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Impact of storage period on different types of bee pollen pigments

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ABSTRACT



Pigments are anti-oxidants, which play an important role in protecting against many diseases. In this study, pigments of different bee pollen types (Sunflowers, Clover, Sesame and Maize) were determined with ethanol solvent and stored under freeze condition. Sunflower bee pollen had the highest amounts of the studied 23 pigments except three pigments. Maize bee pollens were superior to sunflower bee pollen in its content of the three exception pigments (Isozeaxanthin, Zeaxanthin and B- Zeaxanthin). In Sunflower bee pollen there were no significant differences between fresh bee pollen and those stored for 6 months in most of pigments as compared with 12 months storage. Sunflower bee pollen either fresh or stored for 6 months produced the highest values of Isozeaxanthin, Lutein, Lycopene, Violaxanthin, Zeaxanthin, 8- APO- B- Carten- 8- al, α – Carotene, β – Carotene and γ – Carotene compared to 12 months storage. However pigments of clover or sesame bee pollen were the lowest compared to sunflower and maize.

Keywords: Bee pollen types, Sunflower, Clover, Sesame, Maize, storage periods, Pigments.

INTRODUCTION

Pollen is an important product of the honeybee colony that is harvested by bees. It's transmitted to hives in the form of pollen loads and called bee pollen. This product contains many important substances such as volatile amino acids, phenolic compounds, vitamins and pigments that play a powerful role as antioxidants (Lazaridou *et al.*, 2004 and Nayik *et al.*, 2014).

The nutritional value of pollen is assessed based on protein concentration and amount of essential amino acids (Roulston and Cane, 2000). Pollen is the only source of protein where bees collect them from plants and store them inside the cells until needing in the development of offspring and thus the development of the whole colony (Anđelković *et al.*, 2012). Bee pollen have antioxidants that differently changed according to the types of flowers, geographical area, climatic conditions and storage (Zuluaga *et al.*, 2015).

Many of the biological properties of pollen are associated with the antioxidant effect, which results from the presence of many polyphenols, vitamins E and C, and beta-carotene where antioxidant activities can disrupt free radicals and protect the body from the negative effects of its activity. (Gulcin, 2012)

The appearance of yellow and red colors in pollen load is mainly estimated by the presence of pigments such as flavonoids and carotenoids (Stanley and Linskens, 1974). The importance of pigments is not only to the fact that they distinct colors of bee products, but to the greatest importance as antioxidants especially carotenoids (Owayss *et al.*, 2004). Also Bee pollen is rich in natural carotenoid pigments as lycopene and eaxanthin (Pascoal *et al.*, 2014).

* Corresponding author. E-mail address: supersemsema@gmail.com DOI: 10.21608/jppp.2020.68178 The principal carotenoids found in bee pollen after saponification are lutein and β -cryptoxanthin, while β carotene is detected in small or trace amounts (Mărgăoan *et al.*, 2014). Carotenoids have important roles in human health, β -Carotene frame is the main source of vitamin A and its dietary intake may reduce the risk of different types of degenerative diseases (Milani *et al.*, 2017). The bee pollen is a healthful food product with a high nutritional profile and therapeutic characteristic. The storage conditions or period may affect its composition and characteristics Campos et *al.*, 2003; Anjos *et al.*, 2019. The aim of this investigation is study the effects of storage periods on pigments of four bee pollen types

MATERIALS AND METHODS

Six honey bee, *Apis mellifera* L., colonies headed with queens of 1 st Carniolan hybrid were used in Fayoum governorate, during 2017. Pollen grains of Sunflower *(Hilianthus annuus)*, clover *(Trifolium alexandrinum)*, Sesame *(Sesamum indicum)* and maize *(Zea maize)* were collected by placing pollen traps at the entrance of Langstroth's bee hives. Collected pollen pellets were separated (depending on color) and then identified microscopically according to Louveaux *et al.*, 1978 technique.

Identification of pollens

For identification a representative sample of each pollen type was examined by a light microscope slide where it mounted in glycerin jelly and compared with already prepared slides of pollen made from nearly opened anthers of flowering plants growing at the research region. **Spectrophotometric determinations of pigments**. All the tested pigments were done at food safety and quality control lap. Fac. Agric., Cairo Univ., Giza, Egypt. Using calorimetrically determined by spectrophotometer (Spectronic 20, Bausch & Lomb) for different types of bee pollen either fresh or stored at -20 °C for 6 and 12 months. The following parameters were determined:

Anthocyanin. Anthocyanin was estimated using a method described by Fuleki and Francis (1968) as follows: Samples of 3g of bee pollen were mixed with ethanol 95% and HCL 1.5N (85:15 v/v), made up to 100ml then filtered. The absorbance of the extracted solution was measured at 535 nm against a blank. Total anthocyanin was calculated (μ g/g) by the following equation:

O.D. 535 x D.V. x 100 / F.W. x 1 / 98.2

Where; O.D. = optical density (absorbance) of the diluted sample, D.V. = diluted volume (ml) of the extract prepared for O.D. measurements and F.W. = fresh weight (g) of the sample.

Xanthophyll. These pigments were determined according to the modification of the spectro colorimetric procedure

given by Bacot (1954): In a porcelain mortar, 3g of bee pollen were well mixed with 30 ml of 80% ethanol then ground and filtered. The filtrate was made up to 100ml with 80% solvent.

Xanthophyll = 2026.1 × O.D. 474 – 2288.6 x O.D. 485 + 0.0036 (A) – 0.06518 (B).

Where;

O.D. = optical density (absorbance) of the diluted sample.

Carotenoids: Carotenoids were also estimated according to the methods of Britton (1995); the amount of each present pigment was calculated from the equation:

$$X = Ay / (A^{1/6}_{1cm} \times 100)$$

where X = the mass of carotenoids (g), y = the volume of solution (ml), A= the measured absorbance, and A $_{1\%}$ = the specific absorption coefficient, that is, the absorbance (nm) of a solution of 1g of that carotenoids in 100ml of solution. Absorptions used were listed in Table (1).

Table 1. Light absorption m	axima of some caroteno	ids determined in tested be	ee pollen types		
Pigments	$\lambda \max(nm)$	Pigments	$\lambda \max(nm)$		
Antheraxanthin	444	Isozeathanyhin	451		
Astaxanthin	485	Isocryptoxanthin	450		
8° -APO- β -carotene 8° - al	463	Lactucaxanthin	440		
Canthaxanthin	474	Lutin 5,6-epoxide	442		
α – Carotene	444	Lycopene	472		
β-carotene	450	Neoxanthin	439		
γ- Carotene	460	Violaxanthin	440		
ε-Carotene	440	β-Zeacarotene	428		
ξ-Carotene	399	Zeaxanthin	450		
β-Cryptoxanthin	450	Lutein	445		
Crocetin	423	Neurosporene	440		
Citranxanthin	335	*			

Statistical analysis

Data were analyzed using SAS software program (SAS, 2001). Randomized complete design with three replications was used. Duncan's test was used to separate treatment means at $P \le 0.05$.

RESULTS AND DISCUSSION

Pigments extracted with ethanol from different Bee pollen types

According to results in Table (2) there were significant differences in amount of pigments of sun flower, clover, sesame and maize bee pollen. Sun flower bee pollen recorded the highest values in most of pigments expected Isozeaxanthin, B- Zeaxanthin and Zeaxanthin wher maize bee pollen revealed the highest values of these exceptions as 265.40 ± 2.07 mg/g, 219.61 ± 4.27 mg/g and 312.43 ± 2.07 mg/g, respectively.

Moreover, clover bee pollen showed the lowest values in Isocryptoxanthin (31.18±1.46 mg/g), Isozeaxanthin (38.06 ±1.02 mg/g), Lactucaxanthin (31.71±1.26 mg/g), Lutein (41.02±1.30 mg/g mg/g), Neoxanthin (44.53±2.72 mg/g), Neurosporene (19.06±0.41 mg/g), Antheraxanthin (40.12± 0.75 mg/g), Astaxanthin (36.88±1.03 mg/g), ξ – Carotene (44.95±0.94 mg/g) and Citraxanthin (56.96±1.09 mg/g).

While sesame showed the lowest Lutein-5, 6epoxide (40.91 \pm 0.38), Violaxanthin(48.32 \pm 0.69), Canthaxanthin (45.05 \pm 0.58 mg/g), α – Carotene (59.37 \pm 1.34 mg/g), β – Carotene(60.94 \pm 0.49 mg/g) and -Carotene(53.81 \pm 0.54 mg/g) values.

Pigments of bee pollen types as affected by storage period

Results in Table 3 clarify revealed significant differences between fresh Sunflower bee pollen and 12 months storage in Isozeaxanthin, Lutein, Lycopene, Violaxanthin, Zeaxanthin, 8- APO- B- Carten- 8- al, α – Carotene, β – Carotene and γ - Carotene values which recorded mean values as 87.48±0.18, 92.96±0.58, 121.51±2.25, 105.99±1.25, 103.29±0.22, 126.33±0.36, 130.82±0.97, 152.97±0.73 and 128.71±0.57 mg/g, respectively for fresh bee pollen. For 12 months storage the same bee pollen pigments recorded 82.53±1.22, 87.89±1.54, 102.60±3.75, 97.02±1.65, 97.22±1.23, 114.97± 3.35, 115.63 ±3.17, and 132.78±2.20 and 117.70±2.18 mg/g, respectively.

No significant differences between fresh bee pollen and six months storage in all sunflower bee pollen pigments.The results of clover bee pollen showed significant differences in values of Neurosporene, β – Carotene, ξ – Carotene and Crocetin between fresh and 12 months storage. Fresh bee pollen recorded the highest values of above pigments as follow 19.06±0.41 mg/g, and 17.05±0.53 mg/g , 134.18±3.03 mg/g and 116.93±2.52 mg/g, 44.95±0.94 mg/g and 40.88±0.52 mg/g, 69.39±0.62 mg/g and 50.87± 4.53 mg/g, respectively. Furthermore, no significant differences were observed in the rest pigments between fresh and 12 months storage. Also no significant differences in pigments were recorded among fresh and six months storage.

Table 2. Pigments	(mg/g)) of four	bee pol	llen types

Pigments	Bee pollen types								
	Sunflower	Clover	Sesame	Maize					
β -Cryptoxanthin	85.42±0.53a	43.40±1.63c	40.77±0.74c	63.21±0.62b					
Isocryptoxanthin	80.59±0.28a	31.18±1.46d	37.65±0.30c	59.50±1.97b					
Isozeaxanthin	87.48±0.18b	38.06±1.02d	46.30±0.91c	265.40±2.07a					
Lactucaxanthin	97.94± 1.00a	31.71±1.26d	39.92±0.25c	60.77±1.25b					
Lutein	92.96±0.58a	41.02± 1.30d	$48.31 \pm 0.77c$	60.10±0.28b					
Lutein-5,6- epoxide	89.87±0.39a	49.18±1.53c	40.91±0.38d	60.12±0.22b					
Lycopene	121.51±2.25a	81.78±1.66b	38.44±1.14c	78.92±1.24b					
Neoxanthin	87.80±1.21a	44.53±2.72d	51.29±1.27c	72.19±0.73b					
Neurosporene	50.62±0.88a	19.06±0.41c	29.29±0.19b	50.01±0.71a					
Violaxanthin	105.99±1.25a	58.86±1.52c	48.32±0.69d	$66.28 \pm 0.22b$					
B- Zeaxanthin	93.25±0.64b	60.28±1.02c	54.12±0.58c	219.61±4.27a					
Zeaxanthin	103.29±0.22b	48.79±1.65c	52.82±1.38c	312.43±2.07a					
Antheraxanthin	91.46±0.79a	$40.12 \pm 0.75 d$	43.44±0.62c	67.68±1.37b					
APO- B- Carten- 8- al	126.33±0.36a	62.53±1.34c	61.84±0.43c	89.43±1.01b					
Astaxanthin	90.12±0.46a	36.88±1.03d	42.88±1.52c	50.44±0.24b					
Canthaxanthin	96.12±0.40a	51.30±0.92c	45.05±0.58d	56.41±0.71b					
α – Carotene	130.82±0.97a	100.63±2.09b	59.37±1.34d	88.20±0.76c					
β – Carotene	152.97±0.73a	134.18±3.03b	60.94±0.49d	96.45±0.27c					
γ – Carotene	128.71±0.57a	76.71±1.86c	53.81±0.54d	90.92±0.39b					
ξ – Carotene	115.81±0.58a	44.95±0.94d	56.20±1.09c	$93.04 \pm 0.59 b$					
ε – Carotene	123.91±0.52a	58.06±4.95c	58.54±0.66c	78.45±0.94b					
Citraxanthin	111.16±0.22a	56.96±1.09d	62.05±0.65c	90.58±0.48b					
Crocetin	89.35±0.72a	69.39±0.62b	48.06± 0.21c	70.14±0.35b					

Means followed by the same litter(s) in the same row are not significant differences (P≤0.05).

Table 3.	Effect of	f storage ⁻	period o	1 pigments	(mg/g) on [bee pol	llen types
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Diamonto	Fresh	6 months	12 months	Fresh	6 months	12 months	Fresh	6 months	12 months	Fresh	6 months	12 months
Pigments	Sun Flower			Clover			Sesame			Maize		
β-Cryptoxanthin	85.4±0.5a	85.1±3.1a	$81.5\pm 3.8a$	43.4±1.6a	42.9±1.5a	39.6±1.3a	40.7±0.7a	40.7±1.6a	38.5±1.7a	63.2±0.6a	63.0±1.1a	59.5±0.5b
Isocryptoxanthin	80.6±0.3a	80.2±1.8a	76.4±1.5a	31.1±1.4a	31.1±1.4a	28.8±1.4a	37.6±0.3a	37.5±1.6a	$36.0\pm0.5a$	59.5±1.9a	59.3±1.5a	56.3±1.3a
Isozeaxanthin	87.5±0.2a	87.3±1.9a	82.5±1.2b	38.0±1.0a	37.8±1.0a	35.0±0.3a	46.3±0.9a	46.2±1.4a	43.9±1.1a	265.4±2.0 a	262.9±2.3a	220.8±7.4b
Lactucaxanthin	97.9±1.0a	97.4±3.4a	92.4±1.7a	31.7±1.2a	31.5±1.3a	30.1±0.1a	39.9±0.2a	39.8±1.9a	38.7±0.0a	60.7±1.2a	60.5±2.0a	56.4±1.2a
Lutein	93.0±0.5a	92.7±1.4a	87.8±1.5b	$41.0{\pm}~1.3{a}$	40.9±1.3a	39.0±1.5a	$48.3 \pm 0.7a$	48.1±1.8a	45.8±0.6a	60.1±0.2a	59.9±0.9a	56.5±2.0a
Lutein-5,6- epoxide	89.8±0.3a	89.4±1.1a	87.5±1.7a	49.1±1.5a	49.0±1.5a	45.1±2.2a	40.9±0.3a	40.8±1.6a	39.0±0.6a	60.1±0.2a	60.0±1.4a	57.1±1.1a
Lycopene	121.5 <u>+</u> 2.2a	119.4±2.4a	102.6±3.7b	81.7±1.6a	81.3±1.5a	76.9 <u>±</u> 0.9a	38.4±1.1a	38.2±1.3a	36.5±1.1a	78.9±1.2a	78.7±1.8a	74.6±0.6a
Neoxanthin	87.8±1.2a	87.7±1.7a	83.9±3.0a	44.5±2.7a	44.4±2.7a	40.9±3.2a	51.2±1.2a	51.1±2.5a	$48.2 \pm 1.7a$	72.1±0.7a	71.9±2.0a	69.3±1.3a
Neurosporene	50.6±0.8a	50.9±2.2a	47.3 <u>+</u> 2.1a	19.0±0.4a	18.9±0.3a	17.0±0.5b	29.2±0.1a	29.2 <u>+</u> 0.6a	27.3±1.1a	50.0±0.7a	49.8±1.2a	47.2±1.5a
Violaxanthin	105.9±1.2a	105.3±3.1a	97.0±1.6b	58.8±1.5a	58.4±1.4a	55.7±2.6a	48.3±0.6a	48.2±1.9a	46.4±0.7a	66.2±0.2a	64.9±1.3a	61.5±0.3b
B-Zeaxanthin	93.2±0.6a	93.1±1.7a	90.4±0.2a	60.2±1.0a	60.0±0.9a	57.9±1.2a	54.1±0.5a	54.1±1.6a	50.6±0.3a	219.6±4.2a	216.7±3.0a	196.5±1.2b
Zeaxanthin	103.2±0.2a	103.2±1.6a	97.2±1.2b	48.7±1.6a	48.5±1.7a	45.9±0.2a	52.8±1.3a	52.5±1.5a	$51.3 \pm 2.2a$	312.4±2.0a	307.5±3.3a	257.5±32.8b
Antheraxanthin	91.4±0.7a	91.3±1.2a	87.0±1.5a	$40.1\pm0.7a$	40.0±0.7a	37.7±1.4a	43.4±0.6a	43.3±1.4a	$41.7 \pm 0.7a$	67.6±1.3a	67.5±1.8a	64.0±2.0a
8- APO- B- Carten- 8- al	126.3±0.3a	125.8±2.8a	114.9±3.3b	62.5±1.3a	62.2±1.3a	58.8±0.8a	61.8±0.4a	61.4±1.3a	58.5±0.3b	89.4±1.0a	89.3±1.5a	86.6±1.9a
Astaxanthin	90.1±0.4a	90.0±1.2a	85.4±2.6a	36.8±1.0a	36.6±1.0a	34.3±2.0a	42.8±1.5a	42.7±1.3a	41.1±1.4a	50.4±0.2a	50.3±0.7a	46.7±1.4b
Canthaxanthin	96.1±0.4a	95.9±1.7a	91.1±2.4a	51.3±0.9a	$51.1\pm0.9a$	48.8±1.3a	45.1±0.5a	44.8±1.7a	42.4±0.8a	56.4±0.7a	56.4±1.8a	52.0±0.9a
α -Carotene	130.8±0.9a	130.2±3.1a	115.6±3.7b	100.6±2.0a	100.1±1.7a	96.2±1.6a	59.3±1.3a	59.1±1.2a	55.2±1.9a	88.2±0.7a	88.1±1.2a	84.2±2.2a
β -Carotene	152.9±0.7a	152.5±2.8a	132.7±2.2b	134.1±3.0a	132.8±2.6a	116.9±2.5b	60.9±0.4a	60.7±0.5a	57.6±1.4a	96.4±0.2a	96.3±2.1a	83.0±6.5a
γ - Carotene	128.7±0.5a	128.3±1.4a	117.7±2.1b	76.7±1.8a	76.2±1.7a	73.0±1.9a	53.8±0.5a	53.7±1.4a	51.4±0.7a	90.9±0.3a	90.8±0.5a	86.8±1.5b
ξ-Carotene	115.8±0.5a	115.2±3.2a	$107.1{\pm}5.5a$	44.9±0.9a	44.7±0.8a	40.8±0.5b	56.2±1.1a	56.7±1.9a	54.6±0.5a	$93.0\pm0.5a$	92.9±1.6a	88.6±1.8a
ϵ -Carotene	123.9±0.5a	123.2±4.1a	114.1±3.0a	58.0±4.9a	57.0±4.9a	52.9±1.7a	58.5±0.6a	58.3±1.1a	55.6±0.6a	78.4±0.9a	78.4±2.2a	73.5±2.4a
Citraxanthin	111.1±0.2a	110.9±4.5a	102.0±4.5a	56.9±1.0b	56.7±0.9b	66.9±2.1a	62.1±0.6a	61.6±1.8a	58.9±0.9a	90.5±0.4a	90.5±1.5a	87.7±1.5a
Crocetin	89.3±0.7a	89.3±2.2a	$84.6\pm 2.7a$	69.3±0.6a	69.0±0.5a	$50.8 \pm 4.5b$	48.1±0.2a	47.8±1.3a	44.4±1.5a	70.1±0.3a	70.0±0.8a	66.5±1.3b
Means followed by the same litter(s) in the same row in each bee nollen type are not significantly different ($P < 0.05$)												

Means followed by the same litter(s) in the same row in each bee pollen type are not significantly different ($P \le 0.05$).

In case of sesame bee pollen there were significant differences between fresh $(61.84\pm0.43 \text{ mg/g})$ and 12 months storage $(58.50\pm0.33 \text{ mg/g})$ in 8- APO- B- Carten-8- al only. While the rest pigments were not significantly different. No significant differences were observed between fresh and six months storage.

Maize bee pollen showed significant differences in β - Cryptoxanthin, Isozeaxanthin, Violaxanthin, B-Zeaxanthin, Zeaxanthin, Astaxanthin, γ - Carotene and Crocetin for fresh and 12 months storage. For fresh and 12

months storage the values of above-mentioned pigments were as following 63.21 ± 0.62 mg/g and 59.51 ± 0.58 mg/g, 265.40 ± 2.07 mg/g and 220.81 ± 7.44 mg/g, 66.28 ± 0.22 mg/g and 61.58 ± 0.35 mg/g, 219.61 ± 4.27 mg/g and 196.59 ± 1.21 mg/g, 312.43 ± 2.07 mg/g and 257.56 ± 32.85 mg/g, 50.44 ± 0.24 mg/g and 46.78 ± 1.44 mg/g, 90.92 ± 0.39 mg/g and 86.82 ± 1.52 mg/g, 70.14 ± 0.35 mg/g and 66.50 ± 1.36 mg/g, respectively. However, the rest pigments were not significantly different. Also, no significant

differences were observed between fresh and six months storage.

Through the obtained results in this study about impact of storage period on bee pollen pigments of different types it was found no significant effects of storage till six months on bee pollen pigments in comparison with fresh bee pollen of all types. Sunflower bee pollen was the best in terms of containing more pigments than other bee pollen types. These results are in line with those of Keller, *et al.*, 2005 a, b. which revealed that white and red clover (*Trifolium, repens and pratense*), corn (*Zea mays*), rape (*Brassica napus*) and sunflower (*Helianthus sp.*) yielded on average more than 60% of the total collected pollen from different locations of Switzerland.

Muniategui *et al.*, 1990 in four samples of fresh bee-collected pollen reviewed 50–150 μ g/g of carotene and 140-400 μ g/g of xanthophylls. B-carotene content reached to 0.05-0.08% of dry weight. B-carotene content ranged from 0.8 to 315 mg /100g lipids. Bogdanov *et al.*, 2003 showed that from a microbiological and sensory point of view pollen remains stable until 1.5 years of storage at room temperature. Under these conditions pollen keeps its sensory and microbiological quality for a storage period of 2 years, if stored in a cool, dry and dark place.

Owayss *et al.*, 2004 referred to the importance of pigments (colorants) as antioxidants and markers of the origin of bee products. Stojko A., *et al.* 2012 concluded that, 12-month storage of bee pollen decreased the concentration of polyphenols in all three types of extracts, and these changes depend on the storage conditions. Pollen loses a considerable amount of its antioxidant activity (about 59%) after one year. This loss might be due to the decrease of phenolic compounds. Campos *et al.*, 2016 stated that the nutritional value of bee pollen varies considerably between the plant species and is also highly influenced by the storage method.

Anjos et al., 2019 indicated to the conditions of bee pollen storage may affect its composition. Carotenoids have been successfully gained more popularity because of their diverse functions particularly in food and pharmaceutical industries; including their role as provitamin A ingredients, effective antioxidants (Moreira et al., 2018), anti-tumor and anti-cardiac (Rostamabadi et al., 2019), anti-ageing and anti-inflammatory properties (Neville et al., 2013 and Kaulmann and Bohn, 2014). Vegetables and fruits are the main sources of carotenoids which are necessary for immune system activity, antioxidant activities, and intracellular communication (Khalid et al., 2018). Carotenoids not only in plants but also play vital functions in human body such as antioxidant and anti-inflammatory properties, enhancing immune response and preventing from many chronic diseases (Hernández et al., 2018).

CONCLUSION

Bee pollen is a product of the honeybee colony, high in nutritional value and has high medicinal value and used as a dietary supplement for food and pharmaceutical industries. From the obtained results of storage different bee pollen sources. It could be concluded that, among the four tested bee pollen types (Sunflower, clover, sesame and maize) Sunflower bee pollen was the best type contained pigments. For the effect of storage period on pigments, it was noted that no significant differences appeared among the bee pollens stored 6 months and fresh pollens. On the other hand, there are significant differences in pigments of 12 months storage and fresh pollens.

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تأثير فترات التخزين لأنواع مختلفة من حبوب لقاح النحل على محتواها من الصبغات أسماء المتولى عبد الله¹ و رشا عادل سالم² ¹قسم الحشرات الإقتصادية والمبيدات- كلية الزراعة- جامعة القاهرة - الجيزة¹ ²قسم بحوث النحل- معهد وقاية النبات - الدقى -الجيزة²

تعتبر الصبغات من المواد المضادة للأكسدة والتى تلعب دور هام فى الوقاية من العديد من الأمراض. فى هذه الدراسة تم دراسة تأثير فترات التخزين تحت ظروف التجميد على الصبغات الموجودة فى حبوب لقاح النحل لأربع مصادر نباتيه وهى عباد الشمس، البرسيم، السمسم والذرة. وتم تقدير المحتوى من الصبغات بطريقة التقدير اللوني وقد استخدم فى هذه الدراسة مذيب الايثانول لأستخلاص الصبغات عند أطوال موجيه مختلفة. أوضحت النتائج المتحصل عليها أن حبوب لقاح عباد الشمس كانت الأعلى في معظم الصبغات التي تم تقدير المحتوى من الصبغات بطريقة التقدير اللوني وقد استخدم فى هذه الدراسة مذيب الايثانول لأستخلاص الصبغات عند أطوال موجيه مختلفة. أوضحت النتائج المتحصل عليها أن حبوب لقاح عباد الشمس كانت الأعلى في معظم الصبغات التي تم تقدير ها ماعدا ثلاث صبغات عند أطوال موجيه مختلفة. أوضحت النتائج المتحصل عليها أن حبوب لقاح عباد الشمس كانت الأعلى في معظم الصبغات التي تم تقدير ها ماعدا ثلاث صبغات حيث كانت حبوب لقاح الذرة هي الأعلى في محتوى هذه الصبغات (الصبغات والقاح عباد الشمس كانت الأعلى في معظم الصبغات التي تم تشاهد فروق معنوية بين حبوب لقاح عباد الشمس الطازجة وتخزين الستة أشهر الصبغات مع عليها الصبغات مع عليه المحتول في محتوى هذه الصبغات المن من الماحتول من الماح الذي معن عبوب لقاح وما الماح المع في محتوى هذه الصبغات المع في محتواها من الصبغات. حبوب لقاح عباد الشمس الطازجة وتخزين الستة أشهر عالت أخلى معنويه فى محتواها من الصبغات. حبوب لقاح عباد الشمس الطازجه والمخزنة لمدة ستة أشهر كانت أعلى معنويه فى محتواها من الصبغات التاليه: – (10 معنولية معنواليا من الصبغات الألق المحتول الماحين المحتول في المحتول الماحين الماحية الماحين الماحية والما من الصبغات التالية. – (20 محتواها من الصبغات التاليه: – (20 محتواها من الصبغات الألق عباد الألق عباد المحسام معلي والماحين الماحين المحقول في محتول الماحين الماحية الماحين الماحية التالية. حمو الماحي مالم محتواها من الصبغات التالية المحقول ماحين الماحية معنول الماحية الماحية المع معنواها من المع والمحفات التالية: – (20 معام مصادر الماحية الماحية الماحين معوم لقاح المحمو عه من الذرة وعباد الشمس. المحمو مال حلام محمول المحمول المحماحي المحمول المحمول الماحية المحمول المحمو معام الماحي وعاد الشمس. المحمول ماحمو المحموم المحموي ا