Botanical origin and inorganic content of bee honey in Northeast Bulgaria (Shumen region)

Maria A. Lazarova[1], Juliana R. Atanassova[2] & Lilyana D. Yurukova[1]

[1] Institute of Botany, Bulgarian Academy of Sciences, Acad. Georgi Bonchev St., bl. 23, 1113 Sofia, Bulgaria, e-mail: mal@bio.bas.bg; yur7lild@bio.bas.bg

[2] Department of Botany, Faculty of Biology, University of Sofia Kliment Ohridski, 8 Dragan Tsankov Blvd., 1164 Sofia, Bulgaria, e-mail: atanassova_juliana@abv.bg

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Abstract: Honey samples from the Shumen region (NE Bulgaria) collected in 2006 were analyzed palynologically and the preliminary results for the nectar sources of this region were discussed . Four types of unifloral honey were indentified (*Helianthus annuus*, *Robinia pseudoacacia*, *Paliurus* and *Apiaceae*). The most important nectariferous plants for the region are *Robinia pseudoacacia*, *Paliurus spina-christi*, *Helianthus annuus*, *Lotus corniculatus*, *Echium vulgare*, and different species of *Cirsium*, *Carduus*, *Apiaceae* and *Brassicaceae*.

Eight heavy metals and toxic elements (Cd, Pb, Cu, Zn, Cr, Al, As, and V) were analyzed in 11 honey samples by atomic emission spectrometry with inductively coupled plasma (ICP-AES) in a certified lab. The results are assessed by descriptive statistical characteristics and ANOVA analysis.

Key words: bee honey, heavy metals, melissopalynological study, toxic elements

Introduction

Identification of the botanical taxa that are the source of honey is of high practical and scientific importance. Assessment of the botanical origin of unifloral honeys is an important application in the food control (Lieux 1975; Louveaux & al. 1978; Moar 1985). Palynological investigations of Bulgarian honeys are rare (Bozilova & Anchev 1968; Bozilova & Chan 1976; Lazarova & Bozilova 2001, 2002; Atanassova & Kondova 2004; Atanassova & al. 2004). The aim of this study is to establish the plant taxa and inorganic content of honey in the region of the town of Shumen (NE Bulgaria), and to obtain information on the nectariferous plants important for the bees. Pollen analysis was used to designate the honey as unifloral or polyfloral.

Bee honey should be free of heavy metals and toxic elements in concentrations that may represent a hazard to human health. Hygiene norms for such elements in honey are missing in Bulgaria (**Regulation N12** 2002). The norms covered by the standards in various countries should meet the future levels for honey contaminants established by the Codex Alimentarius Commission of the Food Agriculture Organization (FAO) and the World Health Organization (WHO) of the United Nations.

Material and methods

Pollen analysis was carried out on 11 honey samples from the village Zvegor and the Chairdere locality near Shumen (NE Bulgaria) to identify the botanical origin of the honey (Fig. 1). The honey samples were collected in June (numbers 1/6, 2/6, 5/6, 6/6), July, (numbers 3/7, 7/7), August (numbers 4/8, 8/8) and October 2006 (numbers 9/10, 10/10, 11/10). The preparation for pollen analysis followed the harmonized methods of melissopalynology (Louveaux & al. 1978). The fre-

Fig. 1. Location of the analyzed honey samples.

quency of each pollen type is expressed as percentage of the total pollen sum (Table 1).

The inorganic content of eight elements in 11 honey samples was determined. Honey samples of 5 g were wet-ashed with 9.67 M nitric acid and 3 ml portions of hydrogen peroxide and heated until full digestion. The filtrate was diluted with distilled water up to 25 ml. All solutions were stored in plastic flasks. Duplicates of each sample were prepared independently. The elements Al, As, Cd, Cu, Fe, Pb, V, and Zn were determined by atomic emission spectrometry with inductively coupled plasma (ICP-AES), using VARIAN VISTA-PRO instrument. Analytical precision was

Table 1. Detailed pollen spectra of the honey samples from Zvegor and Chairdere, NE Bulgaria (1/6 to 4/8 – unifloral honey; 5/6 to 11/10 – polyfloral honey).

Taxa \ Sample no.	1/6 %	2/6 %	3/7 %	4/8 %	5/6 %	6/6 %	7/7 %	8/8 %	9/10 %	10/10 %	11/10 %
Helianthus annuus			2.1	71.0	1.4		1.8	27.6	16.5	3.3	33.4
Paliurus spina-christi	81.0		2.1		1.8	1.5	1.4		2.4	0.6	
Robinia pseudoacacia	2.5	33.0			13.2	2.4	2.8			0.3	
Apiaceae	1.3	5.0	62.0		3.2	6.8	2.8	2.3	3.5	30.2	3.4
Rosaceae	2.3	4.6	4.3	1.1	12.4	9.7	16.2	4.0	6.6	4.3	4.5
Echium	1.3	0.5	10.0	1.3	1.4	0.6	13.7	2.3	3.7	18.5	1.6
Scrophulariaceae	0.6				3.2	2.4	12.0	6.3	5.0	1.5	1.1
Carduus/Cirsium-type	0.6		1.1	13.0	2.8	1.2	2.5	20.0	14.2	8.2	13.4
Lotus-type	1.5		1.5		9.1	1.5	2.1	4.6	2.2	4.6	3.4
Brassicaceae	1.5	6.2	1.5	2.4	3.6	31.8	3.5	2.3	3.5	2.4	3.4
Taraxacum-type	1.5		1.1	1.1	3.2		1.8	3.4	2.2	0.6	2.3
Achillea/Aster-type	1.3	3.2	0.7	3.4	8.2	1.5	1.4	4.6	2.4	1.3	5.3
Trifolium-type	0.6	7.3	1.1	3.6	1.8	6.8	6.7	1.7	1.7	3.3	3.1
Oleaceae	1.1	4.6	2.1		1.4	4.7	1.4	2.3	1.3		1.9
Mentha/Salvia-type	0.6			1.8	1.4	1.2	1.8	3.4	4.2	1.3	1.3
Fabaceae					1.8	2.1	4.2	2.3	4.6	1.8	3.7
Ranunculaceae	0.6	2.6	1.3		3.6	2.4	4.6	4.0	2.6	1.8	1.6
Boraginaceae		33.0	0.7		1.1	1.8	2.1	1.2	1.3	0.8	1.1
Centaurea depressa	0.6		0.7	1.3	1.4	1.8	1.4	1.2	5.0	1.3	3.7
Convolvulus			0.5				0.7	0.6	1.3	0.6	0.6
Lamiaceae			1.3		2.3	1.2	1.4	0.6	1.1	2.4	1.3
Lavandula			1.1			0.9	0.7	1.2	1.3	1.3	0.6
Primulaceae					8.2	1.2	1.4	0.6	0.6	0.8	0.6
Filipendula			4.3		3.6	2.9	3.2	2.3	7.2	2.4	4.5
Sambucus			0.5			0.9	1.4	0.6	1.3	1.5	1.3
Tilia					3.2	1.2	0.7		1.1	0.3	
Salix	1.1				4.9	7.9				0.6	0.3
Cornus					1.8	1.2	2.1				0.3
Rubus							0.7	0.6	0.6	0.8	
Crataegus-type						0.9	0.7				
Verbascum-type							0.7		1.3	1.3	2.3
Geranium							0.7			0.6	
Vitis							1.4		1.3	1.3	

checked by replicating each sample, blanks and plant reference material (CRM 281). Each final concentration was the average of replicating a separate honey sample and three measurements for each solution and was expressed in mg/kg. Descriptive statistical characteristics and ANOVA analysis were applied.

Results

Four unifloral honeys were established from the eleven bee honey samples, collected in June, July, August and October 2006 from the Shumen region (Zvegor village and Chairdere locality). Two of them were collected in June: N 1/6 with *Paliurus* pollen 81 %, and N 2/6 with *Robinia pseudoacacia* pollen 33 %. One honey sample was collected in July – N 3/7 with 62 % *Apiaceae* pollen and one in August – N 4/8 with 71 % *Helianthus annuus* pollen (Table. 1).

Seven pollen samples were of polyfloral honeys, with predominance (>10 %) of the following pollen taxa:

Zvegor 5/6: *Robinia pseudoacacia* – 13.2 %, *Rosaceae* – 11 %, *Lotus* type 10 %

Zvegor 6/6: *Brassicaceae* – 32 %, *Rosaceae* – 10 %

Zvegor 7/7: *Rosaceae* – 14.4 %, *Echium* – 13.7 %, *Scrophulariaceae* – 12 %

Chairdere 8/8: *Helianthus annuus* – 27.5 %, *Cirsium/Carduus* – 20 %.

Zvegor 9/10: *Helianthus annuus* – 16.5 %, *Cirsium/Carduus* – 14.2 %

Zvegor 10/10: *Apiaceae* – 30.1 %, *Echium* – 21.2 %

Zvegor 11/10: *Helianthus annuus* – 33.4 %, *Cirsium/Carduus* – 13.4 %

Detailed pollen spectra are given in Table 1.

Melissopalynological results have shown that the predominant pollen taxa in the polyfloral honey samples are *Robinia pseudoacacia, Lotus* type, *Rosaceae*, and *Brassicaceae* in the samples collected in June; *Rosaceae*, *Echium*, and *Scrophulariaceae* in July; and *Helianthus annuus*, *Cirsium/Carduus* type, *Apiaceae* in August and October.

The heavy metals Cd, Cu, Fe, Pb, Zn and the toxic elements Al, As, V determined by atomic emission spectrometry with inductively coupled plasma (ICP-AES) registered the following detection limits: Cd, Cu, Zn – 0.004, Al and Fe – 0.04, Pb and V – 0.03 and As – 0.02 mg/l. The concentration of arsenic and vanadium were under the detection limits in the analyzed honey samples: 0.1 and 0.2 mg/kg, respectively (Table 2). The descending order of the maximum values of elements found in the different samples of honey was: Al>Fe>Zn>Cu>Pb>V, As, Cd. The maximum content of elements Al, Cu and Fe was found in the unifloral honey sample 3/7, i.e. with 62 % *Apiaceae*; the maximum Cd in unifloral honey sample 2/6 with 33 % *Robinia pseudoacacia*; and the maximum Pb in sam-

Table 2. Heavy metals and toxic elements in honey samples from the region of Shumen, Northeast Bulgaria (mg/kg).

		Al		As	Cd		Cu		Fe		Pb		V	Zn	
Honey	Site	average n=6	SD		average n=6	SD	average n=6	SD	average n=6	SD	average n=6	SD		average n=6	SD
1/6	Zvegor	2.39	0.84	<0.1	<0.02		0.44	0.07	1.49	0.37	0.45	0.13	<0.2	0.19	0.05
2/6	Zvegor	2.41	0.84	<0.1	0.04	0.01	<0.02		0.89	0.22	0.20	0.06	<0.2	0.21	0.03
3/7	Zvegor	3.10	0.78	<0.1	<0.02		0.60	0.09	2.70	0.27	0.30	0.09	<0.2	0.74	0.11
4/8	Zvegor	2.31	0.81	<0.1	<0.02		0.14	0.04	1.15	0.29	0.32	0.10	<0.2	0.63	0.09
5/6	Zvegor	2.37	0.83	<0.1	<0.02		<0.02		1.21	0.30	0.30	0.09	<0.2	0.18	0.05
6/6	Zvegor	2.58	0.65	<0.1	<0.02		<0.02		1.27	0.32	0.29	0.09	<0.2	0.77	0.12
7/7	Zvegor	2.60	0.65	<0.1	<0.02		<0.02		1.37	0.34	0.29	0.09	<0.2	0.70	0.10
8/8	Chairdere	2.44	0.61	<0.1	0.03	0.01	<0.02		1.15	0.29	0.25	0.07	<0.2	0.43	0.07
9/10	Zvegor	2.50	0.87	<0.1	<0.02		<0.02		1.06	0.26	0.19	0.06	<0.2	0.64	0.10
10/10	Zvegor	2.58	0.64	<0.1	<0.02		<0.02		0.96	0.24	0.40	0.12	<0.2	0.69	0.10
11/10	Zvegor	2.25	0.79	<0.1	<0.02		<0.02		1.10	0.28	0.30	0.09	<0.2	0.71	0.11
Average		2.50							1.30		0.30			0.54	
SD		0.23							0.49		0.08			0.24	
max		3.10			0.04		0.60		2.70		0.45			0.77	
min		2.25			<0.02		<0.02		0.89		0.19			0.18	
median		2.44							1.15		0.30			0.64	

ple 1/6 with 81 % of *Paliurus spina-christi* pollen. The only highest value of Zn was determined in the polyfloral type of honey – sample 6/6, but this maximum was close to the average Zn concentration in unifloral honey samples 3/7. The minimum content of the analyzed elements was found mainly in the group of polyfloral honey samples.

The applied ANOVA analysis confirmed lack of statistically significant difference at a 99.0 % confidence level between the means of the analyzed Al, Fe, Pb, and Zn measured in the group of unifloral honeys and the group of polyfloral honeys (P-values of the F-test were higher than 0.01: Al – 0.612, Fe – 0.215, Pb – 0.571, Zn – 0.352).

Discussion

The honey from different regions has a specific pollen spectrum with different dominants depending on the floristic composition of the region. In this preliminary study it was established that *Robinia pseudoacacia* is an important nectariferous plant for the region of Shumen. In North Bulgaria, especially along the Danube and in Dobrudzha, it yields much more nectar than in the semi-mountainous and mountainous regions. This is explained by the composition of the soils. A single flower can produce daily between 2.5 mg and 3.4 mg of nectar, with about 50 % sugar content (Petkov 2006). The status of *Paliurus spina-christi* as a nectariferous plant was not clear. We have identified *Paliurus* as a good local nectar source. This species is a drought-resisting plant with high nectar production. *Helianthus annuus* and *Brassica napus* were planted near the bee hives and they are very good nectariferous plants visited by bees predominantly for nectar. Many species of *Apiaceae* occur in the region and are also important for the bees.

The group of dominant pollen taxa in the polyfloral honeys are the *Lotus* type, *Echium, Rosaceae*, *Brassica* type, *Cirsium/Carduus* type, *Achillea/Aster* type, *Taraxacun* type, *Trifolium* type, *Lamiaceae, Salix, Boraginaceae*, etc. Thus it can be concluded that those genera and families are preferred by the bees as good nectariferous plants. The *Lotus* type and *Echium* were represented in most honey samples and the *Cirsium/Carduus* type in the samples collected in July and August. *Lotus corniculatus* is an important nectariferous plant. Every flower produces 0.9–1.05 mg of nectar in 24 hours, with 35 % of sugar content and a lot of pollen (Petkov 2006). *Carduus* and *Cirsium* are also excellent honey plants, with high nectar production and long-lasting period of flowering.

According to the published data from some European countries and the maximum admitted levels (e.g. Bogdanov & al. 1986; Barišić & al. 1999; Čelechovská & Vorlová 2001; Matei & al. 2004; Bratu & Georgescu 2005), the inorganic content of the studied honey samples collected in the approximately unpolluted area of Bulgaria is lower, with the content of Fe and Zn threefold and fivefold down, respectively. The lead content is close to the maximum values published in literature.

As honey presents a well composed environmental sample in a given area, the average inorganic content of the bioavailable elements in it could indicate and monitor the background pollution on a very local scale.

The presented data are from a first stage of a long-lasting research that will give an opportunity for juxtaposition and comparison of our results from different years and regions, as well as with the published information from similar studies.

Conclutions

As a result of the pollen analysis of 11 bee honey samples from the region of Shumen (NE Bulgaria), the most important melliferous flora has been identified. Thirty-three pollen taxa have been determined in the honey samples and 19 of them are very important and show the species, genera and families of the plants used by bees as nectar source. They are: *Helianthus annuus*, *Robinia pseudoacacia*, *Paliurus spina-christi*, *Apiaceae*, *Lotus corniculatus*, *Brassicaceae*, *Cirsium*, *Carduus*, *Echium vulgare*, *Rosaceae*, *Achillea*, *Aster, Taraxacum*, *Trifolium*, *Lamiaceae*, *Salix*, *Boraginaceae* (*Cynoglossum officinale*, *Symphytum officinale*), and *Oleaceae* (*Ligustrum vulgare*). As a result of the pollen analysis, we could also recommend future cultivation of *Helianthus annuus* and *Brassica napus*, which are very good nectariferous plants for bee forage.

The melissopalinological analysis of the 11 investigated bee honey samples shows that four are of the unifloral type, and the remaining seven of the polyfloral type, although, in principle, unifloral honey is less frequent.

The concentrations of heavy metals – cadmium, copper, iron, lead, zinc, and of the toxic elements –

aluminum, arsenic and vanadium found in the studied bee honey are approximately low. The statistical analysis of ANOVA showed insignificant differences for all analyzed elements between the group of unifloral type and polyfloral type of honey.

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