662–4688. doi:10.3390/ijms20194662

Lindauer M. 1952. Ein beitrag zur frage der arbeitsteilung im bienenstaat. Zeitschrift für vergleichende Physiologie 34: 299–345. doi:10.1007/BF00298048

Liolios V, Tananaki C, Dimou M, Kanelis D, Rodopoulou MA, Thrasyvoulou A. 2018. Exploring the sugar profile of unifloral bee pollen using high performance liquid chromatography. Jounal of Food and Nutrition Research 57(4): 1–10.

Lortie CJ, Aarssen WL. 1999. The adventage of being tall: Higher flowers receive more pollen in *Verbascum thapsus* L. (Scrophulariaceae). Écoscience 6(1): 68–71. doi:10.1080/11956860.1999.11952207

Louveaux J, Maurizio A, Vorwohl G. 1978. Methods of melissopalynology. Bee World 59: 139–157. doi:10.1080/0005772X.1978.11097714

Luz CFP, Guimarães-Cestaro L, Serrão JE, Message D, Martins MF, Alves MLTMF, Seijo-Coello MC, Teixeira EW. 2018. Using palynological evidence from royal jelly to mediate the spread of *Paenibacillus larvae* in Brazil. Hoehnea 45(3): 512–539. doi:10.1590/2236-8906-45/2018

Maurizio A, Grafl I. 1969. Das Trachtpflanzenbuch. Munchen: Ehrenwirth.

McCaskill A, Turgeon R. 2007. Phloem loading in Verbascum phoeniceum L. depends on the synthesis of raffinose- family oligosaccharides. Proceedings of the National Academy of Sciences 104(49): 19619–19624. doi:10.1073/pnas.0707368104

McLellan AR. 1977. Minerals, carbohydrates and amino acids of pollens from some woody and herbaceaous plants. Annals of Botany 41(6): 1225–1232. doi:10.1093/oxfordjournals.aob.a085413

Morgado LN, Barth OM. 2011. The detection of pollen in royal jelly of honey bees (*Apis mellifera*). Journal of ApiProduct and ApiMedical Science 3(3): 137–139. doi:10.3896/IBRA.4.03.3.05

Naug D. 2009. Nutritional stress due to habitat loss may explain recent honeybee colony collapses. Biological Conservation 142(10): 2369–2372. doi:10.1016/j.biocon.2009.04.007

Niknam V, Lisar YS. 2004. Chemical composition of *Asragalus:* Carbohydrates and mucilage content. Pakistan Journal of Botany 36(2): 381–388.

Özdamar K. 2003. SPSS ile Biyoistatistik. 5th ed. Ankara: Kaan Bookstore.

Palmer-Young EC, Thursfield L. 2017. Pollen extracts and constituent sugars increase growth of a trypanosomatid parsite of bumle bees. PeerJ 5: 1–21.

Piana ML, Belligoli P, Persano Oddo L, Piperno S. 2006. Pollen analysis of royal jelly: Contribution to analytical methods and characterization. Apiacta 41: 28–43.

Prabucki J. 1998. Pszczelnictwo. Wyd. Promocyjne. Szczecin: Albatros.

Renner P, Hrassnigg N, Crassilheim K. 2003. Trophallaxis between nursebees and pollen foragers under laboratory conditions in *Apis mellifera carnica*. Apidologie 34: 492.

Riciardelli d'Albore G, Battaglini Bernardini M. 1978. Originegeagraphique de la geleéroyale. Apidologie 9: 1–17. doi:10.1051/apido:19780101

Roulston T, Cane JH. 2000. Pollen nutritional content and digestibility for animals. Plant Systematics and Evolution 222: 187–209. doi:10.1007/BF00984102

Saavedra-Carhuatocto DM, Aguinaga-Castro F, Rojas-Idrogo C, Delgado-Paredes GE. 2014. Analysis of pollen loads collected by honeybees (*Apis mellifera* L.) from Lambayeque province (Peru): Botanical origin and protein content. Journal of Global Biosciences 3(1): 285–298.

Sajwani A, Farooq SA, Bryant VM. 2014. Studies of bee foraging plants and analysis of pollen pellets from hives in Oman. Palynology 38(2): 207–223. doi:10.1080/01916122.2013.871652

Shuel RW, Dixon SE. 1968. The importance of sugar for the population of the worker honeybee. Journal of Apicultural Research 7: 109–112. doi:10.1080/00218839.1968.11100199

Simpson J. 1955. The significance of the presence of pollen in the food of worker larvae of the honey-bee. The Quarterly Journal of Microscopical Science 96(1): 117–120.

Southwick EE, Heldmaier G. 1987. Temperature control in honey bee colonies. BioScience 37: 395–399. doi:10.2307/1310562

SPSS. 2005. Statistical software package, Version 14.0, Serial No: 9869264, Chicago, IL, USA.

Standifer LN. 1980. Honey bee nutrition and supplemental feeding. Beekeeping in the US Agriculture Handbook 335: 39–45.

Standifer LN, Moeller FE, Kauffeld N, Herbert EW, Jr, Shimanuki H. 1977. Supplemental feeding of honey bee colonies. United States Department of Agriculture, Agriculture Information Bulletin 413: 1–8.

Strant M, Yücel B, Topal E, Puscasu AM, Margaoan R, Varadi A. 2019. Use of royal jelly as functional food in human and animal health. Journal of Animal Production 60(2): 131–144.

Szczęsna T. 2007. Study on sugar composition of honey-bee-collected pollen. Journal of Apicultural Science 51: 15–22.

Takenaka T, Takahashi E. 1980. General composition of the royal jelly. Bulletin of the Faculty of Agriculture Tamagawa University Tokyo 20: 71–78.

Todd FE, Bretherick O. 1942. The composition of pollens. Journal of Economic Entomology 35(3): 312–317. doi:10.1093/jee/35.3.312

Uzun A, Uzun SP, Durmaz A. 2019. Spatial analyses of *Astragalus* species distribution and richness in Kahramanmaraş (Turkey) by geographical information systems (GIS). Turkish Journal of Forest Science 3(1): 37–59. doi:10.32328/turkjforsci.553375

Vaudo AD, Tooker JF, Grozinger CM, Patch HM. 2015. Bee nutrition and floral resource restoration. Current Opinion in Insect Science 10: 133–141. doi:10.1016/j.cois.2015.05.008

Wang Y, Ma L, Zhang W, Cui X, Wang H, Xu B. 2016. Comparison of the nutrient composition of royal jelly and worker jelly of honey bees (*Apis mellifera*). Apidologie 47: 48–56. doi:10.1007/s13592-015-0374-x

Witherell PC. 1978. Activities and behaviour of honey bees. In: Graham M, ed. The hive and the honey bee, 531–558. Carthage: Dadant & Sons.

Wodehouse RP. 1965. Pollen grains. New York: Hafner.

Wright GA, Nicolson SW, Shafir S. 2018. Nutritional physiology and ecology of honey bees. Annual Review of Entomology 63 (1): 327–344. doi:10.1146/annurev-ento-020117-043423

Wykes GR. 1952. The preferences of honeybees for solutions of various sugars which occur in nectar. Journal of Experimental Biology 29: 511–519. doi:10.1242/jeb.29.4.511

Wytrychowski M, Chenavas S, Daniele G, Casabianca H, Batteau M, Guibert S, Brion B. 2013. Physicochemical characterisation of French royal jelly: Comparison with commercial royal jellies and royal jellies produced through artificial bee-feeding. Journal of Food Composition and Analysis 29: 126–133. doi:10.1016/j.jfca.2012.12.002

Xue X, Wu L, Wang K. 2017. Chemical composition of royal jelly. In: Alvarez-Suarez JM, ed. Bee products—Chemical and biological properties, 185–192. New York: Springer.

Yakar ÖY, Fırat F, Bozdağ N, Baydoğan AE. 2014. Sosyal, Kültürel ve Ekonomik yönleri ile Malatya, TC Malatya Valiliği İl Planlama ve Koordinasyon Müdürlüğü, Malatya.

Zaboenko AS. 2000. Sovremennaja ènciklopedija pchelovoda. Doneck 1: 75–76.