

CHEMICAL COMPOSITION AND AMOUNTS OF MINERAL ELEMENTS IN HONEYBEE-COLLECTED POLLEN IN RELATION TO BOTANICAL ORIGIN

El-Kazafy Abdou Taha^{1,2,*}

¹Department of Arid Land Agriculture, College of Agriculture and Food Sciences, King Faisal University, Al-Hasa, Saudi Arabia

²Department of Economic Entomology, Faculty of Agriculture, Kafrelsheikh University, Kafrelsheikh, Egypt

*corresponding author: etaha@kfu.edu.sa

Received 17 August 2014; accepted 07 May 2015

Abstract

This study was conducted at the apiary of the Agricultural and Veterinary Training and Research Station, King Faisal University, Al-Ahsa, Saudi Arabia. The purpose was to study the relationship between the botanical origin and chemical composition of bee-collected pollen. The amount of mineral elements present in bee-collected pollen was also studied. The composition of pollen loads showed the maximum contents of dry matter, ash, glucose, fructose, magnesium (Mg), phosphorus (P), and manganese (Mn) for the date palm; the maximum contents of protein, calcium (Ca), and zinc (Zn) for alfalfa; the maximum contents of fiber, and copper (Cu) for the sunflower; the maximum contents of the lipids and iron (Fe) for summer squash; and the maximum contents of sodium (Na), and potassium (K) for rape. Calcium was found to be correlated in a significantly ($p < 0.01$) positive way with K, Na, Mg, P, Mn, and Zn. Copper, though, was correlated in a significantly ($p < 0.01$) negative way with Ca, Mg, P, Mn, and Zn. The high levels of protein, ash, glucose, and fructose, and low lipid content found in bee-pollen from the date palm and from alfalfa, mean that pollen from the date palm and from alfalfa make an excellent food supplement. It was concluded, that the chemical composition of bee-collected pollen can be correlated with the plant species from which pollen was collected.

Keywords: bee-pollen, botanic origin, chemical composition, minerals.

INTRODUCTION

The natural diet of the honeybee is pollen and nectar. Pollen is more crucial than nectar for the production of brood because it provides the nutritional requirements for developing bees. Pollen constitutes the main source of protein necessary for bee larvae and young workers (Crailsheim et al., 1992; Serra Bonvehi and Escola Jorda, 1997).

Honeybee-collected pollen has an important value not only for bee nutrition, but also for humans. Honeybee-collected pollen provides a rich source of easily digestible protein (Crailsheim et al., 1992; Campos et al., 1996; Serra Bonvehi and Escola Jorda, 1997; Szczesna, 2006a; b). In addition to proteins, pollen also contains carbohydrates, fats and minerals (Orzáez Villanueva et al., 2002; Szczesna et al., 2002; Almeida-Muradian et al., 2005; Human and Nicolson, 2006; Szczesna, 2006a; 2007; Campos et al., 2008; Carpes et al., 2009; Martins et al., 2011; Morgano et al., 2012). The chemical composition of

bee-pollen showed an average of 7.40% moisture, 20.00% protein, 6.00% lipids, and 2.20% ash (Almeida-Muradian et al., 2005). Different values (4.20, 20.50, 4.90, and 2.90%, respectively) of Brazilian bee-pollen were found by Carpes et al. (2009).

The chemical composition of bee-pollen is known to vary according to the plant species from which the pollen was collected (Szczesna et al., 2002; 2006a; Morgano et al., 2012), the geographic origin (Serra Bonvehi and Escola Jorda, 1997; Carpes et al., 2009; Martins et al., 2011; Morgano et al., 2012), the season (Szczesna et al., 2002; Morgano et al., 2012), and the storage and storage method (Human and Nicolson, 2006; Siuda et al., 2012).

Bee-pollen was involved in formulating supplementary food products because of its nutritional and therapeutic properties (Iannuzzi, 1993; Campos et al., 1996; Serra Bonvehi and Escola Jorda, 1997; Pernal and Currie, 2000; Orzáez Villanueva et al., 2002; Kacániová et al., 2012; Morgano et al., 2012).

Bee-pollen was also used in pollen supplements (Iannuzzi, 1993). For the aforementioned purposes, it is very important to select bee-pollen which has a high nutritional value. The present investigation was aimed to classify the bee-pollen from different botanical origins according to their nutritional value, so that high quality pollen may be produced.

MATERIAL AND METHODS

The study was conducted at the apiary of the Agricultural and Veterinary Training and Research Station, King Faisal University, Al-Ahsa (Saudi Arabia) that lies at latitude 25° 25' 46" N and longitude 49° 37' 19" E and an altitude of 121 m above sea level. Throughout the year, five colonies (each of seven combs) of hybrid Carniolan honeybees in the same strength (brood, stored food, and bee strength) were used for pollen trapping.

Pollen traps with a 25% efficiency were fitted onto the entrances of the colonies. Twice a week, from January to December in 2013, the pollen loads were collected from the pollen traps. The pollen loads were dried at room temperature in a shady place for one hour to facilitate the separation and referred to its sources, then dried at 30°C for 2 h and weighed. The collected bee-pollen was hand-sorted by color and appearance, and floral origin of each fraction was identified by microscopical examinations. Pollen types were identified according to their shape and size by comparison with pollen reference slides made by the author. The procedure of preparation of reference slides was described by Louveaux et al. (1978). Pollen samples containing mainly monofloral pollen loads from summer squash (*Cucurbita pepo* Thunb), date palm (*Phoenix dactylifera* L.), sunflower (*Helianthus annuus* L.), rape (*Brassica napus* L.) and alfalfa (*Medicago sativa* L.) were selected as major sources, and stored at -21°C until analysed. Samples used for determination were collected during February-March for summer squash, date palm, rape and sunflower, and during May for alfalfa, summer squash and sunflower.

The determination of the chemical composition of dried bee-pollen was done as follow: dry matter was determined by drying 2.00 g of pollen sample at 105°C to a constant weight (Serra Bonvehi and Casanova, 1987). A sample of 0.07 g was used to determine the nitrogen by the micro-Kjeldahl method (AOAC, 1995) using the 5.60 factor for conversion into crude protein (Rabie et al., 1983). In order to compare our findings with those of other studies which used the factor 6.25 for a nitrogen conversion to protein, we recalculated their values using factor

5.60. A sample of 2.00 g was used for determination of the lipids using a Soxhlet extractor with diethyl ether as the solvent (Folch et al., 1957). The ash determination was made by incinerating 2.00 g in an oven at 550°C until a constant weight was reached (Almeida-Muradian et al., 2005). The crude fiber was quantified by gravimetry from 2.00 g of crushed pollen, in accordance with the fibers method 991.43 (AOAC, 1995). A sample of 2.00 g was used to determine the content of fructose and glucose in bee-pollen by the utilization of HPLC technique as described by Szczesna (2007). Glycemic index (F/G) of bee pollen was calculated. All components were calculated basing on dry matter.

A sample of 1.00 g was used for determination of mineral content. Wet digestion with nitric acid following the method of Taha et al. (2010), was used to prepare the pollen samples for the determination of the mineral content. A blank experiment was carried out by adding the same amount of nitric acid to one mL distilled water. An Atomic Absorption GBC AVANTA Σ GF 3000 was used for the detection of iron (Fe), manganese (Mn), zinc (Zn), and copper (Cu). Sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), and phosphorus (P) concentrations were determined using a flame photometer. The instrument was calibrated by using standard solutions of various metal salts. All determinations were made in triplicate and calculated based on dry matter.

Data were subjected to the one-way analysis of variance (ANOVA). Treatment means were compared using Duncan's Multiple Range Test (Duncan, 1955). The Pearson correlation between minerals was determined using SAS Institute (2003).

RESULTS

In the semi-arid conditions that cover most parts of Saudi Arabia, the temperatures during summer season often exceed 40°C. In January the mean temperature is 16°C which is adequate for growth and blooming of summer squash and rape. Sunflower is grown and flowered more than once in the year. The yearly amounts of trapped pollen loads were 2.50 kg/colony. Five species were recorded as major sources and they contributed 94.76% of the total yearly collected pollen loads. The highest significant ($p < 0.01$) amount of pollen loads was obtained from summer squash, followed by date palm without significant differences between them, while the lowest significant ($p < 0.01$) amount was collected from rape (Tab. 1).

As shown in Table 2, the percentages of ash, protein, lipid, fiber, fructose and glucose content in bee collected-pollen were variable and depended significantly ($p < 0.01$) on the botanical origin. The chemical composition of bee-pollen showed the highest significant ($p < 0.01$) values of dry matter (90.84%), ash (3.88% DM), fructose (21.30% DM) and glucose (17.06% DM) for date palm, protein (19.45% DM) for alfalfa, lipids (5.38% DM) for summer squash, and fibers (1.70% DM) for sunflower.

Data listed in Table 3 show that the amount of mineral elements in bee collected-pollen were variable and depended significantly ($p < 0.01$) on the botanical origin. The highest levels of sodium (Na) and potassium (K); calcium (Ca) and zinc (Zn); magnesium (Mg), phosphorus (P), and manganese (Mn); iron (Fe); and copper (Cu) were obtained from the pollen of rape; alfalfa; date palm; summer squash; and sunflower, respectively.

Table 1.

Yearly average amount of pollen loads (g/colony) collected from the major pollen sources in the Al-Ahsa region in 2013

Botanical origins		Blooming period	Amount (g) of trapped pollen
Common name	Scientific name		
Summer squash	<i>Cucurbita pepo</i> Thunb	January-May, September-October	528 \pm 17.05 ^a
Date palm	<i>Phoenix dactylifera</i> L.	February-March	515 \pm 10.40 ^a
Sunflower	<i>Helianthus annuus</i> L.	February-October	454 \pm 15.12 ^b
Alfalfa	<i>Medicago sativa</i> L.	May-June	446 \pm 10.90 ^b
Rape	<i>Brassica napus</i> L.	January-March	426 \pm 11.40 ^c
Minor sources	-	January-December	131 \pm 18.40 ^d
The total	-	-	2500

Values are the mean \pm S.D. The means of each column followed by a different letter are significantly different at the 0.01 level.

Table 2.

Chemical composition (% DM) of bee-pollen in relation to botanical origin

Component	Botanical origins					Significance
	Sunflower	Summer squash	Date palm	Rape	Alfalfa	
Dry matter	90.81 \pm 0.30a	89.50 \pm 0.50c	90.84 \pm 0.06a	90.27 \pm 0.23b	90.16 \pm 0.16b	**
Protein	14.71 \pm 0.10e	15.82 \pm 0.15d	19.00 \pm 0.11b	18.14 \pm 0.05c	19.45 \pm 0.05a	**
Lipids	4.45 \pm 0.05b	5.38 \pm 0.05a	1.82 \pm 0.02e	3.92 \pm 0.04c	2.89 \pm 0.10d	**
Ash	2.01 \pm 0.03d	1.88 \pm 0.10e	3.88 \pm 0.03a	3.32 \pm 0.02c	3.74 \pm 0.01b	**
Fibers	1.70 \pm 0.02a	0.97 \pm 0.01b	0.15 \pm 0.01e	0.46 \pm 0.01d	0.78 \pm 0.01c	**
Fructose	17.23 \pm 0.08c	17.80 \pm 0.09b	21.30 \pm 0.12a	17.13 \pm 0.05c	19.51 \pm 0.06a	**
Glucose	15.44 \pm 0.11c	16.66 \pm 0.09a	17.06 \pm 0.07a	16.04 \pm 0.10b	16.53 \pm 0.08b	**
F/G	1.12c	1.07d	1.25a	1.07d	1.18b	**

Values are the mean \pm S.D. The means of each row followed by a different letter are significantly different. ** indicate $p < 0.01$.

Table 3.

Mineral contents (mg/kg DM) of bee-pollen in relation to botanical origin

Mineral	Botanical origins					Significance
	Sunflower	Summer squash	Date palm	Rape	Alfalfa	
Na	6345.00 \pm 4.00e	6525.00 \pm 2.50c	6586.40 \pm 4.00d	8350.27 \pm 5.33a	7311.98 \pm 5.00b	**
K	6232.79 \pm 4.00e	6416.55 \pm 4.64c	6402.90 \pm 2.55d	8258.50 \pm 2.00a	7214.00 \pm 1.19b	**
Ca	2086.36 \pm 2.50e	2700.73 \pm 1.43d	5629.85 \pm 2.46b	5246.94 \pm 1.50c	5752.19 \pm 1.97a	**
Mg	2704.62 \pm 1.33d	2353.11 \pm 2.09e	4680.53 \pm 0.76a	3887.63 \pm 2.33c	4557.18 \pm 1.45b	**
P	234.40 \pm 0.88e	235.30 \pm 1.10d	468.05 \pm 1.25a	388.70 \pm 1.14c	455.71 \pm 1.45b	**
Fe	343.46 \pm 1.22d	562.06 \pm 4.00a	338.12 \pm 1.79e	361.32 \pm 1.73c	533.86 \pm 1.87b	**
Mn	18.77 \pm 0.33d	16.60 \pm 0.5e	38.61 \pm 0.50a	22.34 \pm 0.75c	30.26 \pm 0.44b	**
Zn	33.90 \pm 0.33cd	31.92 \pm 1.00d	40.97 \pm 0.90b	34.57 \pm 1.30c	44.18 \pm 1.00a	**
Cu	6.97 \pm 0.67a	6.71 \pm 0.50a	4.24 \pm 0.33c	6.32 \pm 0.20b	5.86 \pm 0.10b	**

Values are the mean \pm S.D. The means of each row followed by a different letter are significantly different. ** indicate $p < 0.01$.

Table 4.

Pearson's correlation coefficients for minerals in bee-collected pollen									
	Na	K	Ca	Mg	P	Fe	Mn	Zn	Cu
Na									
K	0.99**								
Ca	0.60**	0.61**							
Mg	0.35	0.36	0.95**						
P	0.39	0.40	0.98**	0.99**					
Fe	-0.01	-0.01	-0.07	-0.27	-0.14				
Mn	-0.04	-0.04	0.79**	0.90**	0.89**	-0.27			
Zn	0.07	0.08	0.77**	0.87**	0.85**	0.09	0.84**		
Cu	0.10	0.09	-0.69**	-0.77**	-0.77**	0.31	-0.91**	-0.60**	

**Correlation is significant at the 0.01 level (2-tailed).

Calcium was found to have a significant ($p < 0.01$) positive correlation with K ($r = 0.61$), Na ($r = 0.60$), Mg ($r = 0.95$), P ($r = 0.98$), Mn ($r = 0.79$), and Zn ($r = 0.77$). Similarly, Mg had a significant ($p < 0.01$) positive correlation with P ($r = 0.99$), Mn ($r = 0.90$), and Zn ($r = 0.87$). Also, P had a significant ($p < 0.01$) positive correlation with Mn ($r = 0.89$), and Zn ($r = 0.85$). However, Cu had a significant ($p < 0.01$) negative correlation with Ca ($r = -0.69$), Mg ($r = -0.77$), P ($r = -0.77$), Mn ($r = -0.91$), and Zn ($r = -0.60$) (Tab. 4).

DISCUSSION

Protein constitute the second most predominant group in bee-pollen, after carbohydrates. Thus, some investigators refer to the value of pollen as a source of this nutrient for human food (Orzáez Villanueva et al., 2002; Szczesna, 2006a; b; Martins et al., 2011). Bee-pollen had from 14.71 to 19.45% DM protein. These values were significantly ($p < 0.01$) related to the plant source from which bee-pollen was collected. Protein contents could be arranged in a descending order: alfalfa > date palm > rape > summer squash > sunflower. Data published on our monofloral pollen are too poor, so we compared our data with sunflower, date palm and rape from other locations in relation to some determined components. Then we compared the ranges of components contents in bee-pollen from Al-Ahsa, Saudi Arabia with bee-pollen from other countries. The protein content of bee-collected sunflower pollen appears to be deficient and at the bottom of the range for the five major species. Data obtained in the present study confirm the findings of Somerville and Nicol (2006) in Australia, Pernal and Currie (2000) in Canada, Tasei and Aupinel (2008) in France, Nicolson and Human (2013) in South Africa

who also found low protein content in bee-collected sunflower pollen. The protein level (18.14% DM) in bee-collected rape pollen was lower than that obtained by Pernal and Currie (2000) in Canada. Here, the protein content of bee-collected date palm pollen was found to be lower than that of floral date palm pollen grains from Egypt (Hassan, 2011). This might be a result of adding nectar sugars during formation of pellets, which caused increase of the content of carbohydrates, while the protein, lipid and ash contents decreased (Nicolson and Human, 2013).

The average levels of protein found in the current study fall in the range obtained by Szczesna (2006a) for Polish bee-pollen, Carpes et al. (2009) and Martins et al. (2011) in Brazilian bee-pollen, Tasei and Aupinel (2008) in bee pollen from France, and by Mărgăoan et al. (2012) in fresh bee-pollen from Romania. However, the average values were lower than the values found in Polish pollen by Rogala and Szymaś (2004), and by Szczesna (2006b) in Polish bee-pollen, Chinese pollen loads, and in bee-pollen from South Korea.

The values of lipids in bee-pollen could be arranged in an ascending order: date palm < alfalfa < rape < sunflower < summer squash, with significant ($p < 0.01$) differences among them. The high lipid levels in summer squash, and sunflower pollen were confirmed by Singh et al. (1999) for rape and sunflower, and Nicolson and Human (2013) for sunflower. The content of lipids found in our studies ranged from 1.82 to 5.38% DM. The average values were in the range found in Polish pollen (Rogala and Szymaś, 2004) but lower than the values obtained by Carpes et al. (2009) and Martins et al. (2011) for Brazilian bee-pollen, and by Mărgăoan et al. (2012) for Romanian fresh bee-pollen.

The ash contents in bee-pollen could be arranged in a descending order: date palm > alfalfa > rape > sunflower > summer squash, with significant ($p < 0.01$) differences among them. The low ash content in bee-collected sunflower pollen was shown by Nicolson and Human (2013). Ash content of bee pollen from Saudi Arabia ranged between 1.88 and 3.88% DM. This range was similar to the range in Polish pollen (Rogala and Szymaś, 2004) and in pollen from Southern Brazil (Carpes et al., 2009). In comparison to the values found in Spanish samples our values were higher than those obtained by Serra Bonvehi and Escola Jorda (1997) and by Orzáez Villanueva et al., (2002). Our results were also higher from those found in the Romanian fresh bee-pollen (Mărgăoan et al., 2012).

Fructose and glucose concentrations in the bee-pollen were significantly ($p < 0.01$) varied according to the botanical origin. Fructose content ranged between 17.13 and 21.30% DM. This range was higher than the range found in the samples from Poland, Korea, and China (Szczęsna, 2007), and the range shown for Romanian fresh bee-pollen (Mărgăoan et al., 2012). Compared with the content of fructose, the content of glucose was lower. Glucose content ranged between 15.44 and 17.06% DM. This range was higher than the range found in samples from Poland, Korea and China (Szczęsna, 2007), as well as in Romanian fresh bee-pollen (Mărgăoan et al., 2012). It can be noticed that the levels of glucose and fructose in the bee-pollen samples differed and considerably depended on the available honey or nectar that the bees use to form the pellets of pollen. The levels of fructose could be arranged in an ascending order: date palm > alfalfa > summer squash > sunflower > rape, while glucose: date palm > summer squash > alfalfa > rape > sunflower.

According to Mărgăoan et al. (2012), fructose to glucose index (F/G) has been related to the glycemic index in honey. In the present study, the glycemic index of bee-pollen ranged between 1.07 and 1.25. These values could be considered low compared with the values for the samples from Poland, Korea, and China (Szczęsna, 2007), and the values obtained by Martins et al. (2011) for pollen from Sergipe (Brazil). In comparison to the values of Romanian fresh pollen, the values in the present work were higher (Mărgăoan et al., 2012). The value of F/G for bee-pollen from rape (Brassicaceae) was lower than the values for Brassicaceae bee-pollen obtained by Szczęsna (2007).

We detected low levels of fibers in Saudi bee-pollen in contrast to very high level of dietary fiber detected

in Spanish pollen (Serra Bonvehi and Escola Jorda, 1997). To compare, Polish pollen had 2.59% fibers (Rogala and Szymaś, 2004), and the fiber content in Brazilian pollen was between 2.20 and 4.90% (Carpes et al., 2009). Fiber contents in bee-pollen could be arranged in an ascending order: date palm < rape < alfalfa < summer squash < sunflower, with significant ($p < 0.01$) differences among them.

The high levels of protein, ash, fructose, and glucose contents found in bee-pollen from date palm and alfalfa mean that pollen from them is an excellent food supplement. Due to the high nutritional value of them, bee-pollen could be collected and stored to use as pollen supplement to feed honeybee colonies during pollen shortage periods, and as healthy and nutrient food supplement for humans.

Among the determined mineral elements in bee-collected pollen, the mean levels of Na, K, Ca, Mg, P, Fe, Mn, Zn, and Cu were significantly ($p < 0.01$) varied according to the flora from which the pollen was collected. The variation in the mineral contents was mainly due to the differences in the floral origin of pollen, since the plant growth conditions, such as: soil, irrigation water, and geographic origin, and the subspecies of collector bees were the same. These ranges are similar to those obtained by Stanciu et al. (2012) for K, Mn, Zn, and Cu. Meanwhile, Na, Ca, Mg, and Fe tended to be higher than those obtained by Carpes et al. (2009) and Morgano et al. (2012). The differences among our values and the values found in other countries might be correlated to the plant growth conditions, such as: soil, irrigation water, and geographic origin. For example: the electrical conductivity of groundwater used for irrigation in Al-Ahsa oasis ranged between 1.23 and 5.05 dS m⁻¹ with Na as the most abundant cation. The groundwater of Al-Ahsa Oasis is mainly Na and Ca water rather than Ca and Mg dominant water (Al-Zarah, 2008).

The mineral elements in the selected bee pollen showed a predominance of Na (35.54, 35.32, 26.91, 31.50, and 28.44 % of the total mineral quantified), K (34.92, 34.73, 26.53, 31.16, and 28.06 %), Ca (11.69, 14.62, 23.33, 19.80, and 22.27 %) and Mg (15.11, 12.75, 19.39, 14.67, and 17.73 %) for sunflower, summer squash, date palm, rape, and alfalfa, respectively. According to Serra Bonvehi and Escola Jorda (1997) the predominant minerals were K, P, Ca, and Mg. Similarly, Stanciu et al. (2012) found that K occurred at the highest concentrations in all samples collected from Romania, followed by Ca. Similarly, P was found at the highest levels followed by K, while Ca ranked third, in pollen from Brazil (Carpes et al., 2009).

Copper recorded the lowest percentage of all the estimated minerals in pollen from sunflower (0.039 %), summer squash (0.036 %), date palm (0.018 %), rape (0.024 %), and alfalfa (0.023 %). These results are in agreement with those of Carpes et al. (2009), and Stanciu et al. (2012).

Bee-collected pollen from date palm, and alfalfa were found to have high levels of Na, K, Ca, Mg, P, Fe, Mn, and Zn which are considered to be essential for bee and human nutrition.

CONCLUSION

It was concluded that the chemical composition and mineral contents in honeybee-collected pollen can be correlated with the botanical origin. Bee-collected pollen from date palm, and alfalfa were characterised by a high content of crude protein, ash, and high levels of Ca, Na, K, Mg, P, Mn, and Zn. Thus, it can be suggested to collect pollen loads during the blooming periods of date palm (February-March), and alfalfa (May-June) to use it as pollen supplement for feeding bee colonies during the shortage or complete absence of the pollen, or in the presence of only poor quality pollen. Also it can be used as healthy human food supplements.

ACKNOWLEDGEMENT

This work was supported by Deanship of Scientific Research of King Faisal University (Project No. 130275) in the Kingdom of Saudi Arabia.

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