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RESEARCH ARTICLE

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Characterization of *Apis mellifera* honey from different botanical and geographical origins in Egypt

ABSTRACT:

Seven bee honey types identified botanically in terms of their floral origin (Clover, *Cedru*s, Citrus, Banana, Cotton, Brazilian pepper, and Sun flower), and their geographical regions during 2016 - 2017 have been characterized in comparison with artificial honey. Characterization of honeys was based on their physicochemical properties, antimicrobial activity, heavy metal contents and multi-pesticides residues using quantitative analysis methods. All honeys tested were natural and give negative results for adulteration tests. Sun flower and cotton honeys showed the highest sucrose content regardless the artificial honey sucrose content (74 ± 1% (g/100 g). The water content and pH value of different honeys investigated were within the standard limits 20 g/100 g and 3.4 - 6.1, respectively. Hydroxymethylfurfural (HMF) content in most honeys was below the maximum allowable limit (MAL) (40 mg/kg) in honey, except for banana honey. None of the honeys tested showed any antifungal activity. Artificial honey showed no growth inhibition against reference strains of bacteria and fungi tested. Metal content of Fe, Zn, Pb, and Cu in Egyptian honeys fulfill the (MAL) described in the standard codex for honey except for Cd with concentrations exceeding the MAL (0.05 mg/kg) in most types of honey, except for artificial honey. Malathion, chlorpyrifos and tau-fluvalinate were the most frequently detected (25%, n = 2) pesticides in honey samples. It can be concluded that the best Egyptian honeys tested in terms of antibacterial activity were Brazilian pepper honey followed by *Cedrus* honey. The data obtained are extremely important for the public, health officials for medical and nutritional applications of honey.

KEY WORDS:

Honey Adulteration, Metals, Antibacterial activity, Beekeeping, miticides.

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INTRODUCTION:

Honey is a natural sweet food and medicine, and it is of significant economic value (Abeshu and Geleta, 2016)**.** The dominant contributor to production is the domesticated bee, *Apis mellifera* L. Honey is recognized worldwide for its nutritive components that are beneficial for human being. Traditionally Egyptians, Greeks, Romans, and Chinese used it as medicine to heal wounds and diseases of the gut, including gastric ulcers (Pasupuleti *et al.*, 2017). Therefore, it should be natural, free from any contaminants and of high quality.

The quality of honey and its specific character depend on its geographical and specific floral origins, season, environmental factors and beekeepers practices (Kaškoniene *et al.*, 2010; EL-Metwally, 2015). The nectar floral origin predominantly affects the chemical composition of honey in terms of its protein, carbohydrate, enzyme, mineral and organic acid content (Gok *et al.*, 2015). In the EU, the honey regulation states that the geographical and botanical origin of this product must be declared on package labels (Council EU, 2002). A large number of *in vitro* and limited clinical studies have confirmed the broad-spectrum antimicrobial (antibacterial, antifungal, antiviral, and antimycobacterial) properties of honey (Israili, 2014). Consequently, control of honey requires the determination of parameters that could unequivocally establish origin and calls for efforts to improve honey characterization.

Honey bees collect pollen and nectar from the surrounding blossoms covering very large areas. When they return to hives they also carry environmental pollutants (Bogdanov, 2006). The major environmental contaminants of honey and other bee products include heavy metals, radioactive isotopes, organic pollutants, pesticides, pathogenic bacteria, genetically modified organisms and misconducted beekeeping practices (Mullin *et al.*, 2010; Roman, 2010; Al Naggar *et al.*, 2013, 2015, & 2017). Food alerts due to the presence of antibiotics, pesticides or metals in honey have caused some authorities to restrict imports of bee products from some countries, which have damaged the reputation of honey (Juan-Borrás *et al.*, 2015).

Egypt is considered as the most important country for beekeeping among Arab nations, as well as throughout Africa (Al-Ghamdi *et al.,* 2016). Moreover, beekeeping activity is carried out around the year in Egypt with total honey yield about 9112 ton annually (Hussein, 2000), however, only some types of honeys have been extensively studied and characterized in Egypt (Essa *et al.*, 2010; Hegazi *et al.*, 2014; Al Naggar *et al.*, 2017). Moreover, annual reports of physiochemical characteristics, antimicrobial activity and mineral and pesticides residues content of different honey types produced and collected in Egypt are needing. Lacking such these annual reports might affect the reputation of Egyptian honey and other bee products and can adversely affect the beekeeping industry and economy in Egypt.

Accordingly, the aim of this study was to differentiate for the 1st time between seven types of bee honey collected from different botanical and geographical areas in Egypt by their physicochemical properties, pesticide residues, heavy metal contaminants in comparison with artificial and international honey standards of the Codex Alimentarius (Codex Alimentarius, 2001). In addition, antimicrobial properties of tested bee honey were studied against different reference strains of human pathogenic fungi and bacteria.

MATERIAL AND METHODS:

Botanical and geographical origins:

Sampling sites were selected to include different botanical origins and regions of honey production in Egypt during 2016-2017. Geographical locations of honey collection are illustrated (Fig. 1). Information about available floral sources was collected by asking beekeepers to guided identify the floral source of the honey samples. Seven different honey types were provided by beekeepers that operate in different regions. Cotton (*Gossypium sp.*) honey was collected during August 2016 from Desouk city; Kafr El Sheikh Governorate (S1) (31.3°N 30.93°E) in the middle Delta of Egypt. Sun flower (*Helianthus annuus*) honey was obtained during August 2016 from Abu El Matamir city (S2). Citrus honey was collected during April 2017 from new Nubaria city (S3). Both S2 & S3 are locating in Al Behira governorate (30.61°N 30.43°E). Citrus orchard was dominated by orange, *Citrus sinensis*, tangerine, *Citrus reticulate*, bitter orange, *Citrus aurantium* and lemon, *Citrus limon*. Banana (*Musa sp.*) honey was sampled during September 2016 from an apiary in Sadat city, Monufia governorate (S4) (30.52°N 30.99°E). From Qena governorate (S5) (26.143°N 32.728°E), which is a city in Upper Egypt, located in the southern part of the country and covers a stretch of the Nile valley, *Cedrus* (*Cedrus* ssp.) honey was collected during January 2017. Brazilian pepper (*Schinus terebinthifolius*) honey was sampled from an apiary at the Research Center in Alexandria governorate (S6) (31°10′N 29°53′E). Clover (*Trifolium alexandrinum*) honey was collected from an apiary in Aga city, Dakahlia governorate (S7) (31°03′N 31°23′E) of Egypt during June 2017.

Fig. 1. Map of Egypt showing sampling locations of different honeys. (S1) Kafr El Sheikh Governorate, (S2) Abu El Matamir city, (S3) new Nubaria city, (S4) Sadat city, (S5) Qena governorate, (S6) Alexandria governorate and (S7) Dakahlia governorate.

Sample collection:

Fresh honey samples (1 kg each) were squeezed out from honey combs of three randomly selected bee hives at each apiary into a disposable polyethylene container. All samples were stored at refrigerator at 7 - 10°C until analysis.

Artificial honey was prepared as fellow: one kg of high-quality refined sugar was added in a clean container that contained 300 ml of water and 1.1 gm of tartaric acid. Then, heating at 110° C, we continued stirring until the liquid took on a fine golden yellow color; duration of such operation was about 30 to 40 minutes (https://www.scientificamerican.com/article/ artificial-honey/).

The seven honey types surveyed initially underwent to some recommended tests of honey adulteration in Egypt such as detection of: starch and dextrin content, commercial glucose, converted sugar, added sucrose and artificial colors. All honeys studied were natural and gave negative results for all adulterations tests carried out.

Physicochemical characteristics of honey:

Apparent sucrose content:

Sucrose content was determined according to Cantarelli *et al*. (2008).

Moisture content:

Moisture content was determined using the indirect refracting metric method (Bogdanov, 2002).

pH:

The pH was measured using a digital pH meter according to (Bogdanov, 2009).

Total protein content:

Total protein content was measured using the Kjeldahl method as described in AOAC (2005).

Determination of total soluble solids (TSS) (Brix):

The total soluble solids (TSS) were determined according to the method described by Mazumdar and Majumder (2003) using digital-bench-refractometer.

Hydroxymethylfurfural (HMF) analysis:

Hydroxymethylfurfural was determined according to AOAC (1990).

Antimicrobial activity of honey:

Antimicrobial activity of honey was tested against the gram-positive human pathogenic bacteria *Bacillus subtilis* (ATCC-6633), *Staphylococcus aureus* (ATCC-6538) and *Bacillus cereus var. toyonensis* (ATCC-14579) and the gram-negative bacteria *Pseudomonas aeruginosa* (ATTCC-9027), *Escherichia coli* (ATCC-8739) and *Salmonella typhimurium* (ATCC-14028). Antimicrobial activity of honey was also tested against the fungi *Candida albicans,* (*ATCC-90028)* and *Aspergillus niger*, (*ferm-BAM C-21*). Antimicrobial disk diffusion method was performed as described by the national committee for clinical laboratory standard (CLSI, 2006) and the percentages of inhibition were measured.

Heavy metal analysis:

Honey samples were digested for metal analysis according to Fakhimzadeh and Lodenius (2000) Finally, the sample solutions were analyzed for their metal concentrations by a flame Atomic Absorption Spectrophotometer (AAS) (Varian SpectrAAS-400).

Multi-pesticide residues analysis:

Extraction of honey was carried out by using QuEChERS method (Wiest *et al.*, 2011). Then honey samples were screened for 240 different pesticides. Chromatographic multi-residue analysis was performed with a 1200 triple-quadruple GC/MS/MS system (Varian Scientific Equipment, Palo Alto, CA).

Statistical analysis:

Data were analyzed by use of Graph Pad Prism version 5.00 for Windows. Normality of results was assessed by use of the Kolomogrov–Smirnov test, and homogeneity of variance was determined with a Levene's test. If necessary, data were log10 transformed to ensure normality and homogeneity of variance. Data were presented as means and standard deviations. Differences in the quantified variables in different types of honey (except artificial honey) have been evaluated using one-way analysis of variance (ANOVA) with Tukey post hoc Test, $P < 0.05$.

RESULTS:

Sucrose content:

There was significant difference ($P < 0.0001$) in sucrose content between different investigated honeys. Sucrose ratio ranged between 0.2 ± 0.04% in *Cedrus* honey and 74 ± 1.32% (g/100 g) in artificial honey (Table 1). Percentages of sucrose content detected were all within the recommended percentage of sucrose (5 g/100 g) in honey (Codex Alimentarius, 2001) except in artificial, sunflower and cotton honeys.

Moisture content%:

A significant difference ($P < 0.0001$) in the moisture content between the different honeys analyzed were reported (Table 1). The highest moisture content (33 ± 1.15% g/100g) was found in artificial honey sample, as expected, while the lowest moisture content (19 ± 0.5% g/100 g) was in sunflower and cotton honey. Only in artificial, citrus, clover and Brazilian pepper honeys, moisture content exceeded the permissible range for honey (20 g/100 g) (Codex Alimentarius, 2001).

pH:

There were significant differences ($P < 0.05$) in pH of honey samples depending on the honey type (Table 1). The pH values of honey ranged from 3.6 ± 0.05 to 4.8 ± 0.1% which were within the limit (3.4 - 6.10%) recommended in honey by Codex Alimentarius, (2001).

Total soluble solids (TSS):

There was no significant difference ($P > 0.07$) in TSS content between honeys samples investigated. The content of TSS ranged from 67 ± 1 to 81 ± 2% (g/100 g) which was within the accepted percentage of solid materials (80 g/100 g) in honey (Codex Alimentarius, 2001) except for cotton and sunflower honeys (Table 1). The highest TSS content (81 ± 2% g/100 g) was found in sunflower honey while the lowest TSS content (67 ± 1% g/100 g) was reported in artificial honey.

Total protein content:

The protein content of honey types studied ranged from 3.6 ± 0.3 to 10 ± 0.4 mg/g (Table 1). There was a significant difference (*P* < 0.0001) in the protein content between examined samples. The highest protein content (10 ± 0.4 mg/g) was found in sunflower honey while the lowest content (3.6 ± 0.3 mg/g) was in the *Cedrus* honey.

HMF:

The content of HMF in different honeys studied ranged from 1.6 ± 0.7 to 98.6 ± 1.2 mg/kg (Table 1). The highest and lowest concentrations of HMF 98.6 ± 1.21 and 1.6 ± 0.72 were detected in banana and *Cedrus* honeys, respectively. The levels of HMF detected were significantly different (*P* < 0.0001) in honeys studied and were below the standard limit of HMF in honey (40 mg/kg) (Codex Alimentarius, 2001) except in banana honey.

Table1. Physicochemical parameters (Mean ± SD) of different honey types collected (harvested in) from different seasonal, botanical and geographical origins in Egypt during 2016-2017.

Type of honey	Sucrose (%) (g/100g)	Moisture (%) (g/100g)	pH	Total soluble Solids (Brix) (g/100g)	Total protein (mg/g)	HMF* (mg/kg)
Citrus	2.6 ± 0.15[a]	22 ± 0.42[a]	4.4 ± 0.05[ad]	78 ± 1.32	7.1 ± 0.13[a]	5.4 ± 0.73[a]
Cotton	7 ± 0.25[b]	19 ± 0.52[cb]	4.8 ± 0.12[bf]	81 ± 0.53	7.1 ± 0.33[ab]	14.3 ± 0.34[b]
Clover	3.9 ± 0.24[c]	21 ± 0.21[ac]	4.3 ± 0.15[ac]	79 ± 1.52	9.1 ± 0. 22[c]	5 ± 0.53[ac]
Banana	3.2 ± 0.32 [ad]	20 ± 0.31[cd]	4.3 ± 0.3[dc]	80 ± 2.2	5.4 ± 0.30[d]	98.6 ± 1.21[d]
Brazilian Pepper	1.5 ± 0.26 [e]	22 ± 0.71[ae]	4.2 ± 0.12[cd]	78 ± 1.55	7.1 ± 0.22[ae]	18.3 ± 1.42[e]
Sun flower	7 ± 0.55 [bf]	19 ± 0. 32[cf]	4.8 ± 0.05[af]	81 ± 2.23	10 ± 0.43[f]	12.2 ± 2.21[bf]
Cedrus	0.2 ± 0.04[g]	20 ± 0.62[cg]	4.2 ± 0.06[d]	80 ± 1.75	3.6 ± 0.34[g]	1.6 ± 0.72[ag]
Artificial	74 ± 1.32	33 ± 1.15	3.6 ± 0.05	67 ± 1.22	0.0 ± 0.0	19.8 ± 2.31
Standard limit#	5	20	3.4-6.1	80	-	40

* Hydroxymethylfurfural.

Standard limit (recommended content) set by Codex Alimentations (2001).

Means followed by similar letters within each column do not significantly different from each other (p ≤ 0.05).

Antimicrobial activity:

All honey samples tested except artificial honey, exhibited antibacterial activity against *E. coli* and *S. typhimurium*. Significant differences (*P* < 0.05) in antibacterial activities of different honeys against *S. typhimurium* have been noticed (Table 2). While significant difference (*P* < 0.05) in growth inhibition activity against *P. areuginosa* was noticed only in Brazilian pepper, sunflower and *Cedrus* honeys. Brazilian pepper and *Cedrus* honeys were the only honeys that showed growth inhibition against *S. aureus* bacteria. While, the growth of *B. cereus* bacteria was only inhibited by Brazilian pepper honey. None of the honeys tested showed any fungal activity against *C. typhimurium* and *A. niger* (Table 2).

Table 2. Antimicrobial activity of different honeys collected from different seasonal, botanical and geographical locations in Egypt during 2016-2017 and adulterated honey.

Honey type	Diameter of inhibition zone (mm)* (n=3)							
	Gram positive bacteria			Gram negative bacteria			Fungi	
	Bacillus subtilis (ATCC-6633)	*Staphylococcs aureus (ATCC-6538)*	*Bacillus cereus (ATCC-14579)*	*Pseudomonas areuginosa (ATCC-9072)*	*Escherichia coli (ATCC-8739)*	*Salmonella typhimorium (ATCC-14028)*	*Candida typhimorium (ATCC-90028)*	*Aspergillus niger*
Citrus	-	-	-	-	32 ± 0.24	36 ± 0.38[ab]	-	-
Cotton	-	-	-	-	31 ± 0.44	35 ± 0.17[c]	-	-
Clover	-	-	-	-	33 ± 0.35	37 ± 0.49[ad]	-	-
Banana	-	-	-	-	31.5 ± 0.29	33 ± 034[e]	-	-
Pepper	-	14 ± 0.34[a]	13.5 ± 0.55	16 ± 42[a]	30.5 ± 0.32	38 ± 0.22[af]	-	-
Sunflower	-	-	-	24.5 ± 0.19[b]	29 ± 0.52	35 ± 0.41[g]	-	-
Sidr	-	11 ± 0.26[b]	-	23 ± 0.37 [b]	30 ± 0.31	35 ± 0.36[gh]	-	-
Artificial	-	-	-	-	-	-	-	-

* Data are represented as (Mean ± SD) (n = 3).

*Well diameter.1 cm. (100 µl of each one was tested), the sample diluted with distilled water as follow 1: 1 v/v. Means followed by similar letters within each column do not significantly different from each other (p ≤ 0.05).

Heavy metals:

There were significant differences ($P < 0.05$) in heavy metals concentrations detected in different types of honeys (Table 3). The highest concentration of Cu (0.09 mg/kg) was found in citrus honey, while the lowest concentration (0.02 mg/kg) was detected in clover honey. The highest Cu concentration detected in honeys analyzed was 100-fold below the maximum allowable limit (MAL) of Cu (10 ppm) in honey (Piven *et al.*, 2003). Brazilian pepper honey contained the greatest concentration of Cd (0.23 mg/kg) while the least (0.07 mg/kg) was found in *Cedrus* honey. Cadmium content detected exceeded MAL limit (0.05 mg/kg) (Piven *et al.*, 2003) in all honeys except in artificial honey.

Artificial, sunflower and *Cedrus* honeys were free from any lead contamination. Lead contents detected were below the MAL limit for Pb (1.5 mg/kg) in honey (Piven *et al.*, 2003). Cotton honey contained the highest concentration of Fe (1.26 mg/kg) while the lowest concentration (0.04 mg/kg) was detected in artificial honey. Iron concentrations detected in different honeys investigated were below the MAL of Fe (5.2 mg/kg) in honey (Piven *et al.*, 2003). Artificial honey contained the least concentration of Zn (0.02 mg/kg) however; greatest concentration (0.77 mg/kg) was detected in cotton honey. Zinc concentrations detected were below the MAL of Zn in honey (3 mg/kg) (Piven *et al*., 2003).

Table 3. Heavy metals concentrations (mg/kg) (Mean ± SD) (n = 3) in honey samples collected from different seasonal, geographical and botanical origins in Egypt during 2016-2017.

Honey type	Cu	Cd	Pb	Fe	Zn
Citrus	0.09 ± 0.02 ^{a}	0.09 ± 0.02^{a}	0.09 ± 0.01^{ab}	1.12 ± 0.07^{a}	0.76 ± 0.15^{a}
Cotton	0.08 ± 0.02^{ab}	0.12 ± 0.08^{fb}	0.05 ± 0.01^{a}	1.26 ± 0.8^{b}	0.77 ± 0.14^{ab}
Clover	0.02 ± 0.02^{c}	0.18 ± 0.07^{c}	0.08 ± 0.02^{ab}	0.65 ± 0.04^{c}	0.26 ± 0.04^{c}
Banana	0.05 ± 0.03 ^{d}	0.13 ± 0.08 ^{bd}	0.08 ± 0.02^{ab}	1.08 ± 0.04^{d}	0.47 ± 0.09^{d}
Brazilian Pepper	0.03 ± 0.08 ^{e}	0.23 ± 0.09 ^{e}	0.12 ± 0.08^{b}	0.89 ± 0.07^{e}	0.19 ± 0.08^{e}
Sunflower	0.05 ± 0.04^{df}	0.11 ± 0.04^{af}	ND	1.03 ± 0.23^{df}	0.1 ± 0.01^{f}
Cedrus	ND	0.07 ± 0.04^{ag}	ND	0.38 ± 0.24^{g}	0.08 ± 0.01^{gf}
Artificial	ND	ND	ND	0.04 ± 0.01	0.02 ± 0.01
MAL#	10.00	0.05	1.5	5.2	3

*ND= None detected.

Means followed by similar letters within each column do not significantly different from each other ($p \leq 0.05$).

#MAL, maximum allowable limit (Piven *et al*., 2003).

Pesticide residues in honey:

Pesticides detected in honey samples were belonging to organophosphorus (OPs), pyrethroids, organochlorine (OCs), pyrazoles, carbamates and neonicotinoides (Table 4). The most frequently detected pesticides were OPs pesticides; malathion (n = 2), chropyrifos (n = 2) and miticides; tau-fluvalinate (n = 2). Malathion was detected in both citrus and banana honeys at concentration of 0.21 and 0.07 mg/kg, respectively. While residues of chlorpyrifos (0.08 and 0.01 mg/kg) were detected in cotton and clover honeys, respectively. Sunflower honey contained profenofos pesticide at concentration of 0.11 mg/kg.

The residues of tau-fluvalinate that widely used for *Varroa* control were found in both banana and sunflower honeys at concentrations 0.5 and 0.18 mg/kg, respectively. Citrus honey contained residue of the neonicotinoid pesticide thiamethoxam at concentrations 0.01 mg/kg. While dicofol which belongs to OCs pesticides was only detected in Brazilian pepper honey at 0.13 mg/kg. *Cedrus* honey that produced and collected from Upper Egypt was free from any pesticides residues (Table 4).

Table 4. Pesticide residues detected (mg/kg) in different types of Egyptian honey collected from different seasonal, geographical and botanical origins in Egypt during 2016-2017.

Honey type	Pesticide	Pesticides class	Conc. (mg/kg)
Citrus	Malathion	Organophosphorus	0.21
	Thiamethoxam	Neonicotinoid	0.01
Cotton	Chlorpyrifos	Organophosphorus	0.08
	Fenpyroximat	Pyrazole	0.01
Clover	Chlorpyrifos	Organophosphorus	0.01
	Methomyl	Organophosphorus	0.01
Banana	Malathion	Organophosphorus	0.07
	Tau-fluvalinate	Pyrethroids	0.50
Brazilian pepper	Dicofol	Organochlorine	0.13
Sunflower	Tau-fluvalinate	Pyrethroids	0.18
	Profenofos	Organophosphorus	0.11

DISCUSSION:

The physicochemical properties of natural honeys, such as moisture, sucrose, HMF, water-insoluble content and electrical conductivity are strictly defined and constitute the quality indicators which characterize individual honey varieties. The sucrose content of different Egyptian honey was in compliance with national and international regulations, setting upper limit to 5 g/100 g**,** with the exception of artificial, sunflower and cotton honeys. High sucrose content detected in some Egyptian honeys could be might be due to the artificial feeding with sucrose.

The higher the moisture content, the greater the probability of honey fermentation during storage (El Sohaimy *et al.*, 2015). The percentages of moisture contents of Egyptian honey were mostly below the accepted limit (20 g/100 g) except in artificial, citrus, clover and Brazilian pepper honeys. Lower moisture content prolongs honey storage shelf life (El Sohaimy *et al.*, 2015). The variation in moisture content of honeys might attributed to environmental and geographical moisture conditions, content of flower nectar, harvesting season, degree of honey maturity reached in the hive and apiary management (Nanda *et al.*, 2003).

Honey is usually known for its acidic nature. All Egyptian honeys tested in the present study were acidic and within the standard limit (pH 3.40 – 6.10). The pH values of different Egyptian honey types were in range of to those previously reported for Algerian, Indian, and Turkish honeys (Ouchemoukh *et al.*, 2007; Kayacier and Karaman, 2008; Saxena *et al.*, 2010)**.** The presence of organic acids and inorganic ions such as gluconic acid with their lactones or esters, phosphate and chloride are the main determinants of honey's acidity (Terrab *et al.*, 2002).

The protein content of honey was initially utilized to distinguish honey from artificial admixtures and blends. There were significant differences between different honeys studied in their total protein content. This variation occurred as a function of the floral origin (Bath and Singh, 1999). Proteins and amino acids in honeys are attributed both to animal and vegetal sources, including fluids and the secretions of the salivary glands of honeybees (Escuredo *et al.*, 2013).

The higher the HMF value, the lower the quality of the honey is considered to be. It is undoubtedly an excellent indicator of honey freshness and purity (Codex Alimentarius, 2000). All honeys studied contained HMF levels lower than the MAL (40 mg/kg) in honey except in banana honey. High concentrations of HMF in banana honey indicate over-heating or poor storage conditions. HMF is produced when some of the sugars in honey, such as glucose and fructose, begin to break down, specifically when storage temperatures are high over long periods of time (Gomes *et al.*, 2010). In most previous studies, HMF has been reported to have negative effects on human health, such as cytotoxicity toward mucous membranes, the skin and the upper respiratory tract; mutagenicity; chromosomal aberrations; and carcinogenicity toward humans and animals (Lee *et al.,* 1995; Glatt *et al.,* 2005; Monien *et al.,* 2012). In addition to that, it is hypothesized that HMF causes bees to experience dysentery-like symptoms and ulcers in the alimentary canal (Bailey, 1966), leading to their death. Therefore, HMF is considered one of the main quality indexes of different commercial whey proteins, molasses and many other products (Dogan *et al.,* 2005).

The results of this study showed that all honeys studied except artificial honey have a strong antibacterial activity against gram-negative bacteria especially *E. coli* and *S. typhimorium*. Growth inhibition of gram-positive bacteria was noticed only in Brazilian pepper, sunflower and *Cedrus* honeys. The antibacterial activity of honey is due to the presence of hydrogen peroxide generation, presence of other phytochemical constituents such as phenolic acids, lysozyme and flavonoids in bee honey, and also to a naturally low pH, which is unfavorable for bacterial growth (Taormina *et al.*, 2001). The variation in the activity of the different honeys studied might be attributed to several factors such as osmotic properties of honey (Molan, 1992); honey pH (Mairaj *et al.*, 2008) or activity of glucose oxidase and hydrogen peroxide (Efem, 1988) and non-peroxide substances (Radwan *et al.*, 1984) which differ according to the botanical origin of honey and have great effect on the antibacterial activity of honey.

None of the honeys tested in the present study showed any fungal activity against *C. typhimurium* and *A. niger*. These findings are matching with (Estrada *et al.*, 2005) who tested 25 samples of honey and found no inhibitory effect on *A. niger*. Limited antifungal activity of honey is due to the emergence of resistant strains, and also depends on physico-chemical properties, botanical origin and entomological origin of honey (Anyanwu, 2012).

Artificial honey showed no antimicrobial activity as expected because it free from any components that characterize the natural honeys and contribute to its well-known antimicrobial property (Nishio *et al.*, 2016). These findings are of concern to the public, health officials, and to the manufacturers regarding production of honey for medical applications and highlight the importance of frequent characterization of produced, imported and exported honeys to save the nutritional and medical reputation of honey.

The content of Cu, Pb, Zn, and Fe

detected in Egyptian honeys investigated were below the MAL. However, Cd content exceeded the MAL in all honeys except in artificial honey. Generally, Cd as a toxic metal should not be present in food samples. The metal content of bee honey varies with the surrounding environment (major floral and soil contamination) (Al Naggar *et al.*, 2013). Industrial activities and irrigation with waste waters in some areas in Egypt increased the level of both total and available Cd in surface areas of agricultural soils. Additionally, phosphate fertilizers are also an important source of Cd in agricultural sources in Egypt (Abdel-Sabour, 2001). Cadmium is a metal of current toxicological concern and is frequently associated with urbanization and industrial processes (Al Naggar *et al.,* 2014; Jabłońska-Czapla *et al.,* 2016). Its detrimental effects on biochemical, physiological, and behavioral function have been documented in humans and animals (Cao L, Ding G. 2010. Patra *et al.,* 2011; Mirčić *et al.*, 2013). Moreover, the adverse effects of Cd on honey bees have been previously reported (Di *et al.,* 2016; Gauthier *et al.* 2016; Nikolić *et al.,* 2016). Consequently, beekeepers around the world should pay more attention for that and must put their beehives in locations away from any metal pollution.

Pesticides became one of the major contaminants of honey and bee matrices (Mullin *et al.*, 2010). The obtained results indicated that the most frequently detected pesticides in honey were OPs pesticides as previously reported (Malhat and Nasr, 2013; Al Naggar *et al.*, 2015). Detection of malathion and chlorpyrifos pesticides in both clover and cotton honeys investigated in present study were consistent with results of previous studies of pesticides in honey in Egypt (Al Naggar *et al.*, 2015 & 2017). Their presence in honey might attributed to their wide application for controlling pests affecting agricultural crops, ornamentals, green houses, livestock, stored grain, buildings, household and gardens (Abou El Ella, 2008).

The pyrethroid tau-fluvalinate which broadly used as miticide against *varroa* infestation inside honey bee colonies (Johnson *et al.*, 2006) was detected in both banana and sunflower honeys. Since 1988 tau-fluvalinate has been extensively used worldwide by beekeepers to prevent varroatosis (Tsigouri *et al.*, 2001). However, these findings indicate its overuse and carry a risk of direct contamination of honey and other hive products.

Although OCs pesticides usage has been completely prohibited by law since 1986 in Egypt, dicofol was detected in Brazilian pepper honey collected from Alexandria governorate which suggested a recent, direct and illegal use of such pesticides in the fields along the study areas (Gad Alla *et al.*, 2013). Thiamethoxam, a neonicotinoid insecticide, was detected only in citrus honey (0.01 mg/kg). These findings are supported by (Codling *et al.*, 2017), who found thiamethoxam at a concentration of 0.018 mg/kg in clover honeys collected from delta region of Egypt during spring 2013. Thiamethoxam is currently one of the most effective chemicals for the control of sucking pests such as aphids, whiteflies, thrips, some micro Lepidoptera and a number of coleopteran species (Malhat *et al.*, 2014).

CONCLUSION:

It could be concluded that there were differences in physiochemical properties, antibacterial activity, mineral content and pesticides residues detected in different Egyptian honeys studied. The honeys with best antimicrobial properties found were Brazilian pepper, followed by *Cedrus* honey. Beekeepers in Egypt should pay more attention and regulate the use tau-fluvalinate that has been registered for *Varroa* mite control under different trade names (such as Mavrik® and Apistan®). Its adverse effect on drones and queens due to the overuse has been previously reported (Rinderer *et al.*, 1999; Sylvester *et al.*, 1999). This study represents the 1st study to differentiate between different honeys produced in Egypt in comparison with artificial honey and international standers. The data obtained are very important for the public, health officials, and to the manufacturers regarding production of honey for medical and nutritional applications.

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توصيف عسل النحل (أبيس ميلفيرا) من أصول نباتية وجغرافية مختلفة في مصر

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تم تجميع سبعة أنواع من عسل النحل ذو اصول نباتيه مختلفة من حيث أصلها الزهري (البرسيم، السدر، الموالح، الموز، القطن، الفلفل البرازيلي، وزهرة عباد الشمس) ومن أماكن جغرافية مختلفة خلال عامى 2016-2017 بالمقارنة مع العسل الصناعي. استند توصيف عينات عسل النحل إلى خواصها الفيزيائية الكيميائية، والنشاط المضاد للميكروبات، ومحتويات المعادن الثقيلة ومتبقيات مبيدات الافات باستخدام أساليب التحليل الكمي. اظهرت النتائج أن جميع انواع العسل التى تم اختبارها كانت طبيعية وغير مغشوشة. كشفت النتائج ايضا ان عسل زهرة عباد الشمس والقطن يحتوي على أعلى نسبة من السكروز بغض النظر عن محتوى السكروز في العسل الصناعي (74±1٪) (جرام / 100جرام). بينما كانت نسبة محتوى الماء وقيمة الحموضه pH لعينات العسل المختلفة ضمن الحدود المعيارية 20جم/100جم و 3.4-6.1 علي الترتيب. كان محتوى مركب هيدروكسي ميثيل فلورفورال (HMF) في معظم عينات عسل النحل أقل من الحد المسموح به (40 مجم/كجم) في العسل باستثناء عسل الموز. ولم يظهر أي نوع من عينات العسل التى تم اختبارها أي نشاط مضاد للفطريات ولم يظهر العسل الصناعي أي نشاط تثبيط ضد السلالات المرجعية للبكتيريا والفطريات المختبرة. وكانت محتويات المعادن Fe ، Zn، Pb، Cu في الانواع المختلفة اقل من الحد المسموح به (MAL) طبقا للمواصفات القياسية للعسل باستثناء الكادميوم حيث تجاوزت نسبته النسبه المسموح بها (0.05 مجم/كجم) في معظم أنواع العسل ، باستثناء العسل الصناعي . اما بالنسبة لمتبقيات المبيدات كان الملاثيون والكلوربيريفوس وtau-fluvalinate من أكثر المبيدات الحشرية وجودا (25٪، ن = 2) في عينات العسل. ويمكن الاستنتاج أن أفضل انواع عسل النحل المصري التي تم اختبار نشاطها المضاد للبكتريا هو عسل الفلفل البرازيلي ويليه عسل السدر. النتائج التي تم الحصول عليها مهمة للغاية ويمكن للمسؤولين فى مجال الصحة استخدمها في التطبيقات الطبية والغذائية للعسل.