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Studying the inhibitory effect of local honey, black seed oil against some bacterial isolates with a comparisons

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ABSTRACT

The current research aimed to investigate the antibacterial activity of two types of honey alone and in combination with black seed oil and compare their effects. The first type of honey, the mountain type, was obtained from some beehive of Sulaymaniyah city, while the second type (Lowlander) was obtained from beehive in Balad city of Salah Al-Deen.

The bacterial species used were *Staphylococcus aureus, Escherichia coli, Salmonella typhi and Proteus mirabilis* categorized into clinical and environmental groups. Antibacterial activity of the honeys was assayed using the Disc diffusion method. Different concentrations were conducted ranged from 20-100%. Results had revealed that the environmental bacterial isolates were affected mostly toward both honey types. However, the mountainous honey showed inhibitory effect against the Gram negative isolates. Mixture of honey in general with black seed oil had significantly differed and at some points decreased the effect while the black seed alone showed effect toward the environmental isolates at the absolute concentration.

INTRODUCTION

Honey is a sweet natural product produced from the nectar and exudation of plants or excretions of plant sucking insects on the living parts of plants, which honey bees (*Apis mellifera*) collect , transform by combining with specific substances of their own , deposit , dehydrate , store and leave in the honey comb to ripen and mature (Buba *et al*., 2013). The natural honey has been reported to contain about 200 agent , which consist of not only highly concentrated solution of sugars, but also the complex mixture of other saccharides , amino acids , peptides , enzymes , proteins , organic acids, polyphenols , carotenoids substances, vitamins and minerals with antioxidant , bacteriostatic, anti-inflammatory and antimicrobial properties , It is also included in the medical treatment of wounds and sunburn healing effects (Hao Wang ,2011; Alvarez-Suarez *et al*., 2014). Honey is well known for its antibacterial activity, which was first reported in 1892 (as cited by Dustmann in 1919). Since ancient times, honey has been used for treatment and prevention secondary infections of wounds (Paulus *et al*., 2012). A study published by (Kumar, 2015) has linked the nutritious and healing substances of the honey to the plants visited by bees , because it is the raw materials of honey . *Nigella sativa* is known by other names , and its names varying among places (Sufya et al., 2014). *Nigella sativa* oil contains 100 healing components working together in a synergetic manner. That means they all complement each other in the process of aiding our body

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ability to take care of himself which demonstrate the fact that natural products may play a future role by replacing or substituting antibiotics that face a great threat of overall resistance (Ismaeil, 2011). This study aimed to experience the inhibitory effects of honey against a group of clinical bacterial species and with the same species for a second group isolated from environmental sources using two methods of treatment , the first by using *Nigella sativa* oil only and the second by using mixture of honey and *Nigella sativa* oil with dilution rate 1: 1 (v/v).

MATERIALS AND METHODS

Bacterial isolates

Eight bacterial samples were used in this work. Isolates included four species collected from local clinical and environmental sources, the clinical specimens were gathered from different infections of unrelated patients while the environmental bacteria were taken from ground water and contaminated wings of flies. All isolates were purified and identified to the species level following standard growth, biochemical and staining characteristics presented by (Singh, 2009).

Honey types

Honey samples used for this study were purchased from two natural origin ; The first type of honey, the mountainous type, was obtained from some beehive of Sulaymaniyah city , while the second type (Lowlander) was obtained from beehive in Balad city of Salah Al-Deen. Samples were kept in a dark room at 23±25C° until used (Pattamayutanon *et al.*, 2017). Vigorous mixing with vortex was applied to obtain the final mixture of all concentrations used in the study.

Nigella sativa oil (Black seed oil)

Black seed oil was purchased from the local markets of Tikrit city , (manufactured by Hemani Co., Pakistan) , product weight 1.01 FL OZ (30 ML) .The dosage of *N. sativa* and honey with dilution rate 1:1(v/v). Knowing that black seed oil was diluted with hexane solvent.

Inhibitory effect test

The Kirby-Bauer agar diffusion method was used to show the antibacterial effect according to the concentrations (20, 40 , 60, 80,100) % (v/v) of each type of honey as well as of black seed oil and mixture of the honey with oil. Each petri dish had four well with the control in the center. Wells were aseptically cut using a sterile cork borer of about 6 mm diameter allowing at least 30 mm between adjacent wells and between peripheral wells and the edge of the dish (Adam, 2013). Fixed volumes (Aliquots of 200 µl) were poured into the wells made. Activity was assessed by measuring the diameter of the inhibition zone around each well after 24 hours of incubation at 37 ºC using Mueller–Hinton Agar and nutrient agar respectively. (Abd Jalil *et al.*, 2017; Carpes *et al*., 2007).

Statistical analysis

The statistical measurement was based on analysis of variance (ANOVA). Duncan multiple range test was used to evaluate the significant differences between means (comparison of means) at the level of significant $p<0.05$, using the software Mini-Tab version 17 (2017) in order to investigate whether there was a significant difference among the various experiments (Al-rawi, 2000).

RESULTS AND DISCUSSION

This paper discusses the antibacterial effect of two types only of honey bee, black-seed oil and the mixture of honey with oil concerning with clinical and environmental bacterial isolates. The inhibitory effect of honey on bacterial growth was evident at concentrations of (20, 40, 60, 80,100) percentage v/v. with repeating three times to have a definite score.

The results showed clear inhibition for both types of honey against all isolates, particularly at honey concentration of 100% followed by 80 and 60% with equal mean of Con. However the lowlander source of honey scored the highest mean (34.7 and 26.0) versus (21.0 and 28.5) for the mountainous type, illustrated in table (1).

This antibacterial behavior of honey is attributed to its peroxide-related and non-peroxide-related activity and presence of different bioactive compound, as declared by (Alvarez-Suarez *et al.* , 2014 ; Farouk *et al*., 2017) The composition of honey may vary from type to type and depends primarily on its floral source ; seasonal and environmental factors can also influence its constituents with its biological effects. the antioxidant potential of honey is strongly correlated not only with the concentration of total phenolics present, but also with the color, with dark colored honeys being reported to have higher total phenolic contents and, consequently, higher antioxidant capacities

Table (1) The inhibitory effect of two types of honey against clinical and environmental bacterial isolates

Isolates source	Honey type	Bacterial types	Inhibitory concentrations diameter in (mm)					Mean of Honey Type	Mean of Isolates Source	
			20%	**40%**	**60%**	**80%**	**100%**			
Clinical	Mountainous	*Staph. aureus*	R	18	13	20	25	15.2 F	21.0 C	23.5 B
		Salmonella typhi	10	12	28	20	30	20.0 E		
		Proteus mirabilis	R	25	28	37	35	25.0 CD		
		Escherichia. coli	18	20	26	24	30	23.6 DE		
	Mean of con. In Mountainous		7.0 D	18.8 c	23.8 b	25.3 b	30.0 a			
	Lowlander	*Staph. aureus*	35	45	44	50	55	45.8 A	26.0 B	
		Salmonella typhi	R	R	10	22	20	10.4 G		
		Proteus mirabilis	12	20	27	25	30	22.8 DE		
		Escherichia. coli	20	26	25	27	31	25.8 CD		
	Mean of con. In Lowlander		16.8 C	22.8 b	26.5 b	31.0 a	34.0 a			
Environmental	Mountainous	*Staph. aureus*	R	23	30	41	50	28.8 C	28.5 B	31.6 A
		Salmonella typhi	R	R	33	36	45	22.8 DE		
		Proteus mirabilis	R	23	34	32	44	26.6 CD		
		Escherichia. Coli	25	35	35	40	43	35.6 B		
	Mean of con. In Mountainous		6.3 D	20.3 c	33.0 b	37.3 b	45.5 a			
	Lowlander	*Staph. aureus*	35	45	55	50	60		34.7 A	
		Salmonella typhi	R	23	25	44	40			
		Proteus mirabilis	R	25	30	30	45			
		Escherichia. Coli	36	35	40	35	40			
	Mean of con. In Lowlander		17.8 D	32.2 c	37.5 b	39.8 b	46.3 a			
	General Means of Conc.		12.0 D	23.5 c	30.2 b	33.4 b	39.0 A			

*** Variable letters horizontally& Vertically mean that there are significant differences between the probability level of P≤0.05**

Variation of honey composition is related to interactions with plants and other organisms, anthropogenic environmental changes, Land-use change, agricultural intensification and urbanization often destroy and fragment the natural habitats that many pollinators rely on for food and nesting resources (Augul, 2016) and this could attribute directly to the significant differences of inhibitory effect. Among the clinical isolates, lowlander type greatly affected *S. aureus* agreed with (Farouk *et al*.,2017) who claimed the antimicrobial peptides (AMP) is active on Gram positive bacteria.

It is worth to mention that our data showed that means of each bacterial species varied according to types of honey this results is in accordance with the study of (Almasaudi *et al.,* 2017) who published that different types of honey possess different efficacies and mechanisms against the same type of bacteria.

Among the substances produced by bees, apitoxin is one of the most important. Glands located in the abdomen of these insects synthesize this complex chemical. Apitoxin consists of 88% water ; the remaining 12% contains components such as hyaluronidase, phospholipase A2, histamine, melittin, and some other peptides like apamin, secapin , among others. Phospholipase A2 displays antibacterial and anticoagulant actions and plays an active role in the generation of chemical mediators , cell proliferation , muscle contraction, and anti-inflammatory processes **(**Leandro *et al*., 2015).

The seeds of *Nigella Sativa* Linn. Which is commonly and cosmetically known as black seed (traditionally named blessing seed). It is regarded as one of the greatest forms of healing medicine available and included in the medicine of the Prophet Mohammed (Aljawezjjah, 2001). In this study black seed oil was conducted to reveal its effect alone and mixed with honey.

Results illustrated in table (2) indicated that inhibitory effect of mixture honey with black seed oil was at the absolute con. 100% and the rest serial dilution with equal volume rate of both 1:1 scoring an "A" value of means and again the environment bacterial isoltes had have higher mean than the clinical ones.

In view of using black seed oil with honey (mixture), Bakathir and Abbas, (2011) proposed that its antimicrobial action could be attributed to the active ingredients especially thymoquinon and melanin.

Table (2) The inhibitory effect of the mixture of honey with black seed oil against clinical and environmental bacterial isolates

	Isolates source	Concentration used (**inhibition diameter/m**)					Mean of Bacteria	Mean of mixture
		20%	40%	60%	80%	100%		
Staph. aureus	Clinical	R	15	R	18	25	11.6 B	
Salmonella typhi	Clinical	R	R	20	18	23	12.2 B	
Proteus mirabilis	Clinical	R	R	16	22	20	11.6 B	
Escherichia coli	Clinical	15	20	25	20	40	24.0 A	
Mean of Conc. in Clinical		3.8 d	8.8 c	15.3 b	19.5 b	27.0 a		14.9 B
Staph. aureus	Environmental	22	23	25	22	30	24.4 A	
Salmonella typhi	Environmental	R	18	28	26	46	23.6 A	
Proteus mirabilis	Environmental	R	8	25	16	32	16.2 B	
Escherichia coli	Environmental	21	25	30	40	45	32.2A	
Mean of Conc. in Environmental		0.8 d	18.5 c	27.0 b	26.0 b	38.3 a		24.1 A
General Mean of Conc.		7.3 d	13.7 c	21.2 B	22.8 b	32.7 a		

***Dilution rate 1:1 ** type of honey: lowlander**

*** Variable letters horizontally& vertically mean that there are significant differences between the probability level of P≤ 0.05**

The results also showed that *E. coli* isolates were more sensitive than the rest of the species when treated with 100% honey mixture and black seed oil. The sensitivity of the other species was close to each other. The sensitivity of environmental isolates differed among the other three species and *mirabilis Proteus* Was the least sensitive to the combination of honey and black seed oil , which may indicate the possession of resistance to antibiotics and ultimately resistance to the effectiveness of microbial antibody to blend honey and black seed oil , Which confirms that the environmental isolates are more sensitive than clinical , These results have converged with the results of (Hao Wang, 2011) which showed that the phenolic compounds of honey have been known to pose significantly antioxidant activity, including iron-binding and free radical scavenging activity, Manuka honey has been widely used in wound treatment and the antioxidant activity of manuka honey is important in that.

Studies that confirm the results of present study a study of (Paulus and Sebastian , 2012) showed that the antibacterial activity is a major obstacle for clinical applicability. The high sugar concentration, hydrogen peroxide, and the low pH are well-known antibacterial factors in honey and more recently, methylglyoxal and the antimicrobial peptide bee defensin-1 were identified as important antibacterial compounds in honey, The antibacterial activity of honey is highly complex due to the involvement of multiple compounds and due to the large variation in the concentrations of these compounds among honeys.

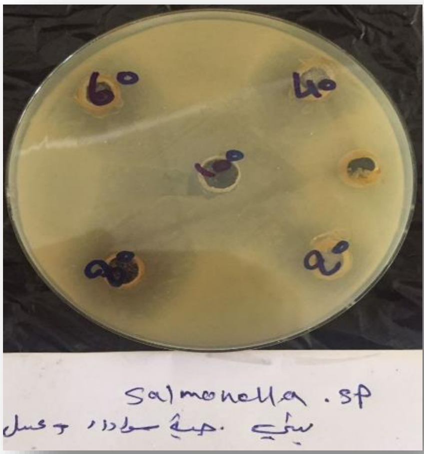

Figure (1) Inhibitory effect of mixture 1:1 black seed oil with honey against *Salmonella typhi* at absolute concentration.

Results of table (3) indicated that black seed oil had potentially inhibited bacterial growth, although mean of each parameter decreased comparing with the effect of honey-black seed oil mixture. Bearing in mind that all the environmental isolates had generally been affected greater than the clinical one with a mean of 14.95 and 10.95 respectively. Moreover among all bacterial isolated *E. coli* was the most one being affected.

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Table (3) The inhibition effect of black seed oil against clinical and environmental bacterial isolates

	Isolates source	Concentration used (inhibition diameter/m) 20%	40%	60%	80%	100%	Mean of Bacteria	Mean of Oil
Staph. aureus	Clinical	R	20	13	15	R	9.6 CD	10.95 B
Salmonella typhi		R	R	11	10	R	4.2 E	
Proteus mirabilis		R	R	R	R	15	3.0 E	
Escherichia. coli		R	22	35	38	40	27.0 B	
Mean of Conc. In Clinicall isolates		0.0 b	10.5 a	14.8 A	15.8 a	13.8 a		
Staph. aureus	Environmental	R	R	18	20	R	7.6 CD	14.95 A
Salmonella typhi		R	16	R	15	20	10.2 C	
Proteus mirabilis		R	R	11	19	R	6.0 DE	
Escherichia. coli		25	35	36	40	44	36.0 A	
Mean of Conc. In Environmental isolates		6.3 c	12.8 b	16.3 B	23.5 a	16.0 b		
General Mean of Conc.		3.2 d	11.7 c	15.6 B	19.7 a	14.9 b		

Variable letters horizontally& Vertically mean that there are significant differences between the probability level of $P \leq 0.05$

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دراسة التأثير التثبيطي لعسل النحل المحلي وزيت الحبة السوداء على نمو بعض الأنواع البكتيرية الخمجية ومقارنتها

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المستخلص

يهدف البحث الحالي إلى دراسة الفعالية الضد بكتيرية لنوعين من العسل ، الجبلي والسهلي ، تارة العسل بمفرده وتارة اخرى بالاقتران مع زيت الحبة السوداء ومقارنة تأثيرهما . وكان أول نوع من العسل هو الجبلي بينما كان الثاني هو السهلي. كانت البكتيريا المستخدمة في هذه الدراسة على اربعة أنواع : المكورات العنقودية الذهبية *Staphylococcus aureus*، الإشريكية القولونية *Escherichia coli*، السالمونيلا التيفية *Salmonella typhi* و بكتيريا المتقلبات الرائعة *Proteus mirabilis* والتي تم تصنيف كل واحده منها إلى مجموعتين وفقا للمصدر الذي جمعت العينات منه إلى سريرية وبيئية . تم اختبار الفعالية الضد بكتيرية للعسل باستخدام طريقة انتشار قرص التثبيط . تراوحت التراكيز المستخدمة بين 20- 100٪ . خضعت جميع النتائج للتحليل الإحصائي باستخدام اختبار ANOVA. وقد كشفت النتائج أن العزلات البكتيرية البيئية تأثرت في الغالب بكلا نوعي العسل . وكان للنوع السهلي الحصة الأكبر في التأثير الضد بكتيري ، ومع ذلك أظهر العسل الجبلي تأثيرا فعال ضد العزلات السلبية لصبغة گرام أكثر منه ضد المكورات العنقودية الذهبية . اختلف تأثير زيج العسل بشكل عام مع زيت الحبة السوداء اختلافاً معنوياً ، وفي بعض الأحيان قلل من تأثيره بينما أظهر زيت الحبة السوداء تأثير اعلى العزلات البيئية مجملا علما ان التركيز المطلق كان له النصيب الاكبر من التثبيط .

الكلمات المفتاحية: التأثير التثبيطي، العسل، النحل المحلي، حبة السوداء، المكورات العنقودية الذهبية، السالمونيلا التيفية، بكتيريا المتقلبات الرائعة، الإشريكية القولونية.