Egypt. Acad. J. biolog. Sci., 4 (1): 13-19 (2011) **A. Entomology**
Email: egyptianacademic@yahoo.com **ISSN: 1687–8809**
Received: 3/11/2010 **www.eajbs.eg.net**

Does the HMF value affect the Antibacterial activity of the Bee Honey?

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ABSTRACT

This study evaluated the antimicrobial activity of different levels of HMF value of honey at different concentration (15,20and 25%honey). Honey and HMF concentrations of 15 %, 20 % and 25 % honey in distilled water were prepared. These preparations were tested for antimicrobial activity against four different types of pathogenic bacteria; *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis* . Antimicrobial activity was determined as an equivalent of the inhibition zones diameters using filter paper discs (in millimeters) after incubation of the cultures at 30°C for 24 hours for bacterial species. The results showed a bactericidal activity of HMF value of honey against a broad spectrum of bacteria with variable degrees. By use of least significant differences test for multiple comparisons, it is apparent that the honey of HMF value (90.24 mg*l*kg) showed the highest inhibitory effect on bacterial growth compared to other honey treatments and HMF concentrations. This value (90.24 mg*l*kg) had the highest bactericidal activity against *Escherichia coli* at concentrations of 15and 20% honey. Similarly, the HMF (90.24 mg*l*kg) value had significantly higher inhibitory effects compared to other treatments on *Bacillus subtilis* growth at concentration 15, 20 and 25% honey and on *Staphylococcus aureus* growth at concentration 15and 25% honey. However, there was no inhibitory effect of HMF value of honey (203.0 mg*l*kg) against all bacterial strains tested at concentration 15% honey. Considerably, no inhibitory effect of all HMF values of honey treatments was noted on *Pseudomonas aeruginosa*. Conclusion, the different levels of HMF value of honey at different concentration (15,20and 25%) showed a bactericidal activity against a broad spectrum of bacteria with variable degrees

Keywords: Antibacterial, Bee Honey, HMF value

INTRODUCTION

Honey, as it is found in the hive, is a truly remarkable material elaborated by bees with floral nectar . Nectar is a thin, easily spoiled sweet liquid that is changed (“ripened”) by the honeybee to a stable, high density and high energy food .The earlier US Food and Drug Act defined honey as “ the nectar and saccharine exudation of plants, gathered, modified and stored in the comb by honey bees (*Apis mellifera* and *A. dorsats*). The limits established in this definition were largely based on a survey published in 1908. Today, this definition has an advisory status only, but is not totally correct, as it allows too high a content of water and sucrose and too low in ash (Molan, 1992).

Honey has been used as a medicine since ancient times in many cultures. Actually honeys vary according to their plant origin and the conditions of their production (Bogdanov,1997) .The use of honey as therapeutic substance has been

rediscovered by the medical profession in more recent times and its gaining acceptance as an antibacterial agent for the treatment of some diseases (Efen,1988)

Honey has antimicrobial properties that discourage the growth or persistence of many microorganisms. The microbes that may be found in honey are primarily yeasts and spore-forming bacteria. The medicinal properties of honey has been reported and documented by beekeepers and medical practitioners alike (Molan, 1992 and Bankova *et al*., 2000). Several studies have been conducted to authenticate this 'forklore' on medicinal properties of honey and there has been a renaissance in the use of honey as medicine in more recent times (Molan, 1992 Bogdanov, 1997, Zumla and Lulat 1989 and Fearnley, 2001). The use of alternative therapies is mostly due to development of antibiotic resistance in bacteria and/or increasing awareness on the adverse side effects of many pharmaceuticals (Fearnley, 2001).

Hydroxymethylfurfural, also called HMF, is a compound that results from the breakdown of simple sugars (such as fructose) at pH 5 or lower. HMF occurs naturally in honey, especially in warm climates.

HMF (5-hydroxymethylfurfuraldehyde) measurement is used to evaluate the quality of honey; generally not present in fresh honey, its content increases during conditioning and storage. Honey processing, requires heating both to reduce viscosity, and to prevent crystallization or fermentation (Singh, Singh, Bawa, & Sekhon, 1988) in air ventilated chambers, at 45–50°C for 4/7 days or by immersion of honey drums in hot water. Heating of unifloral honey leads to different HMF levels in honey (Fallico, Zappal_a, Arena, & Verzera, 2004). HMF is formed during acid-catalysed dehydration of hexoses and, it is connected to the chemical properties of honey, like pH, total acidity and mineral content (Anam & Dart, 1995; Bath & Singh, 1999; Hase, Suzuki, Odate, & Suzuki, 1973; Singh & Bath, 1997, 1998).

Codex Alimentarius (Alinorm 01/25 2000) established that the HMF content of honey after processing and/or blending must not be higher than 80 mg/kg. The European Union (EU Directive 110/2001) fixed a HMF limit in honey of 40 mg/kg with the following exceptions: 80 mg/kg for honey coming from Countries or Regions with tropical temperatures.

However, it is obvious that being able to enter other reactions, this highly reactive compound can make the nutritional value of honey lower by the degradation of its unstable components, or possibly affect its colour if present in higher concentrations (Surh *et al.*, 1994; Kubiš and Ingr, 1998; Gangolli, 1999; Janzowski *et al*., 2000).

Considering all the facts mentioned above, the content of HMF is an important parameter in the evaluation of honey quality. It also enables to detect the damage of honey or its adulteration by invert sugar or starch syrup (Kubiš and Ingr, 1998; Wunderlin *et al*., 1998; Nozal *et al.*, 2000).

This work was conducted in the year (2010), at Beekeeping Research Department , Plant Protection Research Institute, ARC, Giza , Egypt and Faculty of Science, Al-Azhar University.

The present work had an aim; it was to detect which level of HMF value of honey had a potential effect on bacteria.

MATERIALS AND METHODS

Honey preparation

The honey solution was prepared by mixing honey in pure form to sterilized distilled water at various concentrations of 15, 20 and 25%.

The Hydroxymethylfurfural (5-hydroxymethyl-2 furalde-hyde) (HMF) of old honey were analyzed at the Chemical Analysis Laboratory of Honeybee Products, at Beekeeping Research Department, Plant Protection Research Institute, ARC, Giza, Egypt.

Determination of Hydroxymethylfurfural (HMF)

It was determined according to Winkler (1955) as following, the reagents:

Barbituric acid solution: 500 mg barbituric acid was transferred to 100 ml graduated flask using 70 ml water. Then the flask placed in a hot water-bath until all amount of barbituric acid was dissolved, cool and make up to volume.

P-toluidin solution: Weight out 10 g P-toluidin, analytical grade, and dissolved in about 50 ml isopropanol by gentle warming in a water-bath then, transfer to a 100 ml graduated flask with isopropanol and add 10 ml glacial acetic acid. Cool and make up to volume with isopropanol. Keep solution in the dark. Don't use for at least 24 hours.

Distilled water (oxygen free): Nitrogen gas was passed through boiling distilled water. Then water is cooled.

Procedure.

1. Preparation of test sample.

5 g of honey sample is weighted and dissolved without heating with oxygen free distilled water and transferred to a 25 ml graduated flask and made up to volume (honey solution). The sample should be tested after preparation without delay.

2. Photometric determination.

2.0 ml of honey solution is pipette into each of two test tubes and 5.0 ml P-toluidine solution is added to each. Into one test tube 1 ml water is pipette and into the other 1 ml barbituric acid solution and both mixtures are shaken. The tube with added water, serves as the water blank. The addition of reagents should be done without pause and should be finished in about 1-2 min. The extinction of the sample is read against the blank at 550 nm using a 1-cm cell, immediately the maximum value is reached.

3. Calculation and expression of results:

The method may be calibrated by using a standard of HMF standardized by dissolving commercial or laboratory prepared HMF and assying spectrophotometrically. The equation by which results may be roughly worked out is mg /1000 g HMF = absorbance /thickness of layer *192 . Results are expressed as mg HMF/Kg honey.

The antibacterial effects of honey were tested on gram positive (*Staphylococcus aureus,* and *Bacillus subtilis*) and gram negative *Pseudomonas aeruginosa,* and *Echerichia coli* bacteria. .

Assay of Antibacterial Activity

The antibacterial activity of honeys was measured using a classical diffusion method. In general, this method is based on the observation of inhibition of microbial growth on agarized media (Shimanuki and Knox, 1991).

The medium used for bioassay test:

Nutrient agar medium consisted of (g/L) peptone, 5.0; beef extract, 3.0; NaCl, 5.0 and agar-agar, 15.0. The pH was adjusted to 7.0.

Procedure

1- Twenty four hours old culture of each of the test microbial strains was used.
2- Five ml of sterile distilled water were added to the culture tube and mixed by a vortex mixer.

3- Five drops of the suspension were added to 100 ml of nutrient agar medium at 45°C. This was dispensed among Petri dishes, 10 ml for each.
4- Three concentrations (15,20and 25 %) of each honey with different HMF values in distilled water were prepared in clean sterile test tubes.
5- Analytical paper disks 740-E/2 "Diam" were loaded with the honey and aseptically put on the surface of the seeded plates with the two different test organisms.
6- The plates were left for 2 hours in a refrigerator for diffusion after which the plates were incubated at 30°C for 24 hours.

The diameter of a clear zone around the paper disks was an indication of the antagonistic properties of the honey under study and measured in (mm)

Statistical Analysis

Results were analyzed using Analysis of Variance (ANOVA) with the probability p= 0.05 as the critical value for all test. LSD test was used for separation of statistically significant means.

RESULTS AND DISCUSSION

The different level of HMF value of honey at different concentration (15,20 and 25%) showed a bactericidal activity against a broad spectrum of bacteria with variable degrees, Table (1). By use of least significant differences (LSD) test for multiple comparisons, it is apparent that the HMF value of honey (90.24mglkg) showed the highest inhibitory effect on bacterial growth compared to other honey treatments and concentrations. This value (90.24mg*l*kg) was the highest bactericidal activity against *E.coli* at concentration of 15and 20% honey (P=0.000, F=5190.03, LSD=0.215). Similarly, the HMF (90.24mg*l*kg) value had significantly higher inhibitory effects compared to other treatments on *B. subtilis* growth at concentrations 15,20 and 25% honey (P=0.000,F=5597.46,LSD=0.205), and had significantly higher bactericidal activity against *S. aureus* at concentrations 15 and 25% honey compared to other treatments (P=0.000, F=5862.32, LSD=0.219). However, there was no inhibitory effect of HMF value of honey (203.0mg*l*kg) against all bacterial strains tested at concentration 15% honey. Considerable, no inhibitory effect of all HMF value of honey treatment was noted on *P. aeruginosa*.

Table 1: Summary of the antimicrobial activity of varying concentrations of HMF of honey against 4 bacteria strains (, *S. aureus, B. subtilis, P. aeruginosa and E.coli*).

Bacteria strains	Control (HMF5.0 mg/kg)			HMF (76.8 mg/kg)			HMF (90.24mg/kg)			HMF (138.0mg/kg)			HMF (203.0mg/kg)			HMF (288.0 mg/kg)		
	Honey concentration																	
	15%	20%	25%	15%	20%	25%	15%	20%	25%	15%	20%	25%	15%	20%	25%	15%	20%	25%
Staphylococcus aureus	9.1	12.13	16.1	10.13	16.1	20.13	13.1	18.16	22.1	9.1	14.13	20.13	Na	10.16	4.16	6.16	11.13	16.16
Bacillus subtilis	8.1	12.13	14	8.1	13.1	18.16	12.13	17.13	21.16	16.13	8.1	15.1	Na	5.1	14.1	15.13	8.1	13.16
Pseudomonas aeruginosa	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na
Echerichia coli	7.1	12.13	15.16	12.13	14.1	16.16	12.13	14.1	19.1	4.13	6.1	17.16	Na	4.16	12.1	3.16	7.1	10.16

Staphylococcus aureus: P=0.000; F=5862.32 ; LSD=0.219
Bacillus subtilis : P=0.000; F= 5597.46 ; LSD= 0.205
Echerichia coli : P=0.000;F= 5190.03 ; LSD= 0.215

Mixed observations were made regarding the susceptibility of the test microbes to the honey with high value of HMF. *B. subtilis*, *E.coli* and *S. aureus* were susceptible to all HMF value of honey treatments. All HMF values of honey

treatments and concentrations of honey did not inhibit the growth of *P. aeruginosa*. (Demera and Angert, 2004) reported that *S. aureus* was susceptible to honey from *Apis mellifera* and the stingless bee *Tetragonisca angustula.* (Efem, 1993) and (Radwan, *et al*. 1984) also reported that honey from *A. mellifera* inhibits the growth *E. coli*. Mercan[a] *et al.* (2007) studied the antibacterial activity of honey samples from different sources against *Bacillus cereus*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* .The results showed that majority of the honey samples (75%) generally inhibited the bacteria tested. The honey samples which were obtained from Zmir showed more effectiveness as inhibitors against *P. aeruginosa*, *E. coli*, and *S. aureus*. Tharwat and Danial (2007) suggested that most samples of stored honey inhibited the growth of many of the tested microbial species indicating the less importance of diastase activity, HMF content and pH of honey.

Collectively, our findings indicate that the different levels of HMF value of honey at different concentrations (15,20and 25%) showed a bactericidal activity against a broad spectrum of bacteria with variable degrees**.**

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ARABIC SUMMARY

هل يوجد تأثير لقيم هيدروكسى ميثايل فورفورال (HMF)على نشاط عسل النحل كمضاد بكتيرى؟

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تقيم هذه الدراسة التأثير المضاد للبكتريا لتركيزات مختلفة من نحل العسل الذى له قيم مختلفة من HMF. تم تحضير تركيزات مختلفة من العسل باستخدام الماء المقطر واختبرت هذه التركيزات ضد أربعة أنواع من البكتريا الممرضة وهى *سيودومونس إريجنوزا*، *إيشريشيا كولاى* ، *ستافيلوكوكس إيريس و باسيلس ساتيلس* . بينت النتائج أن العسل بقيم HMF المختلفة أظهر تأثير مضاد للبكتريا بدرجات متفاوتة حيث أظهر العسل بقيمة HMF (90.24 مجم/كجم) أعلى تأثير مثبط لنمو البكتريا إذا ما قورن بالعسل بقيم HMF الأخرى بمختلف التركيزات . لقد أظهرت هذه القيمة اعلى تأثير ضد ، *إيشريشيا كولاى* عند تركيزات عسل 15، 20% وكذلك ضد *باسيلس ساتيلس* عند تركيزات 15، 20 و 25% وضد ، *ستافيلوكوكس إيريس* عند تركيزات 15 ، 25%. من ناحية أخرى لم يكن هناك أى تأثير ضد البكتريا المستخدمة للعسل بقيمة HMF (203.0 مجم/كجم) عند تركيز 15% بينما لم تتأثر بكتريا *سيودومونس إريجنوزا* بأى من التركيزات المختلفة لقيم HMF المستخدمة