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The Impact of Protein Content of Pollen on its Collecting by Honey Bees *(Apis mellifera L.)*

Zheko Radev*

Bulgaria

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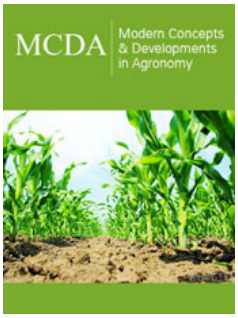

***Corresponding author:** Zheko Radev, Bulgaria

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Abstract

The study results indicate, that honey bees prefer to collect pollen from 2 to 4 plants species during every single month. The honey bees had visited 46 plant species. The analysis not found difference and there is no relation between amount of collected pollen and its protein content. The choice of pollen species for collection is subject to many influences. Different colonies may utilize the local flora in different ways. 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 *Brassica napus, Centaurea cyanus* and *Centaurea solstitialis*, bees collect most of them pollens. The results of the study show that the wide variety of pollen provided the taxons of naturally occurring flora, around 75% and 25% for introduced plants. It is necessary to keep and protect the native flora.

Keywords: Pollen; Protein content; Honey bee; *Apis mellifera* L

Introduction

The pollen loads of honey bees come in various colours, which may vary with weather conditions [1]. The percentage of pollinated plants increases with the increasing visits of the honey bees [2]. Along with this, the flow of pollen into the bee colonies increases [3-4]. Pollen dispersed by insects is commonly heavier, somewhat moist and sticky and coloured in various shades of yellow, brown, orange or red [5]. Individual colonies have a characteristic selection which may differ even between adjacent colonies [6-10]. Authors noted that the pollen collected by colonies side by side sometimes came from predominantly different sources [11,12]. Similar findings have been reported [6,13-15]. No close relationship between pollen collection and pollen phenology [7]. It seems that these differences between colonies arise partly through chance differences in the discoveries of bees-scouts, when searching for new crops. 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 [16]. A genetic influence is involved in preference for some pollen [17]. In France the nitrogen content of the collected pollen undergoes an annual cycle which reaches its maximum in May and June [8]. However, there is no evidence that the bees select pollen for its nutritive value. The pollen contains phytosteroles that attract the honey bees [8]. The bees collect pollen from different plants at different times of the day [18]. Pollen is a source of protein necessary for vital processes of honey bees (*Apis mellifera*) [19-21]. There is a relationship between the nutritional value of pollen and the development, reproduction, and productivity of the bee colonies [22]. The purpose of the study is to identify the impact of protein content of pollen on its collecting by honey bees (*Apis mellifera* L.).

Material and Methods

Pollen traps were placed in five bee hives and the pollen pellets were harvested every 2 days from April till September 2014 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 [23]. 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 [24].

Results and Discussion

The data (Table 1-6) of the quantity collected pollen of honey plants with known protein content for each month during the three years are represented. During the experimental year, the honey bees prefer to collect pollen from 2-4 plants (Figure 1/ Table 1-6) species during every single month, the rest of the flora is 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 [6,8-9,25-26], not all plants in the same area serve as a source of pollen for bees [27]. The choice of pollen species for collection is subject to many influences. Different colonies may utilize the local flora in different ways. 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 5μm to more than 200μm, 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. It is made statistical analysis (Excel) according to the protein content on 22 plant species and their collected amount. According to the result $y=0.002x+20.19$ and $R^2=0.101$, there were not found significant differences between the amount of collected pollen and its protein content (Figure 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. The results of the study show that the wide variety of pollen provide the plants of the native flora, around 75% and 25% for introduced plants. The data confirm the results of previous studies [28-30]. 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.

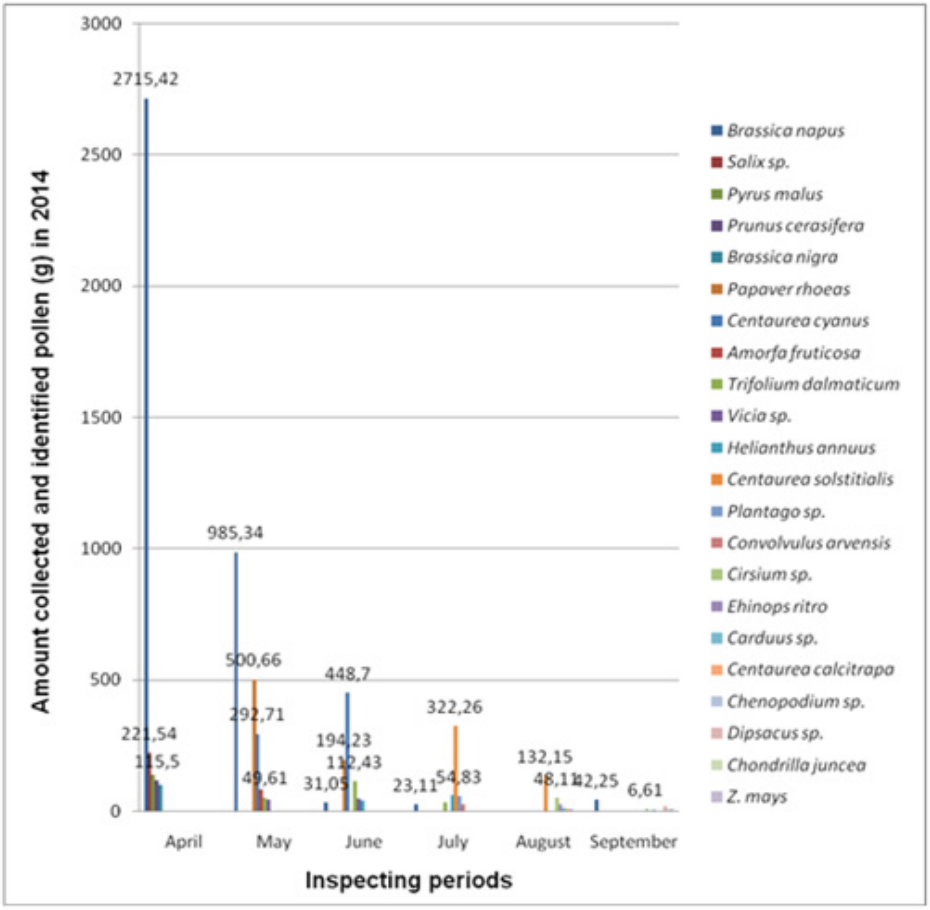

Figure 1: Amount collected and identified pollen (g) in 2014.

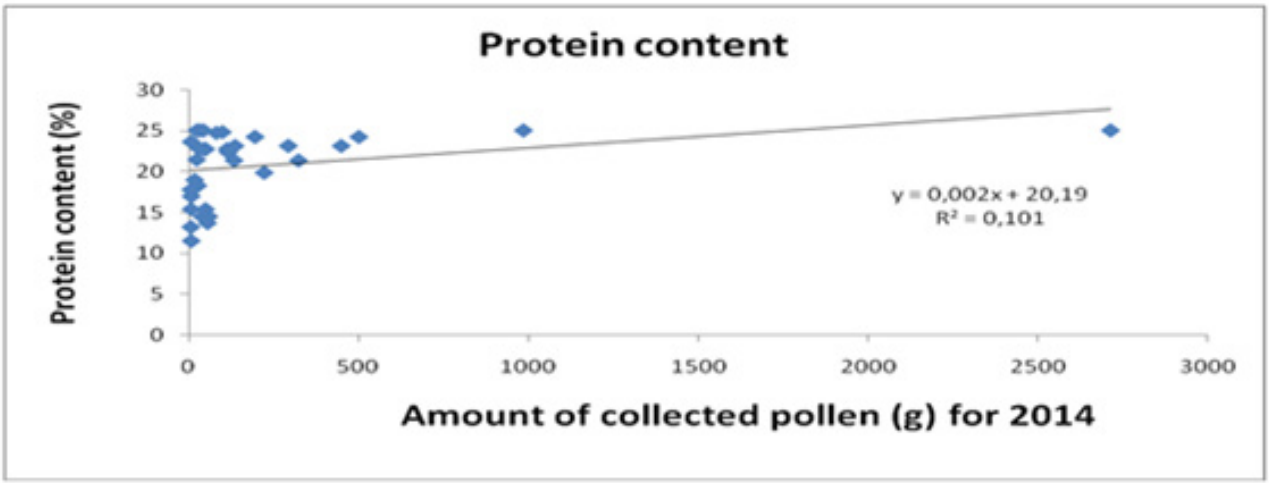

Figure 2: Amount collected pollen (g) according to its protein content (%) for 2014.

Table 1: Amount collected and identified pollen with known protein content (g) in April 2014.

	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)
Bee colony (No) →	**No1**	**No2**	**No3**	**No4**	**No5**	
Plant species						
Brassica napus	495.58	376.72	660.32	300.2	882.6	2715.42
Salix sp.	52.65	52.05	23.87	33.53	59.44	221.54
Pyrus malus	54.57	9.82	15.87	9.47	46.24	135.97
Prunus cerasifera	32.53	23.2	18.46	10.98	30.33	115.5
Brassica nigra	20.31	37.17	16.89	15.73	8.59	98.69
Juglans regia	14.01	0.5	0.27	1.48	0.19	16.45
Morus nigra	2.39	0.67	2.14	7.15	2.72	15.07
Cornus sanguinea	6.25	2.63	0.8	2.68	1.85	14.21
Persica vulgaris	3.19	1.36	2.68	2.7	1.14	11.07
Taraxacum officinale	1.91	0.36	1.21	1.35	1.29	6.12
Lamium purpureum	0.22	0.79	2.65	0.72	0.24	4.62
Veronica longifolia	0.37	0.07	0.31			0.75

Table 2: Amount collected and identified pollen with known protein content (g) in May 2014.

	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)
Bee colony (No) →	**No1**	**No2**	**No3**	**No4**	**No5**	
Plant species						
Brassica napus	188.57	88.09	418.21	68.91	221.56	985.34
Papaver rhoeas	68.24	21.93	80.5	67.26	262.73	500.66
Centaurea cyanus	111.21	45.67	106.55	26.45	2.83	292.71
Amorfa fruticosa	6.99	18.45	18.41	19.24	17.77	80.86
Trifilium dalmaticum	5.59	14.12	11.4	11.7	6.8	49.61

Vicia sp.	9.22	4.83	9.8	5.32	13.87	43.04
Robinia pseudoacacia	4.08	8.9	3.5	1.14	3.97	21.59
Trifolium repens	0.39	1.22	7.26	0.04	0.05	8.96
Brassica nigra	0.51	1.45	1.18	0.19	0.49	3.82
Convolvulus arvensis	0.29	1.6	0.95	0.18	0.44	3.46
Pyrus malus	0.12	1.49	0.4	0.57	0.34	2.92
Salix sp.	0.25	0.06	0.73	0.05	0.24	1.33
Plantago sp.	0.28	0.14	0.31		0.4	1.13
Ranunculus sp.	0.17	0.06	0.26	0.26	0.26	1.01

Table 3: Amount collected and identified pollen with known protein content (g) in June 2014.

	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)**
Bee colony (No) →	**No1**	**No2**	**No3**	**No4**	**No5**	
Plant species						
Centaurea cyanus	93.94	66.93	203.58	51.35	32.9	448.7
Papaver rhoeas	35.45	1.89	54.73	19.21	82.95	194.23
Trifolium dalmaticum	33.68	20.25	26.35	13.95	18.2	112.43
Vicia sp.	9.71	8.3	15.34	6.79	5.76	45.9
Helianthus annuus	7.32	9.18	3.28	12.5	5.98	38.26
Brassica napus	4.53	10.2	15.1	0.39	0.83	31.05
Cirsium sp.	13.59	1.55	7.6	1.45	5.17	29.36
Plantago sp.	8	10.59	4.12	0.5	2.88	26.09
Melilotus officinalis	1.15	3.52	1.67	0.56	1.85	8.75
Amorfa fruticosa	0.09	2.49	2.26	0.24	0.59	5.67
Carduus sp.	0.18	1.17	0.43	0.14	0.33	2.25
Coriandrium sativum	0.94	0.61			0.35	1.9
Trifolium repens	1.46	0.08	0.27	0.07		1.88
Tilia sp.	0.1	0.16	0.1	0.18	0.64	1.18
Crepis sp.	0.07	0.1	0.42		0.08	0.67
Cucumis melo	0.47					0.47
Verbascum sp.	0.24	0.04				0.28
Convolvulus arvensis	0.09		0.14			0.23
Zea mays				0.13		0.13

Table 4: Amount collected and identified pollen with known protein content (g) in July 2014.

	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)**
Bee colony (No) →	**No1**	**No2**	**No3**	**No4**	**No5**	
Plant species						
Centaurea solstitialis	52.77	24.85	77.5	107.9	59.23	322.26

Helianthus annuus	5.24	6.55	5.77	14.72	26.33	58.61
Plantago sp.	10.19	8.37	7.4	9.66	19.21	54.83
Trifolium dalmaticum	6.85	3.74	10.51	6.48	4.33	31.91
Cirsium sp.	1.03	2.38	11.7	3.38	4.62	23.11
Brassica napus	2.03	5.58	9.53	2.55	3.23	22.92
Convolvulus arvensis	2.61	0.55	0.23	8.84	5.34	17.57
Citrullus lanatus	0.05	0.48	1.74	14.1		16.37
Centaurea cyanus	1.72	1.16	3.55	0.07	0.13	6.63
Ehinops ritro	1.41	0.14	0.33	1.41	0.37	3.66
Zea mays	0.52	0.38	0.06	1.8	0.74	3.5
Vicia sp.	0.08	0.79	0.06	0.74	1.28	2.95
Cichorium intybus		0.03	0.1	1.83	0.67	2.63
Carduus sp.	0.12	0.19	0.45	0.78	0.33	1.87
Verbascum sp.	0.83	0.12	0.22	0.35	0.14	1.66
Melilotus officinalis		0.3	0.26	0.67		1.23
Crepis sp.	0.04	0.54	0.05	0.26	0.1	0.99
Portulaca grandiflora	0.62	0.06		0.19		0.87
Centaurea sp.			0.16	0.13	0.29	0.58
Centaurea calcitrapa	0.04	0.02		0.44	0.06	0.56
Papaver rhoeas	0.25	0.12	0.16			0.53
Cucumis melo	0.06	0.1	0.02	0.15	0.03	0.36
Eryngium campestre					0.27	0.27

Table 5: Amount collected and identified pollen with known protein content (g) in August 2014.

	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)**
Bee colony (No) →	**No1**	**No2**	**No3**	**No4**	**No5**	
Plant species						
Centaurea solstitialis	41.6	4.62	20.76	39.73	25.44	132.15
Cirsium sp.	1.19	2.87	0.04	26.19	17.82	48.11
Ehinops ritro	0.48	1.83	0.15	6.72	15.98	25.16
Carduus sp.	1.13	1.22	2.84	0.48	3.75	9.42
Centaurea calcitrapa	2.08	0.22	0.1	2.05	2.1	6.55
Chenopodium sp.	2.14	0.09	0.12	0.62	2.59	5.56
Plantago sp.	2.23	0.61	0.61	0.09	1.38	4.92
Portulaca grandiflora	0.55	0.56		1.36	0.25	2.72
Dipsacus sp.	0.13	0.21	0.21	0.23	1.29	2.07
Brassica napus	0.52		0.08	0.75	0.36	1.71
Atriplex patula		0.17	0.35	0.53	0.22	1.27
Chondrilla juncea	0.48	0.28	0.12	0.18	0.21	1.27

Centaurea sp.	0.06	0.14	0.42	0.06	0.07	0.75
Vicia sp.	0.27	0.07	0.06		0.2	0.6
Cichorium intybus	0.19	0.12	0.06	0.05		0.42
Convolvulus arvensis			0.32	0.09		0.41
Trifolium dalmaticum	0.07	0.11	0.05		0.14	0.37
Melilotus officinalis	0.05			0.15	0.06	0.26
Cucumis melo		0.02	0.11	0.02	0.03	0.18
Eryngium campestre					0.17	0.17
Centaurea cyanus			0.14			0.14
Lythrum salicaria	0.05		0.06	0.03		0.14
Zea mays	0.04					0.04

Table 6: Amount collected and identified pollen with known protein content (g) in September 2014.

	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)**
Bee colony (No) →	**No1**	**No2**	**No3**	**No4**	**No5**	
Plant species						
Brassica napus	1.76	2.25		35.88	2.36	42.25
Dipsacus sp.	3.77			0.6	11.96	16.33
Chondrilla juncea	0.33	0.02	3.2	2.91	0.34	6.8
Cirsium sp.	0.67	3.01	0.08	1.39	1.46	6.61
Zea mays		0.93	0.48	5.12		6.53
Carduus sp.	0.69		3.84	0.07	0.47	5.07
Chenopodium sp.	0.16	0.12	2.24	0.47	0.26	3.25
Centaurea calcitrapa	0.31	0.2	0.85	0.88	0.77	3.01
Cucumis sativus	0.09	0.06	0.1	1.01	0.63	1.89
Centaurea solstitialis	0.69		0.45	0.29	0.03	1.46
Portulaca grandiflora		0.1	0.04	1.07	0.13	1.34
Ehinops ritro	0.08	0.18	0.03	0.52	0.34	1.15
Centaurea sp.	0.45		0.1	0.07		0.62
Cichorium intybus			0.25	0.12	0.15	0.52
Verbascum sp.	0.07		0.19		0.19	0.45
Convolvulus arvensis	0.07		0.22	0.06		0.35
Plantago sp.			0.19		0.05	0.24
Atriplex patula				0.2		0.2
Cucumis melo		0.02	0.11	0.02	0.03	0.18
Eryngium campestre					0.17	0.17
Centaurea cyanus			0.14			0.14

Lythrum salicaria	0.05		0.06	0.03		0.14
Zea mays	0.04					0.04

Conclusion

The study results indicate, that honey bees prefer to collect pollen from 2 to 4 plants species during every single month. The honey bees had visited 46 plant species. 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, *Brassica napus, Centaurea cyanus* and *Centaurea solstitialis*, bees collect most of them pollens. The results of the study show that the wide variety of pollen provided the taxons of naturally occurring flora, around 75% and 25% for introduced plants. It is necessary to keep and protect the native flora.

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