Списание за наука „Ново знание“
ISSN 2367-4598 (Online)
Академично издателство „Талант“
Висше училище по агробизнес и развитие на регионите - Пловдив

New Knowledge Journal of Science
ISSN 2367-4598 (Online)
Academic Publishing House „Talent“
University of Agribusiness and Rural Development - Bulgaria

http://science.uard.bg

DYNAMIC OF THE PROTEIN CONTENT IN MIXED HONEY-BEE COLLECTED POLLEN DURING APICULTURAL SEASON

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Abstract: The knowledge of the protein content of pollen in the area of beekeeping is important about the bee colonies. Pollen collected by honey bees has different protein content at different seasons of the year and for each year, which depend of distribution of the plants and the climate condition. The statistical analysis of the data proved a difference in the protein content in pollen between the summer, spring and autumn. In the study area the percentage of protein content in the examined mixed pollen samples has different value: in 2012 ranged from 13.9% to 25%, in 2013 it ranged from 15.6% to 25.1% and for 2014 it was from 18.4% to 27.8% and the average value is 21%. The study results showed that the protein content of mixed pollen varied for each reporting period of each month and for each single month. The reason of the differences probably came from the dynamic change of the ecological factors and the urban environment, which influenced flowering of the plants. The florospecialization of the honey bees also play major role.

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

INTRODUCTION

Pollen is the source of protein necessary for vital processes of the honey bees (*Apis mellifera*) (Dietz, 1975). The protein contained into the pollen is extremely important and depends on the plants which are visited by the bees. It is not proven difference and 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 (Radev, 2018). According to Tyurner et al. (1972) before the massive nectar collection, the protein is needed for the bees to increase the strength of the colonies, and during the nectar collection for rearing new worker bees. Pollen is essential for the development of honey bees (*Apis*

mellifera L.), providing nutrients such as proteins, lipids, minerals and vitamins (Brodschneider and Crailsheim, 2010; Wright et al., 2018; Radev, 2018). There is a relationship between the nutritional value of pollen and the development, reproduction, and productivity of the bee colonies (Zherebkin and Mironova, 1976; Avetisyan, 1983; Radev et al., 2014; Radev, 2018). According to Stanley and Linskens (1974), Herbert and Shimanuki (1978) and Loper and Berdel (1980), the amount of protein ranges from 3.8% to 40.8% with the average of about 20% for the most species. Radev et al. (2014) found protein content ranged from 13.88% to 25.02% and average value 19.45%, while Kleinschmidt and Kondos (1977) established from 7% to 37%. Research by Liolios et al. (2016) showed that the protein content of pollen from 54 different plants ranged from 12.8% for pollen from *Smilax* sp. to 30.1% for that of *Fallopia* sp., with an average of 20.8%. The result from 50 plants according to Radev (2018) underline the fact that pollens from various botanical species have greatly different protein content. The percentage of the total protein content in the examined bee-collected pollen samples ranges from 11.5% for *Chondrilla juncea* to 27.4% for that of *Cucumis melo*, and the average value was 19.9%.

The objective of this study was to discover the dynamic of the protein content in mixed honey-bee collected pollen in the area of Belozem during Apicultural season.

MATERIALS AND METHODS

Pollen traps were placed at five bee hives and the pollen pellets harvested every 2 days from April till September 2012-2014 in area of Belozem (42,2°.25,033333°) Bulgaria, with a transitional-continental climate. Every month has two reporting periods: I period from 1st to 15th day of the month and II period from 16th to 30/31st day of the month. The collected pollen from the bee colonies was mixed together according to the accurate period and were analysed carefully. Thirty five samples of bee-collected pollen pellets were analysed.

The random sample, which was used for analysis for each period, was taken using the quarter method of sampling. In this method the pollen was spread on paper, and divided it into four equal quarters then the two diagonally opposite quarters were removed. The remaining material was mixed and quartered until the sample was reduced to the desired size. The labeled mixed pollens were stored in separate vials in a freezer at -20ºC.

For nitrogen content determination, pollen was analysed using the Kjeldahl method, which is separated into three steps. During the first step, digestion, a quantity of 1g of pollen was placed into the tubes of a digestion unit K-435 Buchi with 20 ml of strong sulphuric acid (H_2SO_4, 95-98%) in the presence of a catalyst – a Kjeldahl tablet KT-240-A OPSIS LiquidLINE^R (47.17% Sodium sulphate, 47.17% Potassium sulphate, 2.83% Copper (II) sulphate 5-hydrate, 2.83% Titanium (IV) dioxide), which assists the conversion of nitrogen in proteins to ammonium ions. When the digestion was complete, the samples were removed and left to cool at room temperature. After cooling, the ammonia was distilled in the presence of NaOH (KjelFlex 360 Buchi) and collected in a solution of boric acid (H_3BO_3, 99.5-100.5%, Merck), which was then titrated against 1 M HCl in a Mettler Toledo T–50. The crude protein content was estimated using the factor 5.60 (Rabie et al., 1983) by the volume of HCL added to the pollen. In this case the following formula applied: Protein (%) = 0.791*V/m, where:
V = the volume (ml) of HCl used for titration and m = the amount of pollen (g).

RESULTS AND DISCUSSION

The study results shows that the percentage of protein content in the examined mixed pollen samples in 2012 ranged from 13.9% to 25%, in 2013 it ranged from 15.6% to 25.1% and for 2014 it was from 18.4% to 27.8% (Table 1). The study results showed that the protein content of mixed pollen varied for the two reporting periods of each month and for each single month (Table 1). There was a tendency to increase the protein content every year, but the

statistical analysis (Anova: Single factor) (at $p \leq 0.05$, $p= 0.87$) of the results of the experimental work during the three years of research showed no significant differences. When comparing the same monthly results all together for the three years to each other (Anova: Single factor), was found only between April-July and May-July significant differences (at $p \leq 0.05$, $p= 0.01$; $p= 0.00$). Between the other months was not found significant differences (at $p \leq 0.05$, $p > 0.05$). The reasons of the differences in the statistical analysis probably came from the dynamic change of the ecological factors (Fig. 1, 2 and 3) and the urban environment, which influenced flowering of the plants.

Table 1. Protein content (%) of mixed honey bee-collected pollen in different harvesting periods

	Protein content (%) of mixed honey bee-collected pollen in different harvesting periods for three years		
Harvesting periods of pollen	**2012**	**2013**	**2014**
April 1-15		21.1	27.8
April 16-30	25	23	25.5
April (average value)	**25 a**	**22 a**	**26.7 a**
May 1-15	24.6	23.9	24.8
May 16-31	23.4	24.4	23
May (average value)	**24 a**	**24.2 a**	**23.9 a**
June 1-15	23.5	15.6	22.5
June 16-30	21.5	15.7	21.6
June (average value)	**22.5 ab**	**15.6 ab**	**22.1 ab**
July 1-15	17	16	19.2
July 16-31	18.3	19.9	21
July (average value)	**17.7 b**	**17.9 b**	**20.1 b**
August 1-15	17.1	22.8	20.7
August 16-31	13.9	21.9	18.4
August (average value)	**15.5 ab**	**22.4 ab**	**19.6 ab**
September 1-15	15.1	22.6	19.9
September 16-30	16	25.1	24.2
September (average value)	**15.6 ab**	**23.9 ab**	**22 ab**

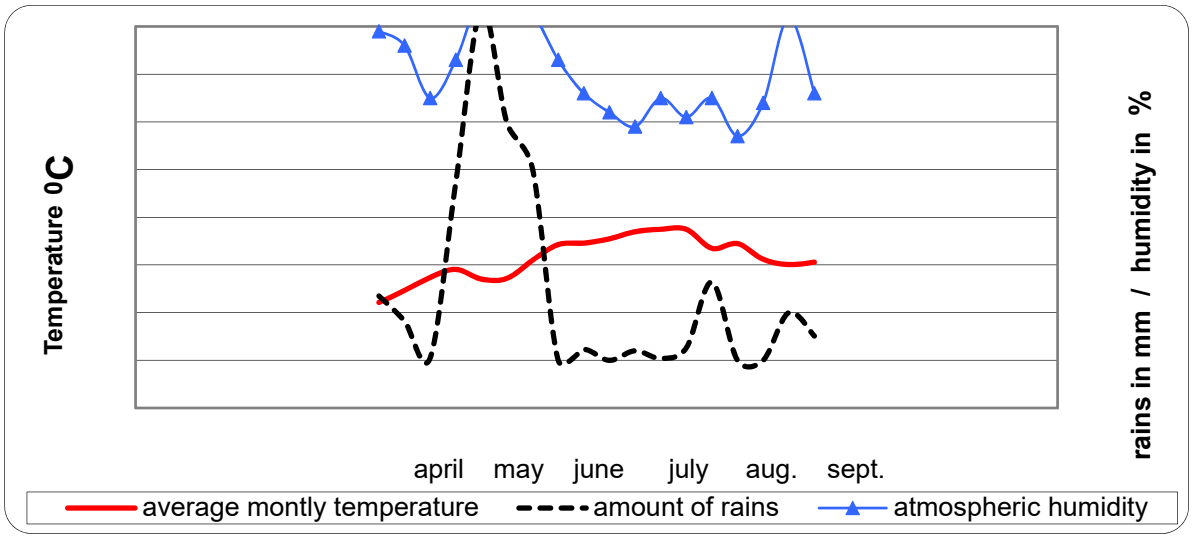

Figure 1. Climatic characteristics of Belozem area for 2012

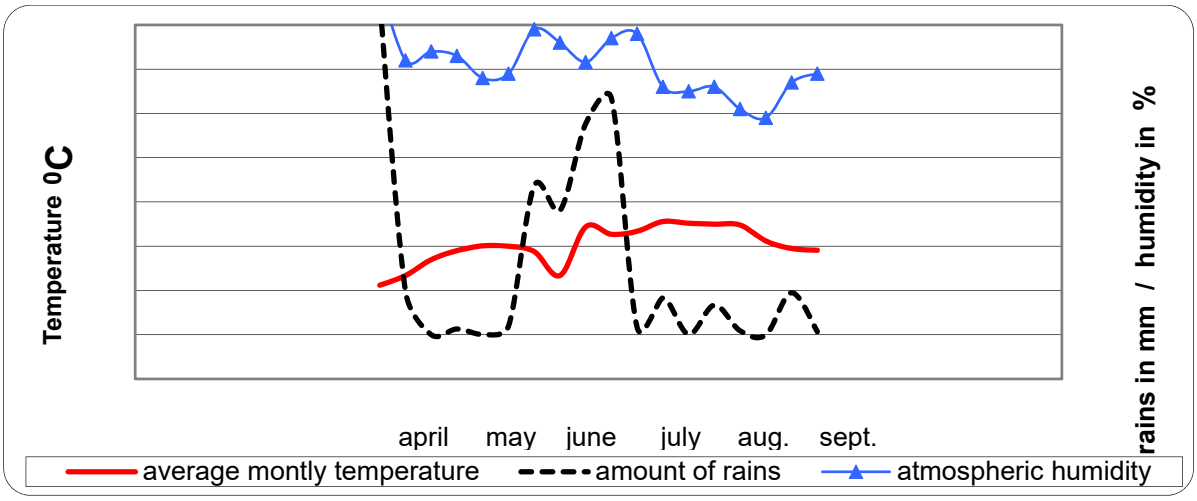

Figure 2. Climatic characteristics of Belozem area for 2013

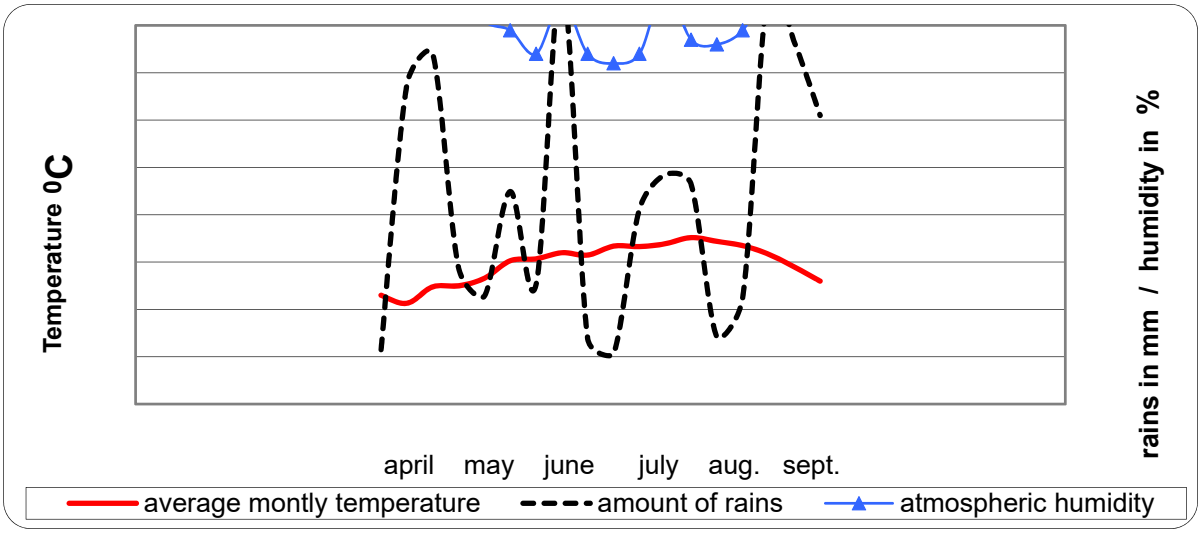

Figure 3. Climatic characteristics of Belozem area for 2014

The results of the seasonal dinamyc of the protein content in the mixed pollen in 2012 showed differences. During the spring (April to May) the protein content was 24.4% on average, during the summer (June-August) it was 18.6% and during the autumn (September) it was 15.6% as shown on Table 2. There is a tendency to reduce the crude protein content of pollen in summer-autumn compared to spring. Statistical analysis of the data in Table 2 according to Duncan's Multiple Rangetest proved a difference between the spring season compared to the summer and autumn, while the summer and autumn results coincided. It can therefore identifiable two periods with different protein content of pollen: spring and summer-autumn. The overall average protein content in the analysed samples was 19.5%. The results of this study agree with Stanley and Liskens (1974), Herbert and Shimanuki (1978) and Szczesna (2006b) who found that average protein content approaches 20%. During autumn, the protein content of pollen significantly reduced to an overall average of 15.6%, which corresponds to results derived from the research of Bonvehi and Jorda (1997), who found an average protein content of pollen samples from Spain close to 16%.

The season results in 2013 gave a different spectrum according to the results of the previous year. During the spring (April to May) the protein content was 23.1% on average, during the summer (June-August) it was 18.6% and during the autumn (September) it was 23.9% as shown on Table 2. During 2013 there is a tendency to reduce the crude protein content

of the pollen in the summer especially in June and first half of July (Table 1) compared to the spring and autumn. From the second half of July (Table 1) the crude protein content of the mixed pollen began to increase essentiality towards autumn. In 2013 (Table 2) the autumn crude protein was even higher than the one in the spring, probably the bees had collected pollen richer on protein. The statistical analysis of the data in Table 2 according to Duncan's Multiple Rangetest proved a difference between the summer season in comparison to the spring and autumn, while the spring and autumn coincided. Therefore, during this year we can identify also two periods with different nutritional value of pollen, namely: spring-autumn and summer were compared to each other in 2012. The overall average protein content in the analysed samples was 21.9%, which was 2.4% more compared to 2012 (19.5%). In addition, that result was close to Liolios (2010), who found an average protein content of 20.8%.

During the spring (April to May) of 2014 the protein content was 25.3% on average, during the summer (June-August) it was 20.6% and during the autumn (September) it was 22% as shown on Table 2. This result is similar to the previous year -2013. During that year there was also a tendency to reduce the crude protein content of the pollen in the summer season in comparison to the spring-autumn period. Statistical analysis of the data in Table 2 for the two years 2013 and 2014 according to Duncan's Multiple Rangetest proved a difference between the summer season and the spring and autumn while the spring and autumn results coincided. Therefore, two periods of different pollen nutritional value could be identified again: spring-autumn and summer.

The overall average protein content in the analysed samples was 22.6%, with 0.8% higher than the protein content of 2013, and it was 3.1% of 2012. The overall average protein content was icreasing every year- in 2012- 19.5%, in 2013- 21.9%, in 2014- 22.6% (Table 2). In 2013 and 2014 during summer-autumn (Fig. 2 and 3), the rainfall influenced the flowering of the high variety of meadow, weed and introduced honey plants contained pollen with a high protein content.

Table 2. Protein content (%) of mixed honey bee-collected pollen in different seasons

	Dynamic of the protein content (%) of mixed honey bee – collected pollen in different seasons for three years		
Harvesting periods of pollen	**2012**	**2013**	**2014**
Spring	24.4 **a**	23.1 **a**	25.3 **a**
Summer	18.6 **b**	18.6 **b**	20.6 **b**
Autumn	15.6 **b**	23.9 **a**	22 **a**
mean for year	**19.5**	**21.9**	**22.6**

CONCLUSION

Pollen collected by honey bees has different protein content at different seasons of the year and for each year, which depend of distribution of the plants and the climate condition. The statistical analysis of the data proved a difference in the protein content in pollen between the summer, spring and autumn. In the study area the percentage of protein content in the examined mixed pollen samples has different value: in 2012 ranged from 13.9% to 25%, in 2013 it ranged from 15.6% to 25.1% and for 2014 it was from 18.4% to 27.8% and the average value is 21%. The study results showed that the protein content of mixed pollen varied for each reporting period of each month and for each single month. The reason of the differences probably came from the dynamic change of the ecological factors and the urban environment, which influenced flowering of the plants.

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