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PAPAVER RHOEAS L. BEE POLLEN

Метою наших досліджень було встановити морфологічні і спектрометричні характеристики, вміст фенольних сполук та антиоксидантну активність бджолиного обніжжя з *P. rhoeas*. Зразки монофлорного і поліфлорного бджолиного обніжжя були зібрані в районах Київської області (Україна) в літній період 2016 року за допомогою навісних пилковловлювачів. Ботанічне походження, монофлорність, сформованість і морфологічні параметри пилкових грудочок визначали в лабораторії кафедри конярства і бджільництва Національного університету біоресурсів і природокористування України. Спектрометричні параметри й антиоксидантну активність бджолиного обніжжя з *P. rhoeas* досліджували в лабораторії Інституту охорони біорізноманіття і біологічної безпеки Словацького аграрного університету в Нітрі. Біохімічні аналізи проводили в лабораторії кафедри зберігання та переробки рослинних продуктів Словацького аграрного університету в Нітрі.

Установили морфологічні параметри пилкової грудочки бджолиного обніжжя з *P. rhoeas*: довжина – $3,31 \pm 0,033$ мм, ширина – $2,97 \pm 0,044$ мм; маса – $9,87 \pm 0,25$ мг. Чистота монофлорного бджолиного обніжжя з *P. Rhoeas* знаходилася в межах від 85 до 91 %. Поліфлорне бджолине обніжжя завжди містило менше 80 % пилкових грудочок з *P. rhoeas*; у середньому в поліфлорному збірі обніжжя пилкові грудочки з *P. rhoeas* траплялись у кількості 38 %. Визначили параметри кольору *P. rhoeas* бджолиного обніжжя для його ботанічної ідентифікації. Методом вимірювань із виключенням дзеркальної складової з освітлювачами D65/10 ° і A/10 ° відповідно: L* – $33,88 \pm 0,25$ і $33,91 \pm 0,25$; a* – $0,04 \pm 0,07$ і $0,14 \pm 0,12$; b* – $4,42 \pm 0,13$ і $4,45 \pm 0,12$; C* – $4,43 \pm 0,13$ і $4,47 \pm 0,11$; h° – $89,34 \pm 0,87$ і $88,01 \pm 1,68$. Методом вимірювань з урахуванням дзеркальної складової з освітлювачами D65/10 ° і A/10 ° відповідно: L* – $41,09 \pm 0,13$ і $41,11 \pm 0,13$; a* – $-0,04 \pm 0,03$ і $0,03 \pm 0,08$; b* – $3,28 \pm 0,07$ і $3,31 \pm 0,07$; C* – $3,29 \pm 0,07$ і $3,32 \pm 0,07$; h° – $90,76 \pm 0,56$ і $89,4 \pm 1,39$. Находження одна на одну ліній, які показують результати кожного вимірювання монофлорного бджолиного обніжжя з *P. rhoeas*, на спектральному графіку свідчить про гетерогенність пилкових грудочок. Антиоксидантна активність бджолиного обніжжя з *P. rhoeas* у водному і спиртовому розчинах становить $68,61 \pm 6,712$ % і $55,80 \pm 1,492$ % відповідно. Вміст фенольних сполук – $419,16 \pm 9,356$ мг ТЕАС/г; фенольних кислот – $2,40 \pm 0,052$ мг САЕ/г; поліфенолів – $16,47 \pm 0,339$ мг ГАЕ/г; флавоноїдів – $13,34 \pm 1,533$ мг QE/г.

Ключові слова: бджолине обніжжя, *Papaver rhoeas* L., монофлорність, спектрометрія, антиоксиданти, фенольні сполуки.

Formulation of the problem. The use of bee pollen in the food, pharmaceutical and medicine industries causes the need of a deep morphological research and of the spectrometric parameters for interspecific product identification and further determination of its biochemical and microbiological characteristics. The popularization of functional nutrition manufacturers to review the requirements for quality and safety of products, improve technologies, environmentally friendly production and processing.

Analysis of recent researches and publications. The most scientific information and study of the species *Papaver rhoeas* L. its characteristics as a harmful weed in the crops of agricultural plants [1, 2, 3]. However, due to the considerable distribution of this species on meadows, forests and animal wings, *P. rhoeas* is gaining importance as polliniferous plant.

Scientists also convinced of the effectiveness of using *P. rhoeas* as a drug substance. It has been established that seed of the species contains readine, protopin, papaver rubin, A, B, C, D, E, regenine, isoregenin, isoradin, allocryptopin, coridine, stylopin, isocoridine, berberine and other alkaloids; sitosterol, higher aliphatic alcohols and fatty acids, anthocyanins, pectin, iron salts and magnesium [4, 5].

P. rhoeas anatomical and morphological features of peduncle and self-incompatibility pollen of plant were studied [6, 7, 8]. Detailed studies were of pollen grains of this species. Thus, M. Cresti, C. Milanesi, P., Salvatici и A. C. Aelst, (1990) point to such features of mature pollen grains – «The mature pollen grain

of *Papaver rhoeas* is bicellular. The vegetative cell contains numerous mitochondria; endoplasmic reticulum is not very extensive and there are few ribosomes and plastids. Golgi bodies are in a very active state. The generative cell is lobed and spindle-shaped. The cytoplasm contains many, generally longitudinally arranged, bundles of microtubules. Other organelles are few in number, and include mitochondria, Golgi bodies and short cisternae of endoplasmic reticulum» [9].

P. rhoeas pollen grains morphological features had been studied earlierly. Shape was defined elliptic in the polar view and circular in the equatorial view (Al-Quaran, 2010). According to others, shape is circular in the polar view, lobate in the dry pollen (PalDat). Exine sculpture was scabrate, verrucate, psilate, perforate. Length of polar axis – 39,7 μm , length of equatorial axis – 28,4 μm [10, 11].

Given that the *P. rhoeas* vegetable raw material is valuable for the pharmaceutical industry, so it will be relevant to investigate bee pollen.

It is known [12, 13, 14], that bee pollen has a high content of biologically active substances depending on its botanical origin. Recently, researchers have established the morphological structure of pollen lumps: weight – 10,11 mg, height – 2,86 mm, width – 2,45 mm [10].

Scientists have presented a lot of results on biologically active compounds in polyfloral bee pollen [10, 12], however, monofloral pollen informations is very few. Comparing monofloral bee pollen from other plant species has met the following data. It has been determined [15], 75 wt. % ethanol/water extracts of *Schisandra chinensis* (Turcz.) Baill., *Brassica napus* L., *Phellodendron amurense* Rupr., *Prunus armeniaca* L. and *Taraxacum officinale* L. monofloral bee pollen had stronger antioxidant activities. And *Prunus armeniaca* L., *Camellia* spp. and *Helianthus annuus* L. monofloral bee pollen presented excellent tyrosinase inhibitory activities. *Prunus armeniaca* L. pollen exhibits both powerful antioxidant and strong tyrosinase inhibitory activities.

Other scientists have established the antioxidant properties of examined plant species were different and decreasing in the following order: *Brassica napus* subsp. *napus* L. > *Papaver somniferum* L. > *Helianthus annuus* L.

Before that we were identified specific features of bee pollen with *Corylus avellana* L., *Salix* spp., *Acer* spp., *Brassica napus* L. [16, 17, 18, 19]. However, questions remain insufficiently studied morphological and biochemical characteristics of *P. rhoeas* monofloral bee pollen.

Therefore, the aim of our research was to establish morphological and spectrometric characteristics, the content of phenolic compounds and the antioxidant activity of *P. rhoeas* bee pollen.

Materials and methods. *P. rhoeas* bee pollen was taken from locations in Kiev region in the summer period 2016. Bee pollen is selected by outer pollen traps of bee colonies from local populations. Monoflorality ratio of total bee pollen collection was determined by using percentage of *P. rhoeas* pollen lumps to all other [20]. Botanical origin of bee pollen was installed by using pollen analysis [20]. Morphological features of bee pollen were defined in the laboratory of Institute Biodiversity Conservation and Biosafety, Slovak University of Agriculture in Nitra. Weight of individual pollen lumps was determined by using analytical scales ANG 100C (Axis). Length and width of bee pollen were measured with software Ascension Waves Vision on photos of pollen lumps from electron microscope Zeiss SteREO Discovery V20. Color of bee pollen was determined by construct CIEL*a*b* color space model by using spectrometry devices at Nicolet 6700 FT-IR Spectrometer and Lovibond SP62 S/N 044929. Used SCE (Specular Component Excluded) and SCI (Specular Component Included) methods. Bee pollen shaping level was determined by method, which was developed at the Department of beekeeping NULES of Ukraine [21]. The content of phenolic compounds and antioxidant activity of bee pollen were determined using standardized methods on the equipment laboratory of Institute Biodiversity Conservation and Biosafety, Slovak University of Agriculture in Nitra. Obtained numeric data were subjected to the statistical analysis.

Results of the research. Dimensions of the length and width of pollen lumps were determined from the average sample of *P. rhoeas* bee pollen (n=30). The length was in the range from 2,9 to 3,77 mm and averaged $3,31 \pm 0,033$ mm. The correlation coefficient of 7,15 % indicated a low degree of variability of this feature ($C_v \leq 10$ %). The width of the pollen lobes was in the range from 2,26 to 3,47 mm, averaged $2,97 \pm 0,044$ mm. The correlation coefficient of 10,6 %, indicates the average degree of variability of this feature ($C_v \geq 10$ %). Can be assumed that of the pollen load width depends on the level of formation bee pollen and may vary depending on filling capacity of pollen collection basket on bee`s leg. In contrast, the length is stable and depends on the length of pollen collection basket on bee`s leg.

In general, we can state that for *P. rhoeas* bee pollen the average size of pollen loads is 3,31 mm in length and 2,97 mm in width (fig. 1).

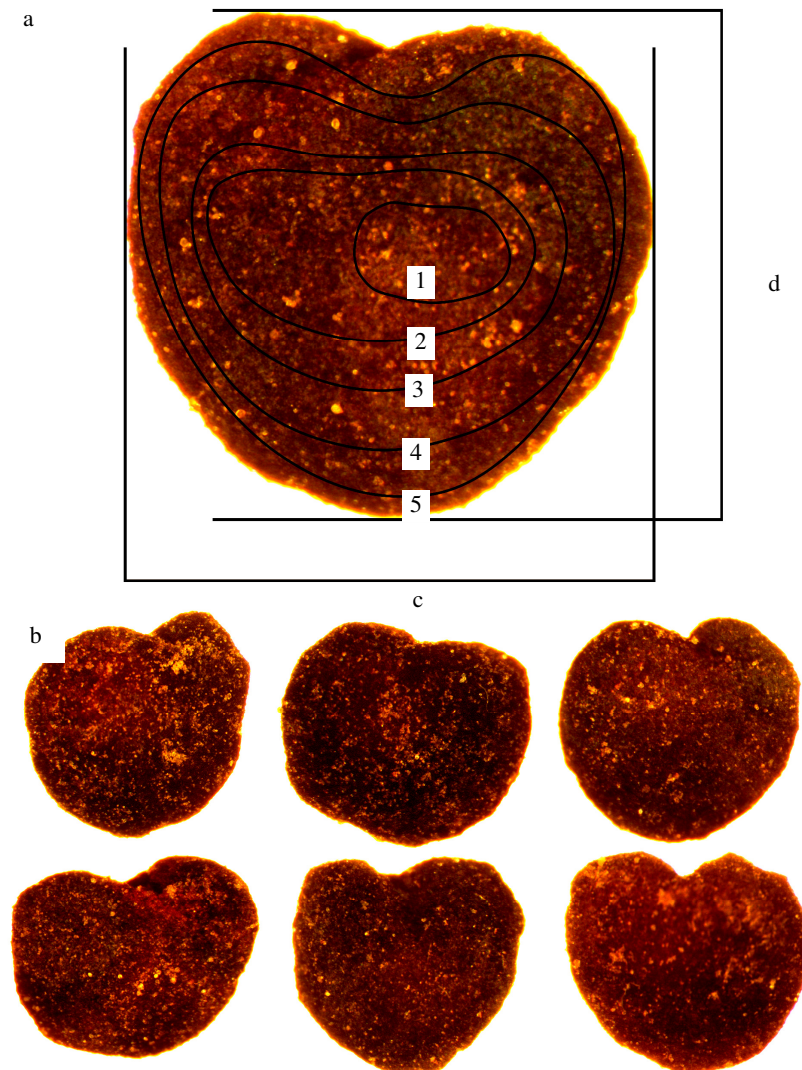


Figure 1. The shape level and morphological parameters bee pollen lumps of *Papaver rhoeas* L.
 (a – morphometric measurements and scale of pollen shape level; b – diversity of bee pollen;
 c – width of pollen lump; d – length of pollen lump, 1-5 – levels of shaping)

Determine the weight of one pollen load from the average sample of *P. rhoeas* bee pollen (n=50). This indicator was in the range from 6,7 to 13,7 mg, average it was $9,87 \pm 0,25$ mg. The coefficient of variation was 18,3 %, which indicates a high degree of variability ($Cv \geq 10$ %). The scope of the data average weight means different density of formation of pollen loads by bees. And consequently, it affects the different concentrations of nutrients in bee pollen, influencing the biochemical characteristics of the product. As a result of the visual assessment, it was found that the color of *P. rhoeas* monofloral bee pollen from was different depending on the collection period. Probably this was due to falling into pollen lumps of pollen of other plant species. The percentage of monoflority were determined in bee pollen collected samples using pollen analysis (fig. 2).

Purity *P. rhoeas* monofloral bee pollen are in the range from 85 to 91 %. Polyfloral bee pollen always less than 80 % *P. rhoeas* pollen loads, and on average, in polyfloral collection pollen gets 38 %.

Color of bee pollen was determined the means of color perception by using the parameters: lightness (L^*); the ratio from green to red color (a^*); the ratio from blue color to yellow (b^*); relative saturation (C^*); hue angle (h°) (tab. 1).

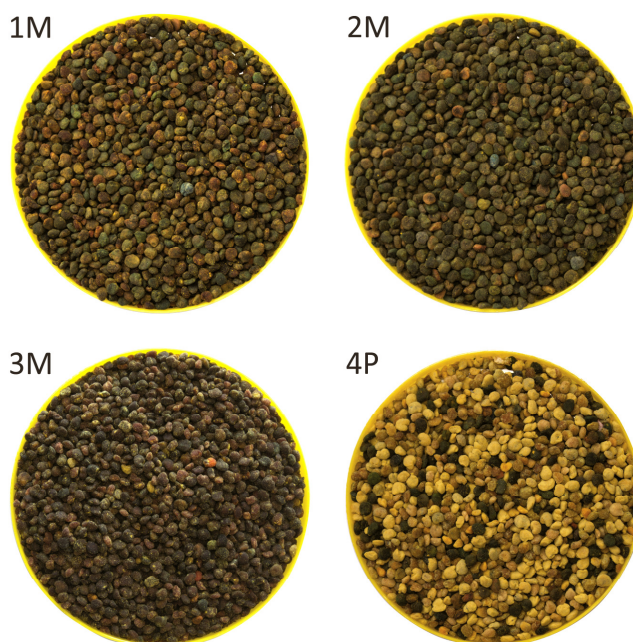


Figure 2. **Samples bee pollen:** 1M – monofloral (sample № 2, 18–12 June, 87 % – *P. rhoeas*, 13 % – others spices); 2M – monofloral (sample № 10, 19–25 June, 85 % – *P. rhoeas*, 15 % – others spices); 3M – monofloral (sample № 58, 7–10 July, 91 % – *P. rhoeas*, 9 % – others spices); 4P – polyfloral (sample № 15, 19–25 June, 38 % – *P. rhoeas*, 62 % – others spices).

Table 1 – *P. rhoeas* bee pollen spectrometric parameters (n=10)

Indicator	Spectrometric parameter				
	L*	a*	b*	C*	h°
Primary Illuminant D65/10°, SCI method					
Min	40,41	-0,19	2,95	2,95	87,73
Max	41,75	0,12	3,65	3,65	93,19
X ± Sx	41,09 ± 0,13	-0,04 ± 0,03	3,28 ± 0,07	3,29 ± 0,07	90,76 ± 0,56
δ	0,42	0,103	0,23	0,23	1,76
C _v (%)	1,03	-232,003	6,99	7,01	1,95
Primary Illuminant D65/10°, SCE method					
Min	32,49	-0,26	3,64	3,65	85,11
Max	35,11	0,36	4,96	4,97	93,14
X ± Sx	33,88 ± 0,25	0,04 ± 0,07	4,42 ± 0,13	4,43 ± 0,13	89,34 ± 0,87
δ	0,79	0,22	0,42	0,42	2,76
C _v (%)	2,33	515,79	9,51	9,503	3,09
Primary Illuminant A/10°, SCI method					
Min	40,41	-0,19	2,95	2,95	77,84
Max	41,75	0,71	3,65	3,65	93,19
X ± Sx	41,11 ± 0,13	0,03 ± 0,08	3,31 ± 0,07	3,32 ± 0,07	89,4 ± 1,39
δ	0,41	0,26	0,22	0,22	4,43
C _v (%)	1,005	763,99	6,57	6,58	4,95
Primary Illuminant A/10°, SCE method					
Min	32,49	-0,26	3,95	4,02	75,0055
Max	35,11	1,06	4,96	4,97	93,14
X ± Sx	33,91 ± 0,25	0,14 ± 0,12	4,45 ± 0,12	4,47 ± 0,11	88,01 ± 1,68
δ	0,78	0,39	0,37	0,35	5,33
C _v (%)	2,29	284,45	8,22	7,75	6,05

Notation. X – arithmetic mean; Sx – error of a measurement; Max, Min – maximum, minimum value sample; C_v – coefficient of variation; δ – standard deviation; L – lightness; a – the ratio from green to red color; b – the ratio from blue color to yellow; C – relative saturation; h° – hue angle; Primary Illuminant D65/10° – is a commonly used standard illuminant defined by the International Commission on Illumination; Primary Illuminant A/10° – is intended to represent typical, domestic, tungsten-filament lighting; SCE – Specular Component Excluded method; SCI – Specular Component Included method.

According to research results of color model parameters with different methods (Specular Component Excluded and Specular Component Included) using the standard illuminant (D65/10°) and typical illuminant (A/10°), the averaged data were received spectrometric parameters, which later can be used for identification of *P. rhoeas* bee pollen.

The difference in monoflora of the studied samples of bee pollen shows Report Color Plot and Report Spectral Plot (fig. 3–4).

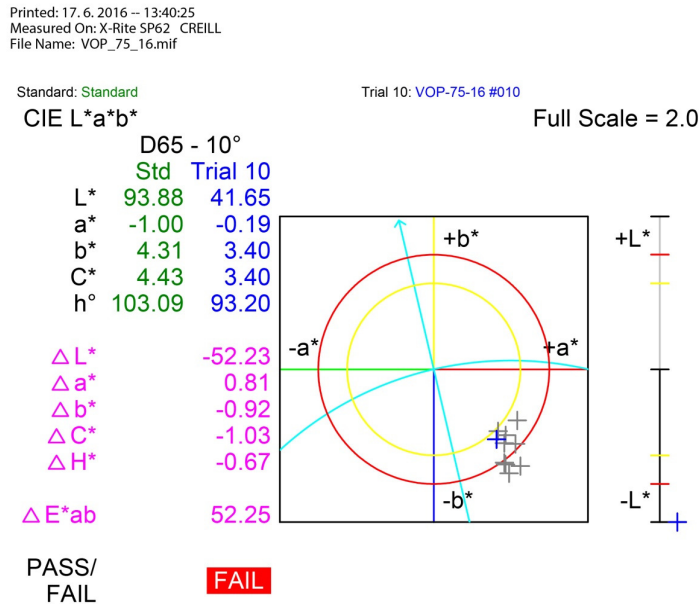


Figure 3. *P. rhoeas* monofloral bee pollen Report Color Plot (n=10)

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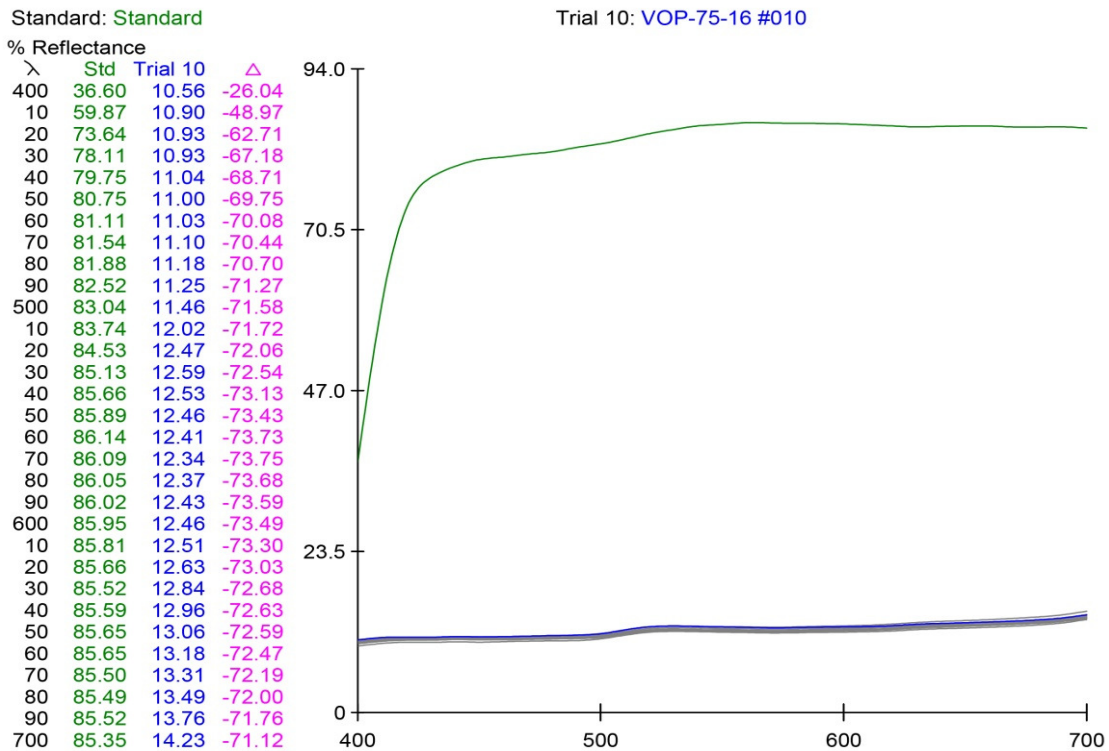


Figure 4. *P. rhoeas* monofloral bee pollen Report Spectral Plot (n=10).

Going one by one lines by *P. rhoeas* monofloral bee pollen Spectral Plot, which show the results of each measurement there is evidence heterogeneous pollen grains in bee pollen. That, pollen loads of bee pollen contain only *P. rhoeas* pollen grains. On Color Plot reflected square identification of the color of bee pollen in the color model CIEL*a*b* color space.

After confirmation of monoflority *P. rhoeas* bee pollen, determined the content of biologically active substances. Namely, antioxidant activity of water and methanol solution (%); phenolic acids (mg CAE/g) and phenolic compounds with phosphomolybdenic method (mg TEAC/g); polyphenols (mg GAE/g) and flavonoids (mg QE/g) (tab. 2).

Table 2 – *P. rhoeas* bee pollen biologically active substances (n=3)

№	Indicator	Value, $X \pm Sx$
1	Antioxidant activity of water solution, %	68,61 ± 6,712
2	Antioxidant activity of methanol solution, %	55,80 ± 1,492
3	Phenolic compounds with phosphomolybdenic method, mg TEAC/g	419,16 ± 9,356
4	Polyphenols, mg GAE/g	16,47 ± 0,339
5	Flavonoids, mg QE/g	13,34 ± 1,533
6	Phenolic acids, mg CAE/g	2,40 ± 0,052

Notation. TEAC – trolox equivalent antioxidant capacity; GAE – gallic acid equivalent; CAE – caffeic acid equivalent.

It was found that in the water solution, the antioxidant activity of *P. rhoeas* bee pollen was higher by 12,81 % compared to methanol. Total content of phenolic compounds using phosphomolybdenic method was on average 419,16 ± 9,356 mg TEAC/g. Of these, phenolic acids were 2,40 ± 0,052 mg CAE/g and polyphenols 16,47 ± 0,339 mg GAE/g. Contents flavonoids was 13,34 ± 1,533 mg QE/g.

Conclusions. *P. rhoeas* bee pollen loads morphometric parameter are length 3,31 ± 0,033 mm, width 2,97 ± 0,044 mm and weight 9,87 ± 0,25 mg. Purity *P. rhoeas* monofloral bee pollen are in the range from 85 to 91 %. Polyfloral bee pollen always less than 80 % *P. rhoeas* pollen loads, and on average, in polyfloral collection pollen gets 38 %.

The color parameters of *P. rhoeas* bee pollen for its botanical identification have been investigated. Specular Component Excluded method with illuminants D65/10° and A/10° respectively: L* – 33,88 ± 0,25 and 33,91 ± 0,25; a* – 0,04 ± 0,07 and 0,14 ± 0,12; b* – 4,42 ± 0,13 and 4,45 ± 0,12; C* – 4,43 ± 0,13 and 4,47 ± 0,11; h° – 89,34 ± 0,87 and 88,01 ± 1,68. Specular Component Included method with illuminants D65/10° and A/10° respectively: L* – 41,09 ± 0,13 and 41,11 ± 0,13; a* – -0,04 ± 0,03 and 0,03 ± 0,08; b* – 3,28 ± 0,07 and 3,31 ± 0,07; C* – 3,29 ± 0,07 and 3,32 ± 0,07; h° – 90,76 ± 0,56 and 89,4 ± 1,39.

Heterogeneous pollen grains in bee pollen are confirmed by the results of each measurement of *P. rhoeas* monofloral bee pollens, which show one over one lines on Spectral Plot.

Antioxidant activity of *P. rhoeas* bee pollen in water and methanol solution were 68,61 ± 6,712 and 55,80 ± 1,492 % respectively. The content of phenolic compounds is 419,16 ± 9,356 mg TEAC/g; phenolic acids – 2,40 ± 0,052 mg CAE/g; polyphenols – 16,47 ± 0,339 mg GAE/g; flavonoids – 13,34 ± 1,533 mg QE/g.

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Пчелиная обножка с *Papaver rhoeas* L.

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Целью наших исследований было установить морфологические и спектрометрические характеристики, содержание фенольных соединений и антиоксидантную активность пчелиной обножки с *P. rhoeas*. Образцы монофлорной и полифлорной пчелиной обножки были собраны в районах Киевской области (Украина) в летний период 2016 года с помощью навесных пыльцеулавливателей. Ботаническое происхождение, монофлорность, сформированность и морфологические параметры пыльцевых комочков определяли в лаборатории кафедры коневодства и пчеловодства Национального университета биоресурсов и природопользования Украины. Спектрометрические параметры и антиоксидантную активность пчелиной обножки с *P. rhoeas* исследовали в лаборатории Института охраны биоразнообразия и биологической безопасности Словацкого аграрного университета в Нитре. Биохимические анализы проводили в лаборатории кафедры хранения и переработки растительных продуктов Словацкого аграрного университета в Нитре.

Установили морфологические параметры пыльцевых комочков пчелиной обножки с *P. rhoeas*: длина – 3,31 ± 0,033 мм, ширина – 2,97 ± 0,044 мм; масса – 9,87 ± 0,25 мг. Чистота монофлорной пчелиной обножки с *P. rhoeas* находилась в пределах от 85 до 91%. Полифлорная пчелиная обножка всегда содержала менее 80% пыльцевых комочков с *P. rhoeas*; в среднем в полифлорном сборе обножки пыльцевые комочки с *P. rhoeas* встречались в количе-