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BIOACTIVITY OF CERTAIN SAUDI ARABIA HONEY AGAINST *Bacillus subtilis* AND *Escherichia coli*

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ABSTRACT: *Seven Saudi Arabia honey types (Sider, Siefy el-taef, Talh, sider el-taef, Somrh el-taef, Besbas-riadh and Blackcumin) were collected from different locations at Saudi Arabia Kingdom and tested against two species of bacteria; Bacillus subtilis and Escherichia coli using undiluted honey as an indicator of unique factors. The diameter of the inhibition zones was used as a criterion of antibacterial spectra (total inhibition zones, mm). The obtained results indicated that the two species of bacteria were significantly affected by the tested honey. The Blackcumin was the highest effective against B.subtilis followed closely by Siefy el-taef, while the lowest effect was recorded by sider el-taef honey. The Talh honey recorded the highest effect against E.coli, while, Sider, Siefy el-taef and Besbas-riadh honey gave the lowest effect. Also B.subtilis was more sensitive to inhibition by Blackcumin honey than E. coli. Siefy el-taef honey induced the lowest effect against both species of bacteria.*

Key words: *Saudi Arabia honey, antibacterial activity, Bacillus subtilis , Escherichia coli*

INTRODUCTION

The antibacterial action of honey was reported for the first time in 1892 (Van ketel, 1892). Honey antibacterial action has two types. The first type is heat- and light- sensitivity, based on hydrogen peroxide produced by honey glucose oxidase in diluted or eaten honey (White *et al.*, 1963). The second type is insensitivity to heat and light which remain intact after storage for longer periods of time (Roth *et al.*, 1986). It called unique factors based on honey component (Lavie, 1968). Many causes were contributed to the unique factor in honey. Sugars: the main honey substances, with its osmotic effect could cause an antibacterial action (Molan, 1992). However, different antimicrobial effects were found at concentrations with no osmotic sugars active. It has been claimed that antimicrobial activity could be referred to lysozyme, a well known antibacterial agent (Mohrig and Messner 1968). In contrast, other studies reported that no lysozyme activity was found (Bogdanov, 1984). The antibacterial flavonoid pinocembrin is present in

honey, but its concentration and involvement to honey's non-peroxide antibacterial activity is small (Bogdanov, 1989). In New Zealand honeys, especially Manuka and viper's bugloss, a number of aromatic acids with antibacterial actions have been isolated (Molan, 1992). The elevated honey osmomolarity besides the low honey PH was responsible for the antibacterial activity (Yatsunami and Echigo, 1984). On the other hand, volatile substances with antibacterial activity have been isolated but their quantitative contribution to the antibacterial action of honey was not examined (Toth *et al.*, 1987). Other workers found non-peroxide activity of honey, extractable by organic solvents, but were not able to identify the chemical nature of the substances (Radwan *et al.*, 1984). The antibacterial unique factors of honey have been widely reviewed by (Molan, 1992).

This study was carried out to determine bioactivity of certain types of Saudi Arabia honey collected from different locations in the kingdom against two species of bacteria; *Bacillus subtilis* and *Escherichia coli*.

MATERIALS AND METHODS

Samples of seven Saudi Arabia honey types (Sider, Siefy el-taef, Talh , Sider el-taef, Somrh el-taef , Besbas-riadh and Blakeumin) were collected from different locations in the Kingdom and tested against two species of bacteria; *Bacillus subtilis* and *Escherichia coli* . The selected bacteria were obtained from Rice Research and Training centre at Sakha, Egypt. The bioactivity of the seven honey against the selected bacteria was carried out using the technique described by Perez *et al.* (1990). Double strength nutrient agar medium (standard nutrient agar) were cooled to 45C° and mixed with bacteria under full sterile conditions until it gives wide good growth ,then pour to sterile Petri dish and cool to 4C° / 24 hour. The tubes were autoclaved for 15 minutes at 15 pounds pressure. Wells were punched in the set agar with an agar punch in regular grid pattern in the middle of the dish under sterile conditions. After incubation for 48 hours, digital calipers were used to measure the inhibition zone by taking the square of the diameter of inhibition area. Each sample as well as the control (only sterile water) was replicated three times.

The obtained data were statistically analyzed using Statistics program (version 9.0) in one–way ANOVA and the means of inhibition zone were compared at 5% level.

RESULTS AND DISCUSSION

The results in Table (1) indicated that the tested honey types cleared significant effects against the two species of bacteria. The Blackcumin honey induced the highest inhibition value (3.97 mm) on *Bacillus subtilis*, while the lowest value (1.93 mm) was recorded by Sider el-taef honey. The rest types of honey were arranged descending according to inhibition value as follows: Siefy el-taef (3.6 mm), Somrh el-taef (2.93 mm), Sider (2.3 mm),

Talh (2.16 mm) and Besbas-riadh (2.1mm). Regarding *Esherichia coli*, the Talh honey was the most effective as the inhibition value was 3.1 mm, followed by Sider, Siefy el-taef and Besbas-riadh with inhibition value of 2.6 mm for each. Meanswhile, the honey of Sider el-taef, Somrh el-taef and Blakeumin induced the lowest effect against *E. coli* with inhibition value of 2.3 mm for each.

Table (1): Bioactivity of seven Saudi Arabia honey types on *Esherichia coli* and *Bacillus subtilis*

Honey type	Mean of inhibition zone (mm)	
	E. coli	*Bacillus subtilis*
Sider (*Zizyphus vulgaris*)	2.60 ab	2.30 d
Siefy el-taef	2.60 ab	3.60 b
Talh (*Acacia ehrenbergiana*)	3.10 a	2.17 de
Sider el-taef (*Zizyphus vulgaris*)	2.30 b	1.93 f
Somrh el-taef (*Acacia tortilis*)	2.30 b	2.93 c
Besbas-riadh(*Anisosciadium lanatum*)	2.60 ab	2.10 def
Blackcumin*(Nigella sativa*)	2.30 b	3.97 a

For each column, means followed by the same letter did not differed significantly at 5% level

Also, it was apparent that, *B. subtilis* was more sensitive to inhibition by Blackcumin honey than *E. coli* as the inhibition value was 3.97 and 2.3 mm , respectively. Meanswhile, *B. subtilis* and *E. coli* were less sensitive to the Sider el-taef honey with inhibition value of 1.93 and 2.3 mm, respectively. Also, the two species of bacteria did not mostly appear obvious differences in sensitivity to inhibition by the rest types of honey.

However, antibacterial activity in undiluted honey was attributed to many factors. Molan (1992) reported that sugars (main honey substances) with its osmotic effect cause an antibacterial action of honey. Yatsunami and Echigo (1984) also, mentioned that the elevated honey osmomolarity besides the low honey PH was responsible for the antibacterial activity. Also, antibacterial activity of honey could be explained by the enzyme glucose oxidase (Weston, 2000).

Finally, it could be concluded that Blackcumin honey was the most effective against *B. subtilis* followed closely by Siefy el taef honey, while the lowest effect was recorded by Sider el-taef honey and the two types of honey gave a moderate effect against *E. coli*.

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النشاط الحيوي لبعض أنواع العسل السعودية ضد بكتريا باسيلس سبتليس ، إشيريشيا كولاي

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الملخص العربي

تم جمع سبعة عينات من العسل (السدر، صيفي الطائف، الطلح، سدر الطائف، السمرة، البسباس، حبة البركة) من مناطق مختلفة داخل المملكة العربية السعودية لاختبار نشاطها المثبط لنوعين من البكتريا هما ***Bacillus subtilis, Escherichia col*** وتم استخدام العسل دون تخفيفه بالماء كمؤشر لوجود العوامل الخاصة. تم قياس مساحة مناطق التثبيط لمزارع البكتريا لكل نوع من الأعسال المستخدمة .

وقد أوضحت النتائج وجود تأثير معنوي للأعسال المختبرة على نوعي البكتريا – وكان عسل حبة البركة أكثر فعاليه ضد بكتريا الباسيلس ويليه مباشره عسل صيفي الطائف بينما أقل تأثير كان لعسل سدر الطائف. وقد سجل عسل الطلح أعلى تأثير ضد بكتريا إيشريشيا كولاي بينما أعطى عسل السدر وصيفي الطائف والبسباس أقل تأثير. أيضا كانت بكتريا الباسيلس أكثر حساسية للتثبيط بعسل حبة البركة مقارنة بالأشيريشيا كولاي , كما أعطى عسل صيفي الطائف أقل تأثير ضد كلا النوعين من البكتريا.