Some Physiochemical and Antimicrobial Characteristics of Honeybee from Colonies Fed with Sugar Syrup and Inverted Sugar

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Abstract

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The present study was performed to determine the impact of feeding honeybee colonies with sugar syrup and inverted sugar on pollen, physiochemical and antimicrobial properties. Raw honeys and bee-processed syrups (sucrose solution or inverted sugar syrup) samples were collected from bee colonies under field conditions in different regions of Egypt. Biological activities were compared between colonies which fed with sugar syrup and inverted sugar. The results obtained clearly showed that the chemical analysis of tested samples indicated that concentration of monosaccharide (fructose and glucose) was the highest in samples from natural honey. In contrast, disaccharide (sucrose) concentration was lowest compared to the samples collected from colonies fed with inverted sugar and sugar syrup. The antibacterial activity of natural honey from the Asyut region was highest against *Staphylococcus aureus* compared to honey obtained from bee colonies fed on sucrose solution and inverted sugar syrup. Only honey samples of colonies which feed with inverted sugar (Qualiubia region), sugar syrup (Arish region) at 100% concentration caused antimicrobial activity against *Staphylococcus aureus.* However, the effect against *Pseudomonas aeruginosa* was observed only with honey sample (100%) harvested from colonies fed with sugar syrup at Arish region. A wide range with highest value of H_2O_2 (201.30 and 139.72 mg/kg) was observed in natural honey samples and honey obtained from colonies fed on inverted sugar from Arish region. The water contents of honey collected from colonies fed with sugar syrup in Qualiubia and Arish regions, or fed on inverted sugar in Arish reached the highest value. The results obtained also showed that the pollen grains content, H_2O_2 and antimicrobial activity were reduced in the harvested honey samples of honeybee colonies fed with inverted sugar and sugar syrup in most regions. In contrast, these diets increased the water content and improved biological activity in colonies fed with inverted sugar, compared to that fed on sugar syrup.

Keywords: Honeybee inverted sugar, sugar syrup, *Pseudomonas aeruginosa*, *Staphylococcus aureus*.

Introduction

European honeybees *(Apis mellifera* L.) are the important insect pollinators of numerous field, fruit, and vegetable crops, and effectively contribute to global food production (Gallai *et al.,* 2009; Southwick & Southwick, 1992). Reproductive success of uncultivated plants depends on honeybee species (*Apis* sp.) and other bee species including those in their native ranges (Gallai *et al.,* 2009; Morse & Calderone, 2000; Potts *et al.,* 2010). In addition to pollination, honeybees also produce economically valuable bee food products such as honey, pollen and propolis. The health and production of a bee colony is dependent on the apiary location. During winter and spring when nectar can be scarce, and when preparing colonies for winter, beekeepers must feed their bees supplementary carbohydrates. Feeding with sucrose, high fructose corn syrup (HFCS) and inverted sugar (a mix of glucose and fructose) often protects the bees from malnutrition, which can lead to immune system impairment (Alaux *et al.,* 2010) and increased pesticide susceptibility (Wahl & Ulm, 1983). Feeding of either sucrose or HFCS influences gene expression by the honeybee fat body that are associated with energy metabolism, and anti-microbial peptide production (Wheeler & Robinson, 2014).

Inverted sugars produced from sucrose hydrolysis which can be carried out by using hydrochloric acid at 70-80ºC or by using invertase (EC.3.2.1.26) at 30-45ºC and pH 4.6. Although, the invertase can be used instead of acid for sucrose hydrolysis, but more costly. Consequently, the use of immobilized invertase would be a viably practical alternative.

Honey is a natural product and functional food added to therapeutic advantages in the treatment of various disorders (Otero & Bernolo, 2020). In addition to honey use as food, it is used topically for wound care for a broad spectrum of injuries and burns (Majtan, 2014). Its biological properties, including antibacterial, anti-inflammatory, anti-biofilm and regenerative activities are essential useful characteristics which may vary from honey to honey and might be significantly affected by technological processing and environmental conditions. Furthermore, a novel synthetic product which mimics the honey's carbohydrate composition and contains fungal glucose oxidase (GOX) has recently been developed as an alternative option to honey-based medical products (Matoke Holdings, 2018). Both these medical products are solely based on the bactericidal activity

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of continuously generated hydrogen peroxide (H_2O_2) produced by fungal GOX in the presence of glucose and water. The GOX found in natural honey is a regular but quantitatively variable bee-derived component of natural honey (Bucekova *et al.,* 2019). The levels of H_2O_2 vary based on the honey source, as many factors may affect the total concentration of H_2O_2 (Farkasovska *et al.,* 2019).

This study aimed to assess some chemical differences among natural bee honey and compared with honey produced by bee hives fed with sugar syrup and inverted sugar. Furthermore, honey antibacterial activity and the presence of pollen grains in honey produced in different regions of Egypt was determined.

Materials and Methods

Experimental procedures

This work was performed in three Governorates of Egypt, Arish, Qualiubia and Asyut from the beginning of January 2021 until the end of April 2021. Eighteen colonies headed by cariniolan queen were selected for this study. Nine, six and three colonies were selected for each apiary in Arish, Qualiubia and Asyut Governorates, respectively. In Arish Governorate six colonies fed with sugar syrup, inverted sugar (three colonies for each) and the rest three colonies left to be fed by natural nectar. Three colonies were used in Qualiubia Governorate for each treatment of inverted sugar and sugar syrup. In apiaries of Asyut Governorate, three colonies were selected and left for natural nectar feeding without any additive sugar solution. Bee feed was provided in bucket feeders equipped with floaters to prevent the drowning of bees. The experimental colonies were fed with sugar syrup and inverted sugars once every week to the end of the experiment. Sugar syrup was prepared by dissolving 1000 gm of sucrose in 1000 ml of water to obtain 1/1 sugar syrup. Also, 1000 ml of inverted sugar was dissolved in 1000 ml water to give 1/1 inverted sugar solution. Inverted sugars were prepared by OrgaPura Company, Egypt, and produced from sucrose hydrolysis by using the enzyme invertase at 52-58ºC and pH 5.5.

Honey samples were extracted from colonies in the investigated apiaries from different Governorates at the end of February 2021. Bee-processed syrup samples (inverted sugar and sugar solution) were collected from Qualiubia and Arish Governorates. The raw honey samples (without any sugar solution additive) were collected only from Asyut and Arish Governorates. One mixed sample per treatment was harvested and all samples were stored at -20±2ºC until use.
The following parameters were measured in the collected bee honey samples:

Determination of sugar contents

The concentrations of fructose, glucose and sucrose in honey samples were determined by high performance liquid chromatography (HPLC) according to the method of Bogdanov & Baumann (1988).

Pollen analysis

Pollen grains of all tested bee honey samples were investigated according to Louveaux *et al.* (1978). Ten-gram honey was dissolved in 20 ml warm water and then centrifuged at 2500 rpm for 10 min. The supernatant was discarded, and the sediment was rewashed with water and centrifuged again for another 10 min. at the same speed. The entire sediment was put on a slide and spread out over an area of 20 × 20 mm. After drying by slight heating at 40ºC, glycerin gelatin was added, and the sample was examination under the light microscope. Melissopalynology was used as a reference of pollen grain frequencies using the following scale: Pollen grains which constitute >45% is considered very frequent, 16 - 45% frequent, 3 - 15% rare, and <3% sporadic (Maurizio, 1975).

Physicochemical analyses

The Moisture content (%) was determined by digital refractometer, and all measurements were performed at 20ºC (A.O.A.C., 1990). pH was measured by a pH meter (Boeco, Germany). Hydrogen peroxide (H_2O_2) was measured through its reaction with 3.5-dichloro-2-hydroxybenzensulphonic (DHBS) acid and 4-aminophenazone (AAP) to form a choromphore (Aebi, 1984) and electrical conductivity of honey samples was determined by conductivity reading meter for a 20% solution (honey weight in water) at 20ºC.

Biological activities

Biological activities of experimental colonies were determined through areas (square inch) of sealed worker brood, stored bee-processed syrup (inverted sugar and sugar solution) measured at 12 days intervals (Fresnay, 1962; Rashid *et al.,* 2012).

Minimum inhibitory concentration (MIC)

Assay was carried out on daily fresh serial honey dilutions (25, 50, 75 and 100%, v/v), aseptically prepared in nutrient broth. Bee honey bacterial cultures were maintained as stock cultures on slants of nutrient agar, with weekly transfers to new tubes. Stock cultures were used to inoculate nutrient broth cultures which served as “working cultures” in the experiments. Cultures were incubated initially for 24 hours at 37°C and then refrigerated to stop growth. The selected bacteria were chosen based on their frequent occurrence in infections. Bacterial isolates were obtained from the Department of Microbiology, the National Research Centre. The bacterium to be tested was swabbed from a broth culture onto a nutrient agar plate. For each trial 0.05 ml of honey, warmed in a 35-40°C water bath, was pipetted into a well-made in the agar plate. The plate was incubated for 24 hours at 37°C. After incubation the zone of inhibition surrounding each well was measured.

Statistical analysis

Statistical analysis was carried out using SPSS program software version18. Paired T test was used to compare the treatment means for significance at P=0.05.

Results

Results obtained (Table 1) indicated that disaccharides of honey samples produced in response to feeding on inverted sugar and sugar syrup diets in Qualiubia Governorate showed a high concentration of sucrose (7.90 and 6.20

g/100g) and apparent sucrose (9.7 and 8.8 g/100g), respectively, whereas honey samples from bee colonies fed on inverted sugar and colonies without any additive diets recorded the lowest sucrose concentration (2.22 and 2.50 g/100g) in Arish Governorate, respectively. Sucrose concentration in honey samples from bee colonies fed on diets without any sugar syrup from Asyut Governorate was 2.70 g/100g, and the apparent sucrose concentration reached 7.05 g/100g. Apparent sucrose concentration reached the lowest value of 4.12 and 5.32 g/100g in honey samples from colonies received inverted sugar and sugar syrup diets, respectively.

Results obtained also showed (Table 1) the presence of both monosaccharides (fructose and glucose) and disaccharides (sucrose and maltose) in the honey samples. Natural bee honey samples without any additive diet indicated a higher amount of fructose (40 and 39.20 mg/100g) for Asyut and Arish Governorates, respectively. Whereas, bee honey samples from El Qualiubia Governorate extracted from colonies fed with sugar syrup showed the lowest fructose concentration (36.00 mg/100g) followed by bee honey samples fed on inverted sugar (36.70 mg/100g) from Arish Governorate. Furthermore, fructose concentration in the two examined honey samples from colonies fed with sugar syrup in El Qualiubia and Arish Governorates reached 37.30 and 37.80 mg/100g, respectively. On the other hand, glucose concentration was high in honey samples from colonies fed on inverted sugar from Arish (33.50 mg/100g), followed by natural bee honey samples for both Arish (30.80 mg/100g) and Asyut (30.00 mg/100g) Governorates. Bee honey samples of colonies fed with inverted sugar in El Qualiubia governorate contained the lowest concentration (27.00 mg/100g) of glucose. In addition, low glucose concentrations were found in colonies that fed with sugar syrup, 28.3 and 27.70 mg/100g for Arish and El Qualiubia governorates.

Water content of honey collected from colonies fed with sugar syrup at Qualiubia and Arish and with inverted sugar at Arish were the highest and ranged from 23.90 to 24.70%. The lowest water content was observed in honey samples of Qualiubia fed on inverted sugar (18.90%), followed by samples from colonies where no sugar solution was added at Arish and Asyut Governorates (19.00 and 19.30%, respectively). The hydrogen peroxide (H_2O_2) concentration in honey samples varied, with highest value (201.30 and 139.72 mg/kg) in honey samples from colonies not fed on sugar syrup or inverted sugar at the Arish region, respectively. Whereas the lowest concentration was found in honey from colonies fed with sugar syrup (9.12 mg/kg) at Qualiubia Governorate followed by honey samples from colonies where no additives were added at Asyut Governorate (31.50 mg/kg) (Table 1).

The pH of Qualiubia honey collected from colonies fed with inverted sugar was lower than that of Arish Governorate. The highest pH was recorded for honey of colonies fed on sugar syrup at Arish Governorate. The electric conductivity values in collected honeybee samples ranged between 0.31 and 0.50 mS/cm according to the source and region. Honey samples with the highest electrical conductivity came from colonies received sugar syrup and that without any additional diet (0.50 and 0.36 mS/cm) at Arish Governorate. Samples with lower conductivity values were those from colonies fed with inverted sugar (0.31mS/cm) at Qualiubia Governorate as well as samples without any sugar syrup (0.33 mS/cm) at Asyut Governorate. The pollen spectrum (%) in collected bee honey samples from colonies received different types of sugar syrup diets is summarized in Table 2. It was observed that honey from colonies fed on inverted or syrup sugars from Qualiubia and Arish showed lower pollen content. However, honey samples of colonies received sugar syrup or without any additive diets (natural honey) from Arish or Asyut Governorates contained more types of pollen grains. Honey samples from Asyut (natural honey) contained higher frequency of pollen (31.70%) from the Family Umbelliferae, followed by *Citrus spp*. (29.16%) in samples of natural honey from Arish region.

Trifolium alexandrinum and *Medicago* sp. pollen in honey samples from hives fed on natural honey and sugar syrup were 25.60 and 24% from Asyut and Qualiubia governorates, respectively. Pollen grains content in honey samples from colonies fed with inverted sugar were 21 and 20% of *Eucalyptus* spp. and *Casuarina* sp., respectively. Several honey samples for each tested feeding sources that contained rare amounts of pollen grains. The lowest values were for *Trifolium alexandrinum* pollen grains found in honey samples from colonies fed with inverted sugar (1 and 2%) at Arish and Qualiubia Governorates, respectively.

Table 1. Chemical analysis of bee honey samples collected from colonies fed with sugar syrup and converted sugars.

	Parameters evaluated								
	Reducing sugars		Disaccharides			Water content	Hydrogen peroxide		Electric conductivity
Type of diet	Fructose g/100g	Glucose g/100g	Sucrose g/100g	Maltose g/100g	Apparent sucrose g/100g	g/100g	mg/kg	pH	mS/cm
Qualiubia									
Inverted sugar	37.30	27.70	7.90	1.80	9.7	18.90	38.44	3.95	0.31
Sugar syrup	36.00	27.00	6.20	2.60	8.8	24.70	9.12	4.02	0.35
Arish									
Inverted sugar	36.70	33.50	2.22	1.90	4.12	24.10	139.72	4.20	0.34
Sugar syrup	37.80	28.3	2.82	2.50	5.32	23.90	99.78	4.50	0.50
Natural honey	39.20	30.80	2.50	4.35	6.85	19.00	201.30	4.14	0.36
Asyut									
Natural honey	40.00	30.00	2.70	4.35	7.05	19.30	31.50	4.21	0.33

Table 2. Pollen spectrum and frequency (%) in tested bee honey samples from three different governorates in Egypt.

	Governorate					
	Qualiubia		Arish			Asyut
Pollen type	Inverted Sugar (%)	Sugar syrup (%)	Inverted sugar (%)	Sugar syrup (%)	Natural honey (%)	Natural honey (%)
Casuarina sp.	20.0	5.0	10.0	12.0	8.30	-
Eucalyptus spp.	21.0	5.0	5.0	4.0	8.30	-
Fam. Compositae	2.0	2.0	-	4.0	-	17.05
Phoenix dactylifera	3.0	5.0	15.0	18.0	18.30	7.31
Trifolium alexandrinum	2.0	2.0	1.0	12.0	5.83	25.60
Medicago sp.	-	-	3.0	24.0	4.16	13.41
Citrus spp.	-	-	-	4.0	29.16	-
Fam. Chenopodoceae	-	-	5.0	4.0	-	-
Fam. Umbelliferae	-	-	-	4.0	-	31.70
Schinius sp.	-	-	-	4.0	-	-
Salix sp.	-	-	-	0.8	4.16	2.43
Nigella sativa	-	-	-	12.0	4.16	-
Prosopis sp.	-	-	-	-	8.30	2.43
Zea maize	-	-	-	-	-	1.21

Table 3 summarizes the antibacterial activity (inhibition zone) of tested different honeybee samples against *Staphylococcus aureus* and *Pseudomonas aeruginosa,* expressed as minimum inhibitory concentration (MIC). Honey samples of colonies which fed on inverted sugar (Qualiubia Governorate), sugar syrup (Arish Governorate) and natural honey (Asyut Governorate) at concentration of 100% caused 1.5, 2.3 and 3.9 cm antimicrobial activity against *Staphylococcus aureus*, respectively. All concentrations of natural honey samples (without any sugar syrup additive) from Asyut Governorate were more effective and exhibited the highest antimicrobial activity at 100% diet concentration (3.9 cm), 75% (3.5 cm), 50% (2.4 cm) and 25% (2.2 cm) against *Staphylococcus aureus*. All the different tested honey samples from the three regions had no effect against *Pseudomonas aeruginosa,* except honey sample of colonies fed with sugar syrup (Arish Governorate) gave 2.3 cm inhibition at 100% diet concentration.

Impact of feeding honeybee colonies with sugar solution and inverted sugar on the biological activities of tested honeybee colonies were summarized Tables 4 and 5. Sealed worker brood, honey and pollen were determined per inch² at 12 days intervals during the experimental period. Measurements indicated that colonies fed with inverted sugar caused a highly significant increase in sealed brood, honey and pollen at almost all dates of the experiment at Arish and Qualiubia Governorates. At the end of experimental season, the total mean of sealed brood, honey and pollen were significantly increased when colonies were fed on inverted sugars. In contrast, the same tested traits of colonies fed with sugar solution had the lowest values in both above mentioned regions.

Table 3. Antimicrobial activity (inhibition zone) of bee honey samples collected from colonies fed with different sugar diets against *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

	***Staphylococcus aureus* inhibition zone (cm)**				***Pseudomonas aeruginosa* inhibition zone (cm)**			
	Diet concentration							
Type of diet	25%	50%	75%	100%	25%	50%	75%	100%
Qualiubia								
Inverted sugar	-	-	-	1.5	-	-	-	-
Sugar syrup	-	-	-	-	-	-	-	-
Arish								
Inverted sugar	-	-	-	-	-	-	-	-
Sugar syrup	-	-	1.9	2.3	-	-	-	2.3
Natural honey	-	-	-	-	-	-	-	-
Asyut								
Natural honey	2.2	2.4	3.5	3.9	-	-	-	-

- = no inhibition

Table 4. Biological activities of honeybee colonies fed with sugar syrup and inverted sugar diets at Arish and Qualiubia governorates.

Biological activity	Date	Sugar solution	Inverted sugar	*T* value	Significance
Arish Governorate					
Sealed brood area (inch²)	March 22	134.33±17.19	179.33±19.68	15.58*	0.004
	April 3	229.67±6.33	313.67±39.84	2.40	0.140
	April 17	298.00±17.79	515.67±23.68	36.91**	0.001
	April 28	336.00±48.95	583.33±47.68	4.18*	0.053
Honey area (inch²)	March 22	14.33±1.20	37.67±11.89	1.96	0.188
	April 3	37.00±11.93	52.00±14.47	1.17	0.360
	April 17	74.33±8.95	98.33±17.63	2.75	0.110
	April 28	105.00±7.64	206.00±18.45	7.70*	0.016
Pollen area (inch²)	March 22	18.67±3.18	31.00±6.43	3.75	0.064
	April 3	28.67±8.41	73.33±18.52	4.32*	0.050
	April 17	32.67±10.09	51.00±20.03	1.75	0.220
	April 28	25.00±7.09	48.67±11.89	2.78	0.109
Qualiubia Governorate					
Sealed brood area (inch²)	March 22	165.67±9.24	404.33±33	8.79*	0.010
	April 3	215.67±28.67	569.00±62.88	7.44*	0.018
	April 17	191.33±12.88	455.00±20.21	12.02**	0.007
	April 28	172.00±15.62	296.33±17.70	7.17*	0.190
Honey area (inch²)	March 22	126.67±7.05	191.33±17.67	2.65	0.120
	April 3	104.33±5.36	210.33±22.56	4.88*	0.039
	April 17	123.33±4.37	339.33±13.87	12.38**	0.006
	April 28	55.33±3.53	75.33±9.96	1.65	0.230
Pollen area (inch²)	March 22	51.33±3.52	80.67±7.51	4.00	0.060
	April 3	58.00±6.43	100.00±2.31	7.00**	0.020
	April 17	52.33±5.36	93.33±4.41	41.00**	0.001
	April 28	40.00±2.30	59.33±1.76	8.04*	0.015

T value from table = 4.303 (*df* at P=0.05), ** Highly significant, * Significant

Table 5. Biological activity means of honeybee colonies feed with sugar solution and inverted sugar at the end of the experimental season.

Biological activities	Region	Sugar solution	Inverted sugar	*T value*	Significance
Sealed brood area (inch²)	Arish	249.50±25.93	398.00±50.62	4.99*	0.000
	Qualiubia	186.17±9.71	431.17±33.36	8.78**	0.000
Honey area (inch²)	Arish	57.67±11.07	98.50±21.01	3.50*	0.005
	Qualiubia	102.42±8.88	204.08±29.11	4.33*	0.001
Pollen area (inch²)	Arish	26.25±3.61	51.00±7.92	4.75*	0.001
	Qualiubia	50.42±2080	83.33±5.06	9.41**	0.000

T value from table = 3.182 (*df* 3 at P=0.05), ** Highly significant, * Significant

Discussion

In the present study we investigated the effect of bee feeding with two different carbohydrate sources (sugar syrup and inverted sugars) on the sugar contents, pollen analysis, physiochemical analysis and biological activities. Results obtained showed that at the Qualiubia Governorate, a high value of apparent sucrose (9.7 and 8.8mg/100g) in bee colonies fed with inverted sugar and sugar syrup was observed. Natural bee honey samples from colonies not fed on additives had a higher amount of fructose (40 and 39.20 mg/100g) for Asyut and Arish Governorates, respectively, as compared to Arish governorate (36.70 mg/100g). A high content of inverted sugar was detected in honey samples from Arish Governorate (33.50 mg/100g). Variability in glucose, fructose, sucrose and maltose contents in Egyptian honey was reported earlier by several workers (Abd Alla & Abd El-Wahab, 2019; El- Sherbiny *et al.,* 1980; Farag, 2013; Nour, 1998).

Water contents of honey collected from colonies fed with sugar syrup in Qualiubia and Arish Governorate, and inverted sugar at Arish reached the highest value and ranged from 23.90 to 24.70%, which is slightly less than what has been reported by Nour (1988). Crane (1979) reported earlier that bee honey showed a marked variation in water content, depending on the atmospheric humidity both before and after honey harvest. Isengard & Schulthei (2003) observed that to

protect honey from microbiological spoilage, the water content must not exceed 23.0%.

In this study, the pH value was more or less the same in all treatments and ranged from 4.02 to 4.14. More variability in pH values in honey has been reported earlier (Abd Alla & Abd El-wahab, 2019; Crane, 1979; Nour, 1988; Rateb, 2005; Vorwhol *et al.,* 1989; White *et al.,* 1962). The electrical conductivity values in this study ranged between 0.31 and 0.50 mS/cm. Lower values of electric conductivity were found in honey from colonies fed with inverted sugar (0.31 mS/cm) at Qualiubia Governorate as well as samples without any sugar syrup (0.33 mS/cm) at Asyut Governorate.

A wide range with highest value of H_2O_2 (201.30 and 139.72 mg/kg) was observed in natural honey samples and inverted sugar from Arish Governorate, respectively. The lowest value was estimated in honey of colonies fed with sugar syrup (9.12 mg/kg) in Qualiubia Governorate followed by samples, without any additives, from Asyut Governorate (31.50 mg/kg). Several factors may affect the total concentration of H_2O_2 in honey (Farkasovska *et al.,* 2019). Although in diluted honey H_2O_2 is considered a key antibacterial compound, some researches have shown that its level in various honeys does not correlate with antibacterial activity (Bucekova *et al.,* 2018; Farkasovska *et al.,* 2019). Natural honey sample (without any additive sugar syrup) from Asyut Governorate were more effective and exhibited the highest value of antimicrobial activity against *Staphylococcus aureus* for all tested concentrations 100, 75, 50 and 25%. Only honey samples of colonies that fed with inverted sugar (Qualiubia Governorate), sugar syrup (Arish Governorate) at concentration 100% caused antimicrobial activity against *Staphylococcus aureus*. Honey samples from the three regions did not have an inhibitory effect against *Pseudomonas aeruginosa,* except the honey sample from colonies fed with sugar syrup at Arish Governorate. The antibacterial efficacy of natural honey samples against both tested bacteria was significantly higher when compared to that of bee-processed syrups. The action of accumulated H_2O_2 in the synthetic honey-like products is more effective in inhibiting bacterial growth than natural honeys. Moreover, phytochemicals, including polyphenols/flavonoids found in honey, can increase the antibacterial activity of natural honey (Bucekova *et al.,* 2018). Interestingly enough, the H_2O_2 level did not differ between honey samples and bee-processed syrups, suggesting that bees add enzymes during the processing of the syrup as compared to those when processing honey. The difference in the antibacterial activities of bee-processed syrups and natural honey must therefore be derived from other compounds of botanical origin (Bugarova *et al.,* 2021).

Biological Measurements were conducted in colonies fed with sugar syrup and inverted sugar at Arish and Qualiubia Governorates. Results obtained showed that colonies fed with inverted sugar caused a highly significant increase in sealed brood, honey and pollen in both regions. Feeding honeybee colonies with sugar syrup has been shown earlier to increase the total amount of pollen collected (Goodwin, 2015; Goodwin *et al.,* 1991; Goodwin & Houten, 1991). In addition, feeding on sugar syrup enhances pollen collection of colonies, mostly due to changes in the behavior of individual foragers (Free, 1965). Feeding on sugar syrup has a greater effect on the collection of target crop pollen than on pollen from other nearby flowers (Goodwin & Houten, 1991). When nectar is scarce when preparing colonies for winter, beekeepers often feed their bee colonies with supplementary carbohydrates. These include sugar syrup, invert sugar (a mix of glucose and fructose) and high fructose corn syrup (Severson & Erickson, 1984). This additional feeding often protects the bees from malnutrition, which can cause the immune system impairment (Alaux *et al.,* 2010) and increased pesticide susceptibility (Wahl & Ulm, 1983).

الملخص

غنية، أيمن محمد محمد عبد الفتاح، حاتم محمد أحمد محفوظ، طارق عيسى عبد الوهاب وأسماء المتولي عبد الله. 2025. بعض الصفات الفسيوكيميائية والمضادة للميكروبات في عسل النحل المستخرج من خلايا مغذّاة على محلولي السكر والسكر المحوّل. مجلة وقاية النبات العربية، 43(2): 194−201. https://doi.org/10.22268/AJPP-001307

أجريت هذه الدراسة لتقدير تأثير تغذية طوائف نحل العسل بالمحلول السكري والسكر المحوّل في توصيف نوعية حبوب اللقاح والتحليل الكيميائي وكذلك التأثير المضاد للميكروبات في عينات العسل. تمّ جمع عينات من العسل الطبيعي وأخرى من عسل طوائف نحل العسل التي تمّت معاملتها بالمحلول السكري والسكر المحوّل تحت الظروف الحقلية في مناطق مختلفة من مصر. تمّت مقارنة الأنشطة الحيوية في الطوائف المغذّاة بالمحلول السكري والأخرى التي تمّت تغذيتها بالسكر المحوّل. أوضحت نتائج التحليل الكيميائي أن عينات العسل الطبيعي سجلت أعلى تركيز للسكريات الأحادية من سكّري الفركتوز والجلوكوز. ووجد أن سكر السكروز، وهو سكر ثنائي، كان أقلّها تركيزاً في عينات العسل الطبيعي قياساً بتلك العينات من الطوائف التي غذيت على المحلول السكري أو السكر المحوّل. كما أبدت عينات العسل الطبيعي من محافظة أسيوط أعلى تأثير مضاد للبكتيريا *Staphylococcus aureus* مقارنةً بالطوائف التي غذيت على المحلول السكري والسكر المحول. كما أنّ عينات العسل التي تمّ جمعها من طوائف مغذّاة بالسكر المحوّل في محافظة القليوبية وتلك التي تمّت تغذيتها بالمحلول السكري في محافظة العريش بتركيز 100% هي الوحيدة التي أنتجت تأثيراً مضاداً للبكتيريا *Staphylococcus aureus*. في حين أنّ عينات الطوائف التي غذيت بالمحلول السكري بتركيز 100% في محافظة العريش كانت هي الوحيدة التي أظهرت تأثيراً مضاداً للبكتيريا *Pseudomonas aeruginosa*. أوضح التحليل الكيميائي وجود أعلى معدل لفوق أكسيد الهيدروجين في عينات العسل الطبيعي والعسل المأخوذ من طوائف غذيت على محلول السكر المحوّل في محافظة العريش. كما وجد أن أعلى معدل للمحتوى المائي كان في عينات العسل المجموعة من الطوائف المغذاة على المحلول السكري في محافظتي القليوبية والعريش أو التي غذيت على السكر المحول في محافظة العريش. علاوةً على ذلك، أوضحت النتائج

المتحصل عليها أن محتوى حبوب اللقاح ومستوى فوق أكسيد الهيدروجين ونشاط التأثير الميكروبي قد تناقص في عينات العسل التي تمّ جمعها من الطوائف التي غذيت على المحلول السكري والسكر المحوّل في معظم الأماكن. وعلى العكس من ذلك، كانت هناك زيادة في المحتوى المائي لعينات العسل وتحسّن في النشاط الحيوي للطوائف المغذاة على السكر المحول مقارنةً بالمحلول السكري.

كلمات مفتاحية: عسل النحل، السكر المحوّل، المحلول السكري، *Pseudomonas aeruginosa*، *Staphylococcus aureus*.

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References

Abd Alla, A.E. and T.E. Abd El-Wahab. 2019. Antimicrobial Activities of some Egyptian Bee Honeys against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Journal of Plant Protection and Pathology, 10(12):597-603. https://doi.org/10.21608/jppp.2019.78003

Aebi, H. 1984. Catalase *in vitro*. Methods in Enzymology, 105:121-126. https://doi.org/10.1016/S0076-6879(84)05016-3

Alaux, C., J.L. Brunet, C. Dussaubat, F. Mondet, S. Tchamitchan and M. Cousin. 2010. Interactions between Nosema microspores and a neonicotinoid weaken honeybees (*Apis mellifera*). Environmental Microbiology, 12(3):774-82. https://doi.org/10.1111/j.1462-2920.2009.02123.x

A.O.A.C. 1990. Official Methods of Analysis, 15th edition. Association of Official Analytical Chemists, Inc., Arlington, USA. 771 pp.

Bogdanov, S. and E. Baumann. 1988. Determination of honey sugars with HPLC. Mitteilungen aus dem Gebiete. Der Lebensmitteluntersuchung undo Hygiene, 79:198-206.

Bucekova, M., M. Buriova, L. Pekarik, V. Majtan and J. Majtan. 2018. Phytochemicals-mediated production of hydrogen peroxide is crucial for high antibacterial activity of honeydew honey. Scientific Reports, 8:9061. https://doi.org/10.1038/s41598-018-27449-3

Bucekova, M., L. Jardekova, V. Juricova, V. Bugarova, G. Di Marco, A. Gismondi, D. Leonardi, J. Farkasovska, J. Godocikova and M. Laho. 2019. Antibacterial activity of different blossom honeys: New findings. Molecules, 24:1573. https://doi.org/10.3390/molecules24081573

Bugarova, V., J. Godocikova, M. Bucekova, R. Robert Brodschneider and J. Majtan. 2021. Effects of the carbohydrate sources nectar, sucrose and invert sugar on antibacterial activity of honey and bee-processed syrups. Antibiotics, 10:985. https://doi.org/10.3390/antibiotics10080985

Crane, E. 1979. Honey: A comprehensive survey. Heinemann, London. 608 pp.

El-Sherbiny, G.A., S.S. Rizk, F.A. El-Ashwah, and H.A. Heikal. 1980. Chemical composition of citrus honey produced in A.R.E. Agricultural Research Review, 58:289-297.

Farag, R.M.A. 2013. Biochemical properties of some Egyptian honeys in relation to their botanical origin. Ph.D. Thesis, Faculty of Agriculture, Cairo University, Egypt. 179 pp.

Farkasovska, J., V. Bugarova, J. Godocikova, V. Majtan and J. Majtan. 2019. The role of hydrogen peroxide in the antibacterial activity of different floral honeys. European Food Research and Technology, 245:2739-2744. https://doi.org/10.1007/s00217-019-03393-y

Free, J. 1965. The behaviour of honeybee foragers when their colonies are fed sugar syrup. Journal of Apicultural Research, 4:85-88. https://doi.org/10.1080/00218839.1965.11100108

Fresnay, J. 1962. A new instrument for brood measurement in a honey bee colony. American Bee Journal, 111(1):20-21.

Gallai, N., J.M. Salles, J. Settele and B.E. Vaissière. 2009. Economic valuation of the vulnerability of world agriculture confronted with pollinator decline. Ecological Economics, 68(3):810-821. https://doi.org/10.1016/j.ecolecon.2008.06.014

Goodwin, R.M., A. Ten Houten and J.H. Perry. 1991. Effect of variations in sugar presentation to honey bees (*Apis mellifera*) on their collection of kiwifruits (Actinidia deliciosa) pollen. New Zealand Journal of Crop and Horticultural Science, 19:259-262. https://doi.org/10.1080/01140671.1991.10421809

Goodwin, R.M. 2015. Feeding sugar syrup to honey bee colonies to improve pollination: a review. Bee World, 78:56-62. https://doi.org/10.1080/0005772X.1997.11099335

Goodwin, R.M. and A.T. Houten. 1991. Feeding sugar syrup to honey bee (*Apis mellifera*) colonies to increase kiwifruit (*Actinidia deliciosa*) pollen collection: effects of frequency, quantity and time of day. Journal of Apicultural Research, 30:41-49. https://doi.org/10.1080/00218839.1991.11101233

Isengard, H.D. and D. Schulthei. 2003. Water determination in honey Karl Fischer titration an alternative to refractive index measurements. Journal of Food Chemistry, 82:151-154. https://doi.org/10.1016/S0308-8146(02)00543-5

Louveaux J., A. Maurizio and G. Vorwhol. 1978. Methods of melis-sopalynology. Bee World, 59:139-157. https://doi.org/10.1080/0005772X.1978.11097714

Majtan, J. 2014. Honey: An immunomodulator in wound healing. Wound Repair and Regeneration, 22:187-192. https://doi.org/10.1111/wrr.12117

Matoke Holdings Ltd. 2018. Antimicrobial Compositions; Matoke Holdings Ltd., Southmoor, Abingdon, UK.

Maurizio, A. 1975. Microscopy of honey. Pp. 240-257. In: Honey: A Comprehensive Survey. E. Crane (ed.). Heinemann in cooperation with the International Bee Research Association, London.

Morse, R.A. and N.W. Calderone. 2000. The value of honey bees as pollinators of US crops. Bee Culture, 128(3):1-15.

Nour, M.E. 1988. Some factors affecting quality of Egyptian honey. Ph.D. Thesis, Faculty of Agriculture, Cairo University, Egypt. 252 pp.

Nour, M.E. 1998. Physicochemical properties of some Egyptian honeys. Journal of Agricultural Science, 23(4):1749-1756.

Otero, M.C.B. and L. Bernolo. 2020. Honey as Functional Food and Prospects in Natural Honey Production. Pp. 197-210. In: Functional Foods and Nutraceuticals: Bioactive Components, Formulations and Innovations, C. Egbuna and G. Dable Tupas (eds.). Springer International Publishing, Cham, Switzerland. https://doi.org/10.1007/978-3-030-42319-3_11

Potts, S.G., J.C. Biesmeijer, C. Kremen, P. Neumann, O. Schweiger and W.E. Kunin. 2010. Global pollinator declines: trends, impacts and drivers. Trends in Ecology and Evolution, 25(6):345-353. https://doi.org/10.1016/j.tree.2010.01.007

Rashid, M., E.S. Wagchoure, A.U. Mohsin, S. Raja and G. Sarwar. 2012 Control of ectoparasitic mites in honeybee (*Apis mellifera* L.) colonies by using thymol and oxalic acid. Pakistan Journal of Zoology, 44(4):985-989.

Rateb, S.H. 2005. Studies on pollen spectrum, chemical and physical characters of some types of honeys. Ph.D. Thesis, Faculty of Agriculture, Assiut University, Egypt. 335 pp.

Severson, D. and E. Erickson. 1984. Honey bee (Hymenoptera: Apidae) colony performance in relation to supplemental carbohydrates. Journal of Economic Entomology, 77(6):1473-1478. https://doi.org/10.1093/jee/77.6.1473

Southwick, E.E. and L. Southwick. 1992. Estimating the economic value of honey bees (Hymenoptera: Apidae) as agricultural pollinators in the United States. Journal of Economic Entomology; 85(3):621-633. https://doi.org/10.1093/jee/85.3.621

Vorwhol, G., M.S. Salem and M.E. Nour. 1989. Chemical and Physical properties of Egyptian honeys. Pp. 240-244. In: Proceedings of the 4th International Conference on Apiculture in Tropical Climates. 6-10 November 1988, Cairo, Egypt.

Wahl, O. and K. Ulm. 1983. Influence of pollen feeding and physiological condition on pesticide sensitivity of the honey bee *Apis mellifera carnica*. Oecologia, 59(1):106-128. https://doi.org/10.1007/BF00388082

Wheeler, M.M. and G.E. Robinson. 2014. Diet-dependent gene expression in honey bees: honey vs. sucrose or high fructose corn syrup. Scientific Reports, 4:5276. https://doi.org/10.1038/srep05726

White, J.W., M.L. Riethof, M.H. Sobers and I. Kushnir. 1962. Composition of American honeys. United States Department of Agriculture Technical Bulletin No. 1261. 124 pp. https://doi.org/10.22004/ag.econ.170887

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