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COLLECTED POLLEN BY THE HONEY BEE (*APIS MELLIFERA* L.) ACCORDING TO ITS PROTEIN CONTENT

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Abstract: The research result indicate, that honey bees prefer to collect pollen from 2 to 8 plants species during every single month. The analysis not found difference and there is no relation between amount of collected pollen and its protein content. The quantity collected pollen by the bees depends on the population of the certain plants that bees visit. We have to take into account the differences in the discoveries of bees-scouts, when searching for new crops, flower type, the number of flowers, bees' direct access pass, the distance to the hives, ecological factors and etc. When flowering introduced agricultural plants, bees collect most of them pollen especially *Brassica napus* and *Helianthus annuus*. The results of the study show that the wide variety of pollen provided the taxons of naturally occurring flora, around 80% and 20% for introduced plants. It is necessary to keep and protect the native flora, which provide to the bees food.

Keywords: pollen, protein content, honey bee, *Apis mellifera* L.

INTRODUCTION

Pollen dispersed by insects is commonly heavier, somewhat moist and sticky and coloured in various shades of yellow, brown, orange or red (Maheshwari, 1950). The pollen loads of honey bees come in various colours, which may vary with weather conditions (Hodges, 1952). According to Hobbs and Lilly (1955) and others authors, the percentage of pollinated plants increases with the increasing visits of the honey bees. Along with this, the flow of pollen into the bee colonies increases (Voskresenska and Lopatina, 1952; Tushmalova, 1958). The choice of pollen species for collection is also subject to many influences. Individual colonies have a characteristic selection which may differ even between adjacent colonies (Maurizio, 1953; Louveaux, 1958/1959; Adams et al., 1979; Raycheva and Radev, 2012). Different colonies may utilize the local flora in different ways. Tod and Bishop (1941) and Eckert (1942)

noted that the pollen collected by colonies side by side sometimes came from predominantly different sources. Similar findings have been reported by Maurizio (1949, 1953), Louveaux (1954), Schwan and Martinovs (1954), and others. It seems that these differences between colonies arise partly through chance differences in the discoveries of bees-scouts, when searching for new crops. No close relationship between pollen collection and pollen phenology was found by Louveaux (1958).

A genetic influence is involved in preference for some pollen (Nye and Mackensen, 1970). The selection of the pollen did not seem to be influenced by their age, colour, moisture or protein content. Some pollen has a greater nutritional and biological value to the honey bees than others, giving greater longevity and greater development of brood, the glands, ovaries and fat body (Maurizio, 1950). In France the nitrogen content of the collected pollen undergoes an annual cycle which reaches its maximum in May and June (Louveaux, 1959). However, there is no evidence that the bees select pollen for its nutritive value. Louveaux (1959) reported that the pollen contains phyosterols that attract the honey bees. Parker (1926) discovered that the bees collect pollen from different plants at different times of the day. Pollen is a source of protein necessary for vital processes of honey bees (*Apis mellifera*) (Dietz, 1975; Haydak, 1935, 1937). According to Radev et al. (2014), there is a relationship between the nutritional value of pollen and the development, reproduction, and productivity of the bee colonies.

The purpose of the study is to identify whether there is a relation between the amount of collected pollen and its protein content.

MATERIALS AND METHODS

Pollen traps were placed in five bee hives and the pollen pellets were harvested every 2 days from April till September 2013 in area of Belozem (Bulgaria) (42,2° .25,033333°). The pollen loads from each hive, were analysed carefully. Four hundred twenty samples of bee-collected pollen pellets are separated over white sheets, according to colour, shape and texture.

The plant species of each pollen pellet is identified through microscopic examination of grains and the amount of collected pollen was weighted by an analytical scale. Melissopalynological analysis was carried out using similar methodology as Louveaux et al. (1978). Each identified pollen sample was placed on a slide with a drop of isoglucose and added fuchsin. The slides were then dried in not above 40°C and fixed with Entelan™ (Entelan Microscopy, Karlsruhe, Germany). To identify the pollen is used the database of the laboratory of Apiculture-Sericulture of the Agricultural school of Aristotle University and self made database of the plants in the study area.

The data on the protein content of pollen from different plant species were available from a previous study (Radev, 2018).

RESULTS AND DISCUSSION

The results of the quantity collected pollen of honey plants with known protein content from experimental colonies for each month during the three years are represented (Tables 1-6). During the experimental year, the honey bees prefer to collect pollen from 2-8 plants (Fig. 1/Tables 1-6) species during every single month, the rest of the flora is probably less preferable. Figure 1 contain the data of the amount collected pollen on the most visited plant species, of which the bees collected the greatest amount of pollen, according to its protein content.

A number of authors have also shown that the large amount of pollen usually comes from a small number of plant species (Maurizio, 1953; Percival, 1955; Louveaux, 1958/ 1959; Murrell, 1981). According to Stanley and Linskens (1974) not all plants in the same area serve as a source of pollen for bees. The pollen grains of different types of flowering plants are distinguished to each other, not only chemically but also on their physical characteristics (size,

structure, adhesiveness and oth.), pollen grains vary in size with species (and to some extent with weather conditions) from less than 5 μm to more than 200 μm , which undoubtedly affects of the speed of collection of bees, therefore the number of flights to individual bees for pollen for the day and during the season, i.e. of the common intensity of pollen-collecting.

Table 1. Amount collected and identified pollen with known protein content (g) in April 2013

Bee colony (№) →	Amount collected and identified pollen with known protein content of each bee colony (g)					Total amount of the collected and identified pollen with known protein content from the bees (g)
	№1	№2	№3	№4	№5	
Plant species						
<i>Brassica napus</i>	259.65	179.10	192.89	106.38	365.15	1103.17
<i>Salix</i> sp.	82.56	52.88	75.66	103.8	176.17	491.07
<i>Prunus cerasifera</i>	67.34	75.01	77.45	44.44	72.72	336.96
<i>Purus malus</i>	20.03	20.80	146.24	1.06	19.70	207.83
<i>Persica vulgaris</i>	35.69	15.83	5.52	6.31	61.97	125.53
<i>Lamium purpureum</i>	6.12	18.92	26.37	5.54	53.30	110.25
<i>Cornus mas</i>	39.57	50.55	0.40	12.64	1.79	104.59
<i>Cornus sanguinea</i>	0.84	96.58		0.14	0.34	97.90
<i>Morus nigra</i>	10.05	15.40	11.71	11.74	3.97	53.32
<i>Taraxacum officinale</i>	7.25	1.15	5.53	2.52	28.21	44.66
<i>Juglans regia</i>	14.61	16.92	1.33	4.88	1.20	38.94
<i>Asphodelus</i> sp.	0.51	0.30	3.62	0.30	4.77	9.50
<i>Veronica longifolia</i>	1.38	3.02	1.10	2.46	0.90	8.86
<i>Brassica nigra</i>	0.22	0.26	6.13	0.54	0.38	7.53

Table 2. Amount collected and identified pollen with known protein content (g) in May 2013

Bee colony (№) →	Amount collected and identified pollen with known protein content of each bee colony (g)					Total amount of the collected and identified pollen with known protein content from the bees (g)
	№1	№2	№3	№4	№5	
Plant species						
<i>Brassica napus</i>	147.38	111.76	227.33	215.62	276.76	978.85
<i>Papaver rhoeas</i>	144.36	45.17	201.4	148.94	158.00	697.87
<i>Amorfa fruticosa</i>	49.41	67.84	178.66	37.20	141.43	474.54
<i>Rosa canina</i>	36.12	5.39	23.58	6.50	4.33	75.92
<i>Trifolium dalmaticum</i>	18.41	1.81	32.30	13.74	3.39	69.65
<i>Robinia pseudoacacia</i>	8.29	4.77	23.79	3.23	2.95	43.03
<i>Pyrus malus</i>	4.41	2.87	24.06	1.56	2.03	35.47
<i>Plantago</i> sp.	0.89	3.04	0.48	7.43	12.46	24.30
<i>Coriandrum sativum</i>	2.69	1.48	9.39	2.22	4.18	19.96
<i>Brassica nigra</i>	0.99	0.26	8.73	1.55	0.49	12.02
<i>Ranunculus</i> sp.	0.40	0.56	0.28	1.92	6.30	9.46
<i>Vicia</i> sp.	0.10	2.05	2.22	3.45	0.77	8.59
<i>Juglans regia</i>	3.68	1.26	0.42	0.99		5.21
<i>Convolvulus arvensis</i>	0.80		1.53	0.70	0.98	4.01
<i>Morus nigra</i>	0.37	0.37	0.39	0.38	0.32	1.83
<i>Trifolium repens</i>			1.42	0.10		1.52
<i>Salix</i> sp.		0.08		0.12	0.10	1.02
<i>Centaurea cyanus</i>	0.09		0.50	0.05		0.64
<i>Crepis</i> sp.		0.05			0.44	0.49

Table 3. Amount collected and identified pollen with known protein content (g) in June 2013

Bee colony (№) →	Amount collected and identified pollen with known protein content of each bee colony (g)					Total amount of the collected and identified pollen with known protein content from the bees (g)
	№1	№2	№3	№4	№5	
Plant species						
<i>Helianthus annuus</i>	152.75	91.73	137.15	29.58	175.23	586.44
<i>Papaver rhoeas</i>	74.46	5.85	117.87	17.65	49.79	265.62
<i>Plantago</i> sp.	35.47	17.99	35.42	79.97	57.98	226.83
<i>Tilia</i> sp.	33.38	11.35	1.40	1.28	19.16	66.57
<i>Coriandrum sativum</i>	5.44	5.85	13.94	4.48	11.28	40.99
<i>Cirsium</i> sp.	30.41	0.10	0.62	0.57	0.98	32.68
<i>Convolvulus arvensis</i>	2.69	0.22	23.03	2.97	1.47	30.38
<i>Trifolium dalmaticum</i>	5.87	2.51	15.27	1.04	4.40	29.09
<i>Crepis</i> sp.	2.06	0.11	8.62	0.08	9.38	20.25
<i>Cucumis melo</i>	1.32	0.46	7.60		0.43	9.81
<i>Amorfa fruticosa</i>	0.38		1.21	0.03	2.89	4.51
<i>Carduus</i> sp.	3.80	0.39		0.07	0.07	4.33
<i>Verbascum</i> sp.			2.85		0.61	3.46
<i>Melilotus officinalis</i>	0.09	0.10	0.39	2.14	0.22	2.94
<i>Brassica napus</i>	0.11		0.58		2.20	2.89
<i>Zea mays</i>	0.03	1.37		0.38	0.71	2.49
<i>Citrullus lanatus</i>			1.21		0.06	1.27
<i>Centaurea cyanus</i>	0.51	0.05	0.15	0.09	0.16	0.96
<i>Ranunculus</i> sp.	0.34			0.16	0.30	0.80
<i>Vicia</i> sp.		0.29	0.46			0.75
<i>Cichorium intybus</i>	0.16		0.20	0.21	0.11	0.68
<i>Cucumis sativus</i>		0.61				0.61
<i>Brassica nigra</i>			0.35			0.35
<i>Amaranthus retroflexus</i>	0.11				0.17	0.28

Table 4. Amount collected and identified pollen with known protein content (g) in July 2013

Bee colony (№) →	Amount collected and identified pollen with known protein content of each bee colony (g)					Total amount of the collected and identified pollen with known protein content from the bees (g)
	№1	№2	№3	№4	№5	
Plant species						
<i>Plantago</i> sp.	187.35	101.56	48.16	159.64	158.18	654.89
<i>Helianthus annuus</i>	97.79	109.73	120.08	34.68	126.82	489.10
<i>Citrullus lanatus</i>	164.10			5.81	73.32	243.23
<i>Centaurea solstitialis</i>	72.85	28.49	37.26	2.89	30.25	171.74
<i>Centaurea</i> sp.	1.29		89.83	16.34	63.6	171.06
<i>Zea mays</i>	47.63	32.02	2.57	13.9	19.20	115.32
<i>Verbascum</i> sp.		1.37	86.01	10.04	13.36	110.78
<i>Convolvulus arvensis</i>	11.57	4.45	6.15	7.46	48.73	78.06
<i>Carduus</i> sp.	54.48	4.45	0.64	8.33	0.34	68.24
<i>Crepis</i> sp.	10.21	2.49	2.29	2.3	41.44	58.73
<i>Cirsium</i> sp.	22.82	12.15	0.88	9.75	7.14	52.74
<i>Centaurea calcitrapa</i>	19.43	14.54	1.50	4.85	6.29	46.61
<i>Portulaca grandiflora</i>	23.61	6.85	0.33	3.76	2.93	37.48
<i>Trifolium dalmaticum</i>	21.93	4.22	0.24	7.89	0.32	34.60
<i>Amaranthus retroflexus</i>	18.43	0.13	9.93	0.78	0.07	32.28
<i>Lythrum salicaria</i>	3.14	16.05		0.15		19.34
<i>Melilotus officinalis</i>	5.39	2.04	0.25	0.74	1.73	10.15
<i>Cichorium intybus</i>	0.09	2.71	6.20	0.12	0.21	9.33
<i>Eryngium campestre</i>	3.65	0.62	0.98	2.53	0.71	8.49
<i>Brassica napus</i>	4.42	1.37	0.30	0.76	0.12	6.97
<i>Melilotus sativus</i>	1.61	0.39	4.39		0.11	6.50
<i>Papaver rhoeas</i>	0.92		0.59		0.19	1.70
<i>Cucumis sativus</i>	0.28		0.13	0.41	0.04	0.86

Table 5. Amount collected and identified pollen with known protein content (g) in August 2013

Bee colony (№) →	Amount collected and identified pollen with known protein content of each bee colony (g)					Total amount of the collected and identified pollen with known protein content from the bees (g)
	№1	№2	№3	№4	№5	
Plant species						
<i>Brassica napus</i>	80.11	68.88	39.33	31.21	73.64	293.17
<i>Centaurea solstitialis</i>	118.67	19.85	51.02	13.02	73.04	275.6
<i>Convolvulus arvensis</i>	5.60	39.99	41.25	15.07	99.43	201.34
<i>Cirsium</i> sp.	30.68	32.12	6.76	2.82	13.82	86.20
<i>Centaurea</i> sp.		0.77	19.92	0.18	19.14	40.01
<i>Portulaca grandiflora</i>	9.60	7.78	3.73	16.02	2.39	39.52
<i>Dipsacus</i> sp.	14.63	3.91	4.21	1.82	12.74	37.31
<i>Carduus</i> sp.	3.30	2.50	19.06	0.93	1.10	26.89
<i>Centaurea calcitrapa</i>	4.05	3.28	1.86	10.35	6.47	26.01
<i>Helianthus annuus</i>	4.58	10.39	3.30	3.73	3.94	25.94
<i>Chondrilla juncea</i>	2.78	0.69	12.43	0.76	2.34	19.00
<i>Atriplex patula</i>	1.02	6.51	0.10	1.12	3.99	12.74
<i>Chenopodium</i> sp.	7.03	0.29	1.17	0.22		8.71
<i>Cichorium intybus</i>	1.58	0.45	5.82	0.13	0.35	8.33
<i>Plantago</i> sp.	0.68	4.37	1.50	1.01	0.36	7.92
<i>Zea mays</i>		0.78		7.14		7.92
<i>Trifolium dalmaticum</i>	4.98	0.85	0.04	0.36	0.07	6.30
<i>Verbascum</i> sp.		0.15	4.41	0.08	0.22	4.86
<i>Vicia</i> sp.	1.94					1.94
<i>Cucumis sativus</i>	0.27	0.52	0.16	0.4	0.18	1.53
<i>Citrullus lanatus</i>	0.98				0.38	1.36
<i>Crepis</i> sp.	0.07	0.27	0.62		0.11	1.07
<i>Melilotus officinalis</i>			0.23	0.26		0.49
<i>Ehinops ritro</i>	0.24			0.09		0.33

Table 6. Amount collected and identified pollen with known protein content (g) in September 2013

Bee colony (№) →	Amount collected and identified pollen with known protein content of each bee colony (g)					Total amount of the collected and identified pollen with known protein content from the bees (g)
	№1	№2	№3	№4	№5	
Plant species						
<i>Brassica napus</i>	5.28	34.94	32.8	25.49	56.31	154.82
<i>Centaurea solstitialis</i>	32.8	7.56	11.15	6.63	21.86	80.00
<i>Cirsium sp.</i>	6.13	15.94	1.35	4.23	6.17	33.82
<i>Convolvulus arvensis</i>	0.53	0.10	2.01	0.45	16.93	20.02
<i>Chondrilla juncea</i>	2.44	0.69	10.58	0.96	1.28	15.95
<i>Portulaca grandiflora</i>	1.19	1.62	0.80	2.80	0.35	6.76
<i>Carduus sp.</i>	0.25	0.61	4.75	0.13	0.75	6.49
<i>Dipsacus sp.</i>	3.10	0.99	0.19	0.73	1.19	6.20
<i>Centaurea calcitrapa</i>	1.43	0.55	0.87	1.16	1.39	5.40
<i>Chenopodium sp.</i>	1.81	0.57	1.49	0.03	0.07	3.97
<i>Atriplex patula</i>	0.57	1.92	0.07	0.81		3.37
<i>Ehinops ritro</i>	2.67	0.04		0.04	0.61	3.36
<i>Cichorium intybus</i>	0.10	0.16	1.77	0.07	0.94	3.04
<i>Cucumis sativus</i>	0.05	0.20	0.10	0.24	0.13	0.72
<i>Centaurea sp.</i>		0.04		0.35		0.39
<i>Lithrum salicaria</i>	0.04	0.11	0.09	0.12		0.36

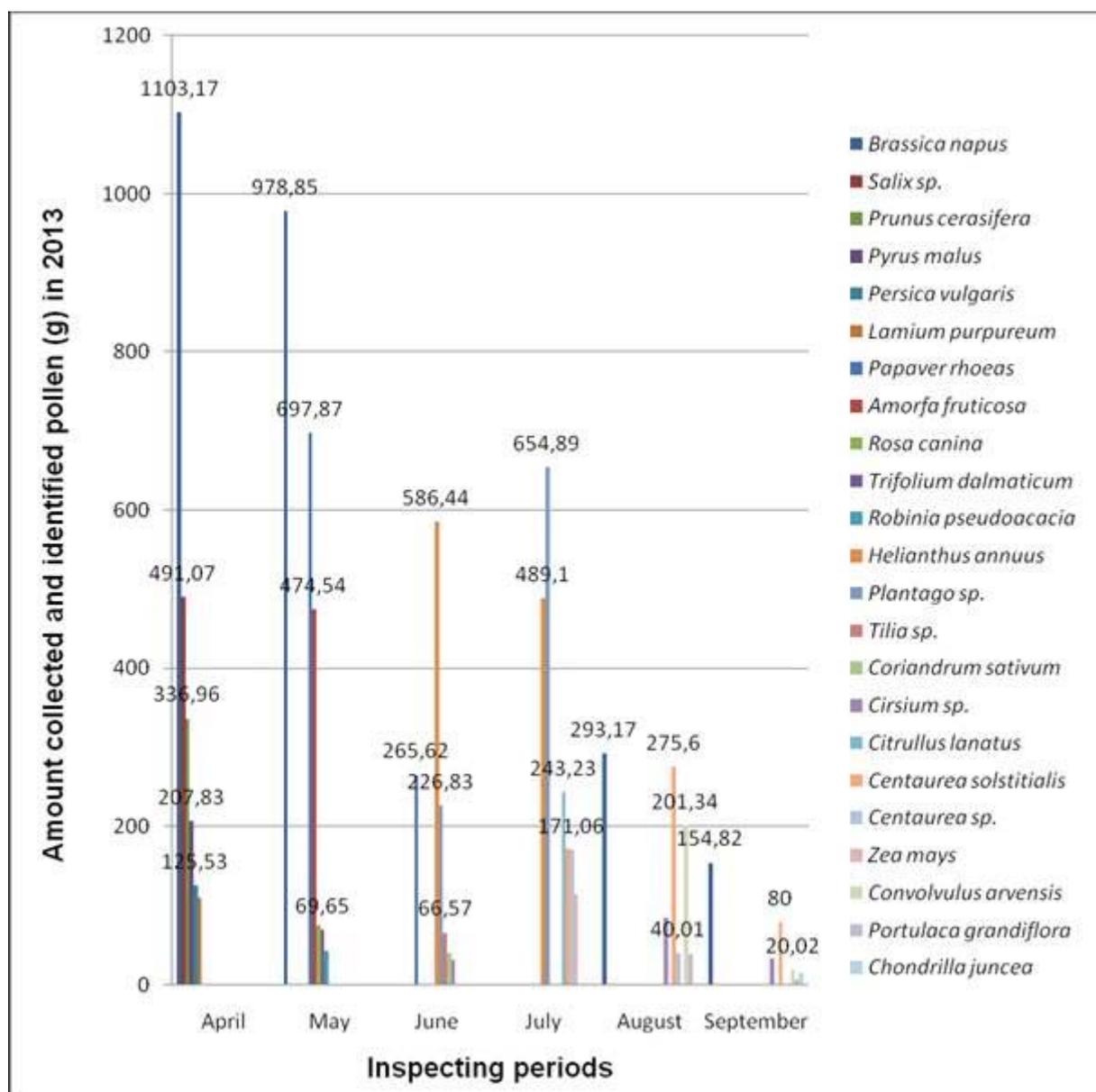


Figure 1. Amount collected and identified pollen (g) in 2013

The amount was analysed of pollen of the most visited plant species from which the honey bees have collected the largest amount of pollen during the year, according to their protein content (Fig. 2).

It is made statistical analysis (Excel) according to the protein content on the first 2-8 plant species and their collected amount. According to the result $y=0.003x+19.51$ and $R^2=0.043$, there were not found significant differences between the amount of collected pollen and its protein content (Fig. 2). It could be said that there is no relation between amount of pollens collection and their protein content. The amount of pollen that the bees collect depends on the population of the certain plants that bees visit.

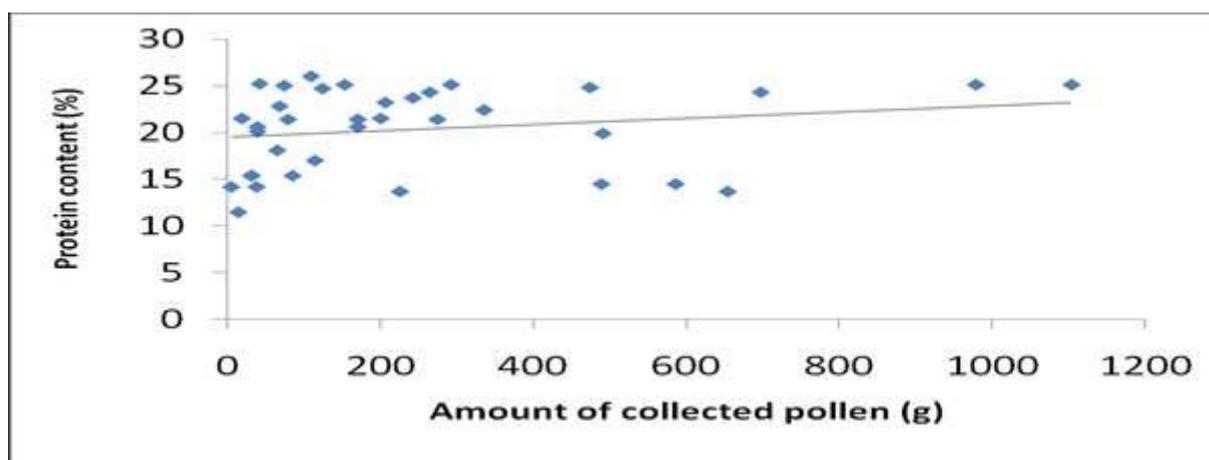


Figure 2. Amount collected pollen (g) according to its protein content (%) for 2013

The results of the study show that the wide variety of pollen provide the plants of the native flora, around 80% and 20% for introduced plants. The data confirm the results of previous studies (Radev and Gospodinova, 2015; Radev, 2015 I, 2015 II). From an ecological point of view, it is necessary to keep the naturally distributed flora in order to ensure the normal feeding of bees and maintenance of the biodiversity.

CONCLUSION

The research result indicate, that honey bees prefer to collect pollen from 2 to 8 plants species during every single month. The analysis not found difference and there is no relation between amount of collected pollen and its protein content. The quantity collected pollen by the bees depends on the population of the certain plants that bees visit. We have to take into account the differences in the discoveries of bees-scouts, when searching for new crops, flower type, the number of flowers, bees' direct access pass, the distance to the hives, ecological factors and etc. When flowering introduced agricultural plants, bees collect most of them pollen especially *Brassica napus* and *Helianthus annuus*. The results of the study show that the wide variety of pollen provide the taxons of naturally occurring flora, around 80% and 20% for introduced plants. It is necessary to keep and protect the native flora, which provide to the bees food.

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